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Comparative Study of Serum Albumin, γGT and LDH levels in HIV Coinfection with Hepatitis B Virus in Relationship with CD4 Count

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Abstract

This study was designed to determine the serum albumin, GGT and LDH level in relationship with the CD4 count and hepatitis B and C coinfection in HIV patient. Sixty HIV patients aged 16 to 75 years classified according to the CD4 cell count value and with reference to HBV/HCV co-infection status were studied. Viral Immunochemical status of the patients was determined by ELIZA and Immunoblotting. CD4 cell count was estimated by flow cytometry while the plasma LDH, GGT and Albumin was estimated biochemically by spectrophotometry. The results showed a significantly higher mean value in the plasma level of albumin in HIV-monoinfected patients with CD4 count of between 251-399cells/µl and those with values of ≥400 cells /µl than those with CD4count between 0-250 cells/µl. with $p<0.05$. The results showed a significantly higher mean value in the plasma level of albumin in HIV-HBV patients with CD4 count of between 251-399cells/µl and those with values of ≥400 cells /µl than those with CD4count between 0-250 cells/µl. with $p<0.05$. In conclusion there was a biochemical alteration in the plasma level of Albumin in the HIV monoinfected and HIV-HBV patients and this significant alteration was also found to be directly proportional to CD4 cell count hence the need for the estimation of these parameters in the management of HIV infection. None of the patients was coinfected with HCV.

Keywords: : Serum Albumin, γGT, LDH HIV Coinfection, Hepatitis B Virus, CD4

1. Introduction

HIV patients with chronic HBV and/or HCV are more likely to die of liver disease and have a more rapid progression to Acquired Immunodeficiency Syndrome (AIDS) than patients solely infected with HIV. Blood samples were assayed for the presence of HIV 1/2antibodies, Hepatitis B Surface Antigen (HBsAg) and Hepatitis C antibodies HCVab [1]. The prevalence of HBV and HCV among HIV patients was 13% and 10%, respectively. This calls for integration of HBV and HCV prevention, and treatment into HIV programs.

Liver disease caused by chronic hepatitis B Virus (HBV) and hepatitis C Virus (HCV) is emerging as a significant cause of morbidity and mortality among immunocompromised Human Immunodeficiency Virus (HIV) infected individuals [2]. HBV, HIV, and HCV have similar modes of transmission, and a high proportion of HIV infected adults are at increased risk of developing chronic HBV and HCV infections. This co-infection may result in serious medical complications such as increased risk for liver-related morbidity and mortality.

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 [3].

Biochemical parameters could serve as pointers for early detection of liver disease and renal malfunction in HIV patients. Ruttmann *et al*., [4] Serum gamma-glutamyl transferase (GGT) has been widely used as an index of liver dysfunction.HIV binds to CD4 receptors on helper T-lymphocytes, monocytes, macrophages and neural cells. CD4 cells migrate to the lymphoid tissue where the virus replicates and then infects new CD4-positive cells. As the infection progresses, depletion or impaired function of CD4 cells predisposes to the development of immune dysfunction.

AIDS appear as severe immunodeficiency and evidence of life-threatening infections and unusual tumors. The initial assessment should also 'stage' the disease. The most widely used staging system is the 1993 revision of the CDC's AIDS Surveillance Case Definition for Adolescents and Adults.

According to this system, individuals are assigned a stage according to both a CD4 cell count category and a clinical one. The CD4 cell count categories are as follows:

- CD4 count greater than or equal to 500 cells/mm^3 or 29%.

- CD4 count equal to 200-499 cells/mm^3 or 14%-28%.
- CD4 count less than 200 cells/mm^3 or less than 14%.

People with high serum GGT have higher mortality, partly because of the association between GGT and other risk factors and partly because GGT is an independent predictor of risk. [4].

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. However, in HIV-positive patients with respiratory symptoms, a very high LDH level (>600 IU/L) indicated histoplasmosis (9.33 more likely) in a study of 120 PCP and 30 histoplasmosis patients[5].The aim of this work is to determine levels of albumin, GGT and LDH in relationship with the CD4 count and hepatitis B and C coinfection in HIV patients.

2. Materials and Methods

2.1. Subjects

A total of sixty subjects which consist of both male and female were recruited for the study. Sixty subjects of sero-positive HIV patients either and not co infected with hepatitis B and C viruses serves as test and no control. The sixty subjects were patients from federal medical center, Owo, Ondo State, Nigeria. This study took place between June 2014 and September 2014.

Information were extracted and data were derived from them such as if drugs are been initiated and used and their demographic informations. The age ranges of these subjects were between 16 and 75years and their CD4 counts was also a major consideration.

2.2. Collection and handling of specimen

Collection of blood samples was implemented according to current practices. Serum was used. Blood samples were collected by venapuncture into a plane red top bottle, centrifuged and separated. Serum was separated soon as possible to avoid haemolysis because extensive haemolysis may affect test performance. Extraction of serum were used for the determination of anti-HCV, HBsAg, using immunoblotting, ELISA, and immunochromatographic methods and Gamma glutamyl transferase, Lactate Dehydrogenase and Albumin were carried out biochemically by spectrophotometry using the reagent kits of Randox.

Samples were stored at 2-8^{o}C not more than 24hours; they may be deep-frozen at -20^{o}C. Repeated freezing/thawing cycles was avoided. Samples that have been frozen and defrosted more than 1-2 times were not used.

2.3. Principle of HEPATITIS B surface Antigen (HBsAg) test Using Quick Profile HBsAg TEST.

Hepatitis B surface Antigen 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. If present, HBsAg will react with 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 in sample; no red line will appear in the Test Zone (T). The gold conjugate complexes will continue to migrate alone until 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 continue to 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.

2.4. Principle of HEPATITIS C VIRUSES (HCV) Using Quick Profile HCV ANTIBODY TEST

Hepatitis Ab Test employs chromatographic lateral flow device in a cassette format. Colloidal gold conjugated goat anti-human IgG are dried and immobilized on the fiberglass strip. HCV antigens are immobilized at the Test Zone (T) and goat anti mouse IgG antibodies are immobilized at the Control Zone (C). When the sample is added, it migrates by capillary diffusion rehydrating the gold conjugate. If present in sample, HCV antibodies will bind the gold conjugate anti-human IgG and/or IgM forming complexes. These complexes will continue to migrate along the strip until the Test Zone (T) zone where they are captured by the HCV antigens to form a visible red line. The un-bound gold conjugate will continue to move and bind with goat anti- mouse IgG at the control Zone (C) forming a visible red line. If no HCV antibodies in sample, only a red line is appeared at the control Zone (C), which indicates the validity of the test.

2.5. Determination of serum activities of GGT

Randox kit was used for the quantitative in vitro determination of GGT activities in the serum.

PRINCIPLE (Colorimetric method):

The substrate L –y- glutamyl- 3-carboxy-4- nitroanilide, in the presence of glycyglycine is converted by y-GT in the sample to 5-amino-2-nitrobenzoate which can be measured at 405nm .

2.6. Determination of serum activities of LDH

This reagent is intended for in vitro quantitative determination of lactate Dehydrogenase in serum or plasma.

PRINCIPLE

Kinetic determination of lactate Dehydrogenase according to the following reaction.

Pyruvate +NADH + $H^+ \rightarrow$ L-Lactate + NAD^+

LDH-P activity (U/L) = (ΔOD/min) x 16030.

2.7. Determination of plasma activities of ALBUMIN

Randox kit was used for the quantitative in vitro determination of albumin activities in the serum and plasma.

PRINCIPLE

The measurement of serum albumin is based on its quantitative binding to the indicator 3,3',5,5'-tetrabromo-m cresol sulphonephthalein (bromocresol green, BCG). The albumin-BCG-complex absorbs maximally at 578nm, the absorbance being directly proportional to the concentration of albumin in the sample.

3. Results

COMPARATIVE STUDY OF SERUM ALB, GGT AND LDH IN HIV MONOINFECTED IN RELATIONSHIP TO CD4 COUNT.

TABLE 1: CD4 (0-250 VS 251-399)

	0-250	251-399	T- VALUE	P- VALUE	SIGNIFICANCE
CD4	147.9±15.3	346.1±9.4	10.983134	<0.00001	SIGNIFICANT
LDH	324.3±14.7	324.4±11.7	0.206988	0.418759	NOT SIGNIFICANT
GGT	24.6±3.5	21.5±3.5	0.585108	0.281667	NOT SIGNIFICANT
ALB	33.8±0.5	39.2±1.0	4.899743	1.8E.05	SIGNIFICANT

TABLE 2: 0-250 VS ≥400

	0-250	≥400	T VALUE	P VALUE	SIGNIFICANCE
CD4	147.9±15.3	518.3±18.2	13.622407	<0.00001	SIGNIFICANT
LDH	324.3±14.7	334.6±18.3	0.379331	0.353335	NOT SIGNIFICANT
GGT	24.6±3.5	23.3±1.4	0.377394	0.354048	NOT SIGNIFICANT
ALB	33.8±0.5	40.7±1.1	4.608326	2.5E-05	SIGNIFICANT

TABLE 3: 251-399 VS ≥400

	251-399	≥400	T VALUE	P VALUE	SIGNIFICANCE
CD4	346.1±9.4	518.3±18.2	7.141739	<0.00001	SIGNIFICANT
LDH	324.4±11.7	334.6±18.3	0.406533	0.343316	NOT SIGNIFICANT
GGT	21.5±3.5	23.3±1.4	0.535511	0.29775	NOT SIGNIFICANT
ALB	39.2±1.0	40.7±1.1	-0.930536	0.178984	NOT SIGNIFICANT

The results showed a significantly higher mean value in the plasma level of albumin in HIV-monoinfected patients with CD4 count of between 251-399cells/µl and those with values of ≥400 cells /µl than those with CD4count between 0-250 cells/µl. with $p<0.05$. However there was no significant difference was obtained in the plasma level of LDH and GGT with $p>0.05$. No significant difference was obtained in the plasma values of LDH, GGT and Albumin in HIV-monoinfected with CD4 value of 251-399cells/µl and those with values between ≥400 cells /µl with $p>0.05$ ($p>0.05$) (Tables 1, 2 and 3).

COMPARATIVE STUDY OF SERUM ALB, GGT AND LDH LEVELS IN HIV+HBV IN RELATIONSHIP TO CD4 COUNT

TABLE 4: 0-250 VS 251-399

	0-250	251-399	T VALUE	P VALUE	SIGNIFICANCE
CD4	147.1±16.3	347.9±8.9	10.608019	<0.00001	SIGNIFICANT
LDH	327.4±13.7	324.7±10.9	0.150982	0.440484	NOT SIGNIFICANT
GGT	25.0±3.2	22.6±3.3	0.495321	0.311933	NOT SIGNIFICANT
ALB	34.8±0.8	39.9±1.2	3.464452	0.000788	SIGNIFICANT

TABLE 5: 0-250 VS ≥400

	0-250	≥400	T VALUE	P VALUE	SIGNIFICANCE
CD4	147.1±16.3	510.9±16.9	14.053985	<0.00001	SIGNIFICANT
LDH	327.4±13.7	341.0±18.3	0.516368	0.304186	N0T SIGNIFICANT
GGT	25.0±3.2	22.6±1.3	0.782236	0.219285	NOT SIGNIFICANT
ALB	34.8±0.8	39.9±1.0	3.55086	0.00049	SIGNIFICANT

TABLE 6: 251-399 VS ≥400

	251-399	≥400	T VALUE	P VALUE	SIGNIFICANCE
CD4	347.9±8.9	510.9±16.9	7.095238	<0.00001	SIGNIFICANT
LDH	324.7±10.9	341.0±18.3	0.647915	0.260283	NOT SIGNIFICANT
GGT	22.6±3.3	22.6±1.3	0.059519	0.476414	NOT SIGNIFICANT
ALB	39.9±1.2	39.9±1.0	0.500748	0.309581	NOT SIGNIFICANT

The results showed a significantly higher mean value in the plasma level of albumin in HIV-HBV patients with CD4 count of between 251-399cells/µl and those with values of ≥400 cells /µl than those with CD4count between 0-250 cells/µl. with $p<0.05$. However there was no significant difference was obtained in the plasma level of LDH and GGT with $p>0.05$.No significant difference was obtained in the plasma values of LDH, GGT and Albumin in HIV-HBV patients with CD4 value of 251-399cells/µl and those with values between ≥400 cells /µl with $p>0.05$ ($p>0.05$). None of the patients was coinfected with HCV.

4. Discussion, Conclusion and Recommendation.

Discussion

The results showed a significantly higher mean value in the plasma level of albumin in HIV-monoinfected patients with CD4 count of between 251-399cells/µl and those with values of ≥400 cells /µl than those with CD4count between 0-250 cells/µl. The results showed a significantly higher mean value in the plasma level of albumin in HIV-HBV patients with CD4 count of between 251-399cells/µl and those with values of ≥400 cells /µl than those with CD4count between 0-250 cells/µl. These imply a direct proportional relationship between plasma albumin and CD4 cell count in HIV mono and coinfection with HBV. The findings agree with the report of Olawumi and Olatunji, [6] that reported a significant positive correlations between pretreatment albumin and both pretreatment CD4 count and pretreatment weight and treatment weight and between post treatment albumin and both post-treatment CD4 cell count up to a count of 700 cells/mL and post treatment weight. Olawumi and Olatunji, [6] concluded that in developing countries where many patients may not be able to afford to pay for CD4 cell counts and viral load tests, which are the traditional markers for HIV disease, serum albumin would be a very useful surrogate test for predicting severity of HIV infection and for clinical monitoring of response to antiretroviral therapy. The findings of this work is also consistent with the report of Kannangai *et al*., [7] that found a significant positive correlation of CD4+ T-cell counts and a negative correlation of viral load with albumin and DHEAS levels. Plasma albumin and, to some extent, DHEAS were reported by Kannangai *et al.,* [7] as a promising prognostic markers in monitoring HIV infection.

The results of this work could also be attributed to the fact that associations between lower serum selenium, lower CD4 count, and higher plasma viral load may be related to the frequent occurrence of low serum albumin and the acute phase response among individuals with more advanced HIV-1 infection [8].

4.1 Conclusion.

This work revealed a biochemical alteration in the plasma level of Albumin in the HIV monoinfected and HIV-HBV patients. This significant alteration was also found to be directly proportional to CD4 cell count.

4.2 Recommendation.

Estimation of plasma albumin, LDH, GGT and CD4 count is recommended in the management of HIV infection.

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