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Assessment of Some Cardiac Biomarkers in Adult HIV Seropositives in Nnewi, Nigeria.

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Abstract

Human Immunodeficiency virus (HIV) infection is associated with increased of developing heart disease. Information on cardiac status in HIV infected in Nigerians is lacking. We assessed the impact of HIV infection on serum Myoglobin, Troponin, total CK, CK-MB, LDH and AST of HIV infected adults presenting at Nnamdi Azikiwe University Teaching Hospital Nnewi, Anambra State.

The aim of this study is to determine some cardiac biomarkers in adult HIV seropositive participants, blood samples collected from the 300 randomly recruited participants were used for HIV screening, $CD4^+$ T cell count, serum Myoglobin, Troponin, total CK, CK-MB, LDH and AST. Standard Laboratory methods were used for the analysis. The results showed that the mean serum Myoglobin and Troponin levels were significantly higher in symptomatic HIV participants not on antiretroviral therapy (ART) compared with asymptomatic HIV participants at $P< 0.05$, in each case. Also, the mean serum Myoglobin and Troponin levels were significantly higher in symptomatic HIV participants not on ART compared with HIV asymptomatic HIV participants at $P<0.05$ in each case. Again, the mean serum total Creatine kinase, CK-MB, LDH and AST were significantly higher in symptomatic HIV participants not on ART compared with asymptomatic HIV participants at $P<0.05$ respectively. Similarly, the mean serum total Creatine kinase, CK-MB, LDH and AST were significantly higher in symptomatic HIV participants not on ART compared with HIV seronegative control subjects at $P<0.05$ in each case. Once again, the mean serum total Creatine kinase, CK-MB and AST were significantly higher in asymptomatic HIV participants compared with HIV seronegative control subjects at $P<0.05$ respectively. Increased levels of some cardiac markers were seen in HIV infected participants.

Keywords: HIV, cardiac markers, participants.

1 Introduction

Human immunodeficiency virus (HIV) is a lentivirus that breaks down the body's immune system, infects CD4 lymphocytes, depletes them and gradually leads to Acquired immunodeficiency syndrome (AIDS) which is a fatal illness (Rasool *et al*, 2008). Infection with HIV leads to a progressive impairment of cellular functions, which is characterized by a gradual decline in blood $CD4^+$ T cell counts thereby predisposes the individual to a wide variety of opportunistic bacterial, fungal, viral, protozoal infections and cancerous growth (Khiangte *et al,* 2007).

Reports have it that individuals infected with HIV have increased risk of developing cardiovascular diseases (Sudano *et al,* 2006) such as pericarditis (Sudano *et al*, 2006), endocarditis (Miro *et al*, 2003). There are some cardiac biomarkers used for measuring heart function especially in heart diseases (Vasadevan *et al*, 2011).

In this study, cardiac protein markers such as Myoglobin, Troponin and cardiac enzyme markers such as total CK, CK-MB, LDH and AST were used to evaluate the cardiac function of adult HIV seropositives in Nnewi.

2 Materials and Methods

2.1 Subjects

The study was conducted in Nnamdi Azikiwe University (NAUTH), Nnewi in Anambra state, South East Nigeria. Three hundred subjects were randomly recruited for this study. Using the World Health Organization (6), staging for HIV as a guide, the participants were grouped, comprising of 100 symptomatic HIV subjects on ART, 100 symptomatic HIV subjects not on ART and 100 (male = 49; female = 51) HIV seronegative control subjects. These participants have no history of any disease which was obtained using questionnaire and they were randomly recruited from the voluntary and counseling unit (VCT) of NAUTH. Ethical approval was sorted and obtained from the NAUTH ethics committee and informed consent was obtained from the participants.

2.2 Sample collection

Six milliliter (6 ml) of fasting blood samples were collected from all the participants in this study. 2ml of blood samples were collected into EDTA sample tubes for HIV screening and CD4+ T cell count. The remaining 4 ml of blood sample were collected into plain tube and allowed to clot, centrifuged, the serum separated and analyzed for serum Myoglobin, Troponin, CK, CK-MB, LDH and AST levels.

2.3 Quality control measures

Quality control sera were run along test in each batch of analysis these were compared with the reference values of the control sera. Standard deviation and coefficient of variation were calculated on them.

2.4 Methods of assaying

2.4.1 Determination of Antibodies to HIV-1 and HIV-2 in Human plasma.

Procedure

Two different methods were used, namely, Abbott determine TM HIV -1 and HIV-2 kit, which is an in-vitro visually read immunoassay (Abbott Japan Co.Ltd.Tokyo, Japan) and HIV-1 and 2 STAT-PAK Assay kit, which is an Immunochromatographic test for the quantitative detection of antibodies to HIV-1 and HIV-2 in Human plasma (CHEMBIO Diagnostic system, Inc, New York, USA). For the Abbott determine TM HIV -1 and HIV-2 kit, the procedure described by the manufacturer was used for the analysis. Briefly, 50 µl of participant serum samples separated from the corresponding whole blood samples in EDTA were applied to the appropriately labeled sample pad. After 15 minutes but not more than 60 minutes of sample application, the result was read. This method has inherent quality control that validates the results. For the Immunochromatographic method for HIV -1 and HIV-2, the procedure described by the manufacturer was used for the analysis. In brief, 5 ml of participant's plasma was dispensed into the sample well in the appropriately labeled sample pad. Three drops of the buffer supplied by the manufacturer was added into the appropriately labeled sample pad. The results of the test were read at 10 minutes after the addition of the running buffer. This method has inherent quality control and validates the results.

2.4.2 Determination of $CD4^+$T cells counts by CyFlows SL-Green

Procedure

200 ml EDTA whole blood was collected into PARTEC test tubes (Rohren tube). Then 20 µl of $CD4^+$ T antibody was added into the tube. The contents was mixed and incubated in the dark for15 minutes at room temperature. 800 ml of CD4 buffer was added into the mixture and mixed gently. Then the Partec tube was plugged on the Cyflow counter and the $CD4^+$ T cells were displayed as peaks and interpreted as figures.

2.4.3 Quantitative determination of Troponin I (cTnI) in human sera

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Standard cTn1 Calibration Curve.

Calibration curve was prepared by the method as described by the manufacturer (Life Sciences Advanced Technologies, Inc, USA). The following concentrations of standards of cTn1 were provided: 0 ng/ml, 2 ng/ml, 7.5 ng/ml, 30 ng/ml and 75 ng/ml. Each standard was treated in the same procedure as described for the test sample. A standard calibrate curve was prepared by plotting concentrations (ng/ml) of these standards against their absorbances.

Procedure

The procedure was as described by the manufacturer (Life Sciences Advanced Technologies, Inc, USA). 100 µl of standards, specimens and controls were dispensed into appropriates microtiter wells. 100 µl enzyme

conjugate reagent was dispensed into each well, gently mixed for 30 seconds and incubated at room temperature for 90 minutes. The incubation mixture was emptied into a sink, rinsed 5 times with deionized water and the residual water droplet absorbed with absorbent paper. Then, 100 µl of tetramethylbenzidine (TMB) reagent was added into each well, gently mixed for 10 secs and allowed to developed colour at room temperature for 20 minutes. The reaction was stopped by the addition of 100 µl hydrochloric acid into each well and gently mixed for 30 seconds.

The absorbance of the standard and test were read within 15 minutes of performing the analysis, after zeroing the machine with blank at 450nm wavelength using spectrophotometer. The concentrations of cTn1 in specimens were extrapolated from the graph.

2.4.4 Quantitative determination of Myoglobin in human sera

Standard myoglobin Calibration Curve.

Calibration curve was prepared by the method as described by the manufacturer (Life Sciences Advanced Technologies, Inc, USA). The following concentrations of standards of myoglobin were provided and they were prediluted 10 fold: 0 ng/ml, 25 ng/ml, 100 ng/ml, 250 ng/ml, 500 n/g and 1000 ng/ml. Each standard was treated in the same procedure as described for the test sample. A standard calibrate curve was prepared by plotting concentrations (ng/ml) of these standards against their absorbances.

Procedure

The procedure was as described by the manufacturer (Life Sciences Advanced Technologies, Inc, USA). 20 µl of already 10 fold diluted standards, 10 fold diluted specimen and 10 fold diluted controls was dispensed into appropriates microtiter wells. 200 µl enzyme conjugate reagent was dispensed into each well, gently mixed for 30 seconds and incubated at room temperature for 45 minutes. The incubation mixture was emptied into a sink, rinsed 5 times with deionized water and the residual water droplet absorbed with absorbent paper. Then, 100 µl of tetramethylbenzidine (TMB) reagent was added into each well, gently mixed for 5 seconds and allowed to developed colour at room temperature for 20 minutes. The reaction was stopped by the addition of 100 µl hydrochloric acid into each well and gently mixed for 30 seconds.

The absorbance of the standard and test were read within 15 minutes after zeroing the machine with blank at 450 nm wavelength using spectrophotometer. The concentrations of myoglobin in specimens were extrapolated from the graph.

2.4.5 Quantitative determination of total Creatine kinase (CK) in Human sera

Procedure

The procedure was as described by the manufacturer (Agappe Diagnostics, Switzerland). 100 µl of standards, specimens and controls were dispensed into appropriates test-tubes. 1000 µl of working reagent (D-glucose 125 mmol/L, N-Acetyl-L-cysteine 25 mmol/L, magnesium acetate, 12.5 mmol/L, NADP 2.4 mmol/L, EDTA 2 mmol/L, Hexokinase > 6800 U/L, creatine phosphate 250 mmol/L, ADP 15.2 mmol/L, AMP, 25 mmol/L, Diadenosine pentaphosphate 103 mmol/L, G-6-PDH > 8800 U/L) were dispensed into each test-tube respectively, gently mixed and incubated at 37 ^{o}C for 1 minute. The change in absorbance per minute was measured within 3 minutes at 340 nm.

Calculation:

Creatine kinase Activity (U/L) = (▲OD/ 3 minutes) x 4127.

2.4.6 Quantitative determination of Creatine kinase (CK)- MB in Human sera

Procedure

The procedure was as described by the manufacturer (Agappe Diagnostics, Switzerland). 40 µl of standards, specimens and controls were dispensed into appropriates test-tubes. 1000 µl of working reagent (imidazole (pH 6.7), 125 mmol/L, D-glucose 25 mmol/L, N-Acetyl-L-cysteine 25 mmol/L, magnesium acetate, 12.5 mmol/L, NADP 2.52 mmol/L, EDTA 2.02 mmol/L, Hexokinase > 6800 U/L, creatine phosphate 250 mmol/L, ADP 15.2 mmol/L, AMP, 25 mmol/L, Diadenosine pentaphosphate 103 mmol/L, G-6-PDH > 8800 U/L) were dispensed into each test-tube respectively, gently mixed and incubated at 37 ^{o}C for 100 seconds. The change in absorbance per minute was measured within 3 minutes at 340 nm.

Calculation:

Creatine kinase –MB Activity (U/L) = (▲OD/ 3 minute) x 8254.

2.4.7 Quantitative determination of Aspartate amino transferase (AST).

Procedure

The procedure was as described by the manufacturer (Agappe Diagnostics, Switzerland). 100 µl of standards, specimens and controls were dispensed into appropriates test-tubes. 1000 µl of working reagent (Tris buffer, 88 mmol/L, L-aspartate, 260 mmol/L, MDH, > 600 U/L, LDH > 900 U/L, NADH, 0.20 mmol/l, α- ketoglutarate 12 mmol/L)) were dispensed into each test-tube respectively, gently mixed and incubated at 37 ^{o}C for 100 seconds. The change in absorbance per minute was measured within 3 minutes at 340 nm.

Calculation:

AST Activity (U/L) = (▲OD/ 3 minutes) x 1768

2.4.8 Quantitative determination serum lactate dehydrogenase

Procedure

The procedure was as described by the manufacturer (Agappe Diagnostics, Switzerland). 10 µl of standards, specimens and controls were dispensed into appropriates test-tubes. 1000 µl of working reagent (Tris buffer, 80 mmol/L, pyruvate 1.6 mmol/L, sodium chloride, 200 mmol/L NADH, 240 mmol/L) were dispensed into each test-tube respectively, gently mixed and incubated at 37 ^{o}C for 1 minute. The change in absorbance per minute was measured within 3 minutes at 340 nm.

Calculation:

LDH Activity (U/L) = (▲OD/ 3 minutes) x 16030.

2.5 Data analysis

The result of the analysis was statistically analyzed. Students't-test and one way analysis of variance (ANOVA) were used to compare means. The analyses were performed with the use of Statistical *Package for Social Sciences* (SPSS) statistical software package, version 16.0. P <0.05 is considered statistically significant.

3. Results

The mean serum Myoglobin and Troponin I (ng/mL) levels were significant different amongst the groups at P = 0.000 (F = 40.18 and 709.44) respectively.

Between group comparison showed that the mean serum Myoglobin and Troponin I levels were significantly higher in symptomatic HIV infected subjects not on ART compared with asymptomatic HIV individuals (P<0.05, in each case).

Also, the mean serum Myoglobin and Troponin I levels were significantly higher in symptomatic HIV infected subjects not on ART compared with HIV seronegative control subjects (P<0.05, in each case).

Similarly, the mean serum Myoglobin and Troponin I levels were significantly higher in asymptomatic HIV infected subjects compared with HIV seronegative control subjects (P<0.05, in each case).

However, the CD4+ T cell counts was significantly lower between symptomatic HIV infected subjects not on ART compared with asymptomatic HIV infected subjects, between symptomatic HIV infected subjects not on ART compared with HIV seronegative control subjects and between asymptomatic HIV infected subjects compared with HIV seronegative control subjects (P<0.05, in each case)

The mean serum activities of total CK, CK-MB, LDH and AST (IU/L) levels were significant different amongst the groups at P = 0.000 (F = 96.98; 245.89, 294.54.60 and 75.65) respectively.

Between group comparison showed that the mean serum activities of total CK, CK-MB, LDH and AST levels were significantly higher in symptomatic HIV infected subjects not on ART compared with asymptomatic HIV infected subjects at $p<0.05$ respectively.

Also, between group comparison showed that the mean serum activities of total CK, CK-MB, LDH and AST levels were significantly higher in symptomatic HIV infected subjects not on ART compared with HIV seronegative subjects ($p<0.05$, in each case).

Similarly, the mean serum activities of total CK, CK-MB and AST levels were significantly higher in asymptomatic HIV infected subjects compared with HIV seronegative subjects ($p<0.05$, respectively). But the mean serum activity of LDL was the same in asymptomatic HIV infected subjects compared with HIV seronegative subjects $p>0.05$. (See table1).

Table 1: Comparison of mean ± SD serum levels of Cardiac markers in symptomatic HIV infected subjects on ART (A), not on ART (B) and (C) control group (D).

Group	Myoglobin (ng/mL)	Troponin (ng/mL)	Ck (IU/L)	CK-MB (IU/L)	LDH (IU/L)	AST (IU/L)	CD4 (/Ml)
A (n=100)	60.40± 32.87	1.60 ± 0.25	122.50 ±17.59	13.96± 4.35	187.22± 25.25	31.63± 9.28	374.78± 121.59
B (n=100)	41.43± 13.74	0.66 ± 0.16	102.28 ± 16.79	4.88± 1.64	157.12 ±25.25	20.23± 8.64	437.20 ±129.75
C (n=100)	30.12± 15.03	0.03 ± 0.16	60.84± 28.32	2.51± 1.51	155.20 ±23.42	12.27± 5.57	940.64± 148.85
F(p)-value	40.18 (.000)	709.44 (.000)	96.98 (.000)	245.89 (.000)	294.54 (.000)	75.65 (.000)	216.22 (.000)
A v B	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
A v C	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
B v C	<0.05	<0.05	<0.05	<0.05	>0.05	<0.05	<0.05

Key:

F (p) value = mean ± SD of parameter compared among groups A, B, C and D (using ANOVA).

A V B p value = mean ± SD of parameter compared between group B and C (using t-test).

A V C p value = mean ± SD of parameter compared between group B and D (using t-test).

B V C p value = mean ± SD of parameter compared between group C and D (using t-test).

4. Discussion

In this study, the serum level of Myoglobin, Troponin and serum activities of total CK, CK-MB, LDH and AST were significantly higher in HIV positive individuals. Generally, the increases in the levels of both cardiac proteins and cardiac enzymes were more marked in symptomatic HIV individuals not on ART. Researchers have reported that the increased risk of developing heart disease in HIV individuals may be due to the direct effects of the human virus on the heart (Malnick and Goland, 1998), the chronic inflammatory effect of the virus itself on the myocardium (Lewis, 2000, Aberg, 2009) and the presence of autoantibodies (Malnick and Goland, 1998).

Also, individuals infected with HIV have been linked with heart problem such as pericarditis (Sudano *et al,* 2006) and endocarditis (Miro *et al,* 2003).

In a study, myocardial infarction was observed in HIV subjects (Carr, 2000; Behrens and Schmidt 2005). In another study, Friis *et al* (2003) shows that old age, current or former smoking, previous cardiovascular diseases were associated with an increased risk of myocardial infarction.

5. Conclusion

In this study, we conclude that the serum levels of Myoglobin, Tropinin I and serum activities of total Creatine, Creatine -MB, Lactate Dehydrogenase and Aspartate amino transferase were significantly increased in adult symptomatic HIV positive subjects. Again, the blood level of CD4+ T cell counts were significantly reduced in adult symptomatic HIV positive subjects. This discovery suggests possible impairment of cardiac function which may lead to cardiovascular disorder in HIV infection, therefore, the study suggests that the prediction of severity and monitoring of disease could be done by evaluating the CD4+ T cell counts, Myoglobin, Tropinin I, total Creatine, Creatine -MB, Lactate Dehydrogenase and Aspartate amino transferase in HIV infected individuals. Therefore, it is necessary to include these cardiac markers as part of routine tests for evaluating the cardiac function of people infected with HIV and malaria. Identification of these biomarkers in these individuals will afford more precise and specific tool for early detection, better treatment, better management and follow-up.

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Conflict of interest

There is no conflict of interest whatever with anyone or group of persons.

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