



Evaluation of Apolipoprotein Profile in Hiv/Aids Subjects in Pre and Post 12 Months Antiretroviral Therapy Using 1.5 Mg/ML Troponin Diagnostic Cut-Off for Myocardial Infarction in Nauth Nnewi, South Eastern Nigeria

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ABSTRACT

Patients infected with HIV have an increased risk of developing heart disease. HIV causes Acquired Immune Deficiency Syndrome AIDS by invading the host cells and depleting the immune system. It has been reported that acute myocardial infarction might occur at 1.5 ng/ml troponin level. Information on cardiac status in HIV infected subjects in Nigeria is scanty. The aim is to evaluate the serum

apolipoproteins in HIV subjects, in pre- and post- 12 months antiretroviral therapy (ART) using 1.5ng/ml troponin diagnostic cut-off for myocardial infarction. A total of 100 HIV subjects not on ART devoid of malaria co-infection were recruited in this study. Apolipoprotein parameters were re-classified based on 1.5 ng/ml troponin diagnostic cut-off for myocardial infection. 30 out of the 100 participants that were within 1.5 ng/ml troponin diagnostic cut-off for myocardial infection were followed up on commencement of ART for 12 months. Their blood samples were drawn at 3, 6, 9 and 12 months for some apolipoproteins and CD4 analyses using standard laboratory methods. The results showed that as months of antiretroviral therapy deepened or lengthened, the numbers of HIV positive subjects with values higher than the troponin diagnostic value reduces when compared with values before therapy ($p<0.05$). Paired-wise comparison showed that there were significantly higher levels of CD4 counts, Apo A2, Apo C2, Apo E but lower levels ApoA1, Apo B and Apo C3 in symptomatic HIV individuals before ART when compared with values after therapy at $p<0.05$ respectively. These lower levels ApoA1, Apo B and Apo C3 in symptomatic HIV individuals after therapy may indicate possible cardio-protective effect of the drug on the heart, which may connote immune recovery. The troponin values were significantly higher than the diagnostic cut off for acute myocardial infarction (AMI) studied amongst the group at $p<0.05$ respectively. This may imply that AMI may occur at any group of study.

Keyword: HIV, apolipoproteins, myocardial infarction, CD4, ART.

INTRODUCTION

HIV infection is a systemic disease that has affected many organs of the body including the cardiovascular system especially in advanced stage of the infection ¹. Patients infected with HIV have an increased risk of developing heart disease. Amongst the most common heart problem associated with HIV are pericarditis ², endocarditis ³, cancer that affects the heart ⁴, pulmonary hypertension due to inflammation and genetic factors ⁵ and coronary artery disease (CAD) ⁶. The pathogenesis of HIV-associated cardiomyopathy includes direct effects of the human virus on the heart ⁴, the inflammatory response of the host myocardium to the virus ⁷ and the presence of autoantibodies ⁴ as well as decreased immunity.

Untreated HIV infection has been documented to progress to AIDS. This is a clinical condition that destroys immune system like the CD4 cells, also called the T- helper cells. This cells play an important role in immune defense system of the host. This Immune depletion leaves the patients susceptible to various opportunistic infections, malignancies, anemia and death ⁸. HIV infection has been documented to influence antiviral drugs and affect many organs of the body. It has been reported that acute myocardial infarction (AMI) might occur at 1.5 ng/ml troponin level ⁹. Information on cardiac status in HIV infected subjects in Nigeria is scanty.

Cardiac markers are available to assess cardiac diseases such as apolipoproteins. Troponin is the most sensitive and specific test for myocardial damage because it has increased specificity compared with creatine kinase (CK-MB) (MANN) ¹⁰. It has been reported that acute myocardial infarction (AMI) might occur at 1.5ng/ml Troponin level ⁹.

Conditions such as congestive heart failure, myocarditis and unstable angina have been documented to cause myocardial cell damage with potentially increased cardiac Troponin I

levels above the expected value ¹¹. The most preferred biomarker for myocardial damage is cardiac Troponin (I or T) ¹². The cardiac Troponins I or T are released within 4 - 6 hours of an attack of MI and remain elevated for up to 2 weeks with peak concentrations reached in approximately 12 to 24 hours after infarction and are now preferred markers for assessing myocardial damage ^{12;13}

In Nigeria, not much has been researched into on the levels of Cardiac markers in HIV subjects, hence their evaluation in this study. ARDs are used as chemotherapeutic interventions of HIV/AIDS infection, many a time on long term basis. The drug may present side effects, HIV itself may present with signs & symptoms that may occur as drug side effects. We therefore hypothesize that biomarkers such as Apolipoproteins may exist in the body fluids of subjects living with HIV/AIDS. Identification of these biomarkers and the effect of ART on them will afford more precise and specific tool for early detection, better treatment, better management and follow-up of subjects.

This is a prospective study, conducted in NAUTH, Nnewi in Anambra State. Based on 3.1 % prevalence rate of HIV in Nigeria ¹⁴ and using the formula of Naing *et al*,¹⁵ for sample size calculation, a total of 100 HIV positive subjects with mean age of 40.70 ±10.56 years were randomly recruited. They all underwent HIV and *Plasmodium falciparum* screening. The serum samples were screened for HIV infection using two Immunochromatographic techniques respectively. Similarly, the blood samples were screened for malaria parasite infection using Giemsa stained thick and thin blood films for microscopic detection of malaria parasite and rapid chromatographic immunoassay for qualitative detection of circulating Plasmodium falciparum antigen in the whole blood. Using the World Health Organization ¹⁶, staging for HIV as a guide, the subjects were grouped as follows:

Out of 100 HIV subjects not on ART, apolipoprotein parameters were re-classified based on 1.5 ng/ml troponin diagnostic cut-off for myocardial infection. A total of 30 HIV subjects not on ART devoid of malaria co-infection were followed up on commencement of ART for 12 months. Their blood samples were drawn at 3, 6, 9 and 12 months for some apolipoproteins and CD4 analyses using standard laboratory methods.

Blood sampling: Five milliliters (5 ml) of fasting blood sample was collected from each of the subject in this study. One milliliter (1 ml) of blood samples were collected into EDTA sample containers for malaria antigen estimation, thick and thin film for malaria microscopy, HIV detection and CD4 counts. The remaining four milliliters (4 ml) of blood samples were collected into plain tubes and allowed to clot, centrifuged, separated and aspirated into plain sample tubes and kept frozen until assay for apolipoprotein (apo A1, A2, B, C2, C3 and E) profiles respectively.

Quality control measures: Quality control sera were analyzed along tests samples in each batch of analysis these were compared with the reference values of the control sera. Also, pooled sera were included as control; mean, standard deviation and coefficient of variation were calculated on them.

Questionnaire: Questionnaires were included to get the bio-data of the participants and other health information that were helpful in the interpretation and analysis of results, in this study.

Ethical Clearance: Ethical approval for the study was obtained from the Ethics Review Committee, Nnamdi Azikiwe University Teaching Hospital (NAUTH), Nnewi. Written and oral informed consent was also obtained from the participants and they were assured of anonymity and confidentiality.

Inclusion and Exclusion criteria: Participants on triple combination of Zidovudine, Lamivudine and Nevirapine based on WHO first line of ART, were included in this study¹⁷. Only participants adjudged as HIV stage 1 (asymptomatic HIV) and HIV stage 11 (symptomatic HIV) according to WHO criteria for HIV staging were included in the study. Individuals presenting with HIV stage 111, IV, pregnant women, subjects presented with history of smoking, hypertension, tuberculosis, diabetes, heart, renal diseases and any other clinical condition apart from HIV were excluded from the study.

METHODS OF ASSAY

Diagnosis of Plasmodium falciparum malaria- using Giemsa stained thick and thin blood films and rapid chromatographic immunoassay for qualitative detection in blood¹⁸.

Estimation of Troponin I by the method of^{19;20} using the principle of ELISA, by kits Life Sciences Advanced Technologies, Incorporated, USA.

HIV screening-All blood samples were double screened for HIV using HIV immunoassay kits provided by Abbott Japan Co.Ltd. Tokyo, Japan and CHEMBIO Diagnostic system, Inc, New York, USA.

Estimation of apolipoprotein profile by the method of Tiez,²¹ using the principle of turbidimetric test, by kits from Spinreact laboratories limited, Spain.

Statistical Analysis: The data generated were statistically analyzed. Student's-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 21.0. $P < 0.05$ is considered statistically significant.

RESULTS

Table: Mean $\pm$ SD serum levels of Apolipoprotein in Symptomatic HIV subjects before and after 12 months therapy using 1.5 ng/mL Troponin diagnostic cut-off for myocardial infarction

Group	ApoA1(g/L)	ApoA2(g/L)	Apo B(g/L)	ApoC2(g/L)	ApoC3(g/L)	ApoE(g/L)	CD4 (/μL)
Before therapy (n=20)(A)	0.42 $\pm$ 0.13	0.83 $\pm$ 0.21	2.30 $\pm$ 0.71	0.05 $\pm$ 0.02	0.01 $\pm$ 0.01	0.23 $\pm$ 0.07	367.50 $\pm$ 96.56
3months therapy (n=15)(B)	1.20 $\pm$ 0.15	0.13 $\pm$ 0.05	3.36 $\pm$ 0.33	0.00 $\pm$ 0.00	0.07 $\pm$ 0.02	0.00 $\pm$ 0.00	285.47 $\pm$ 112.82
6 months therapy (n=11)(C)	1.31 $\pm$ 0.12	0.25 $\pm$ 0.05	3.01 $\pm$ 0.28	0.01 $\pm$ 0.00	0.06 $\pm$ 0.01	0.01 $\pm$ 0.01	350.00 $\pm$ 99.40
9 months therapy (n=4)(D)	1.54 $\pm$ 0.05	0.29 $\pm$ 0.04	2.21 $\pm$ 0.74	0.02 $\pm$ 0.01	0.04 $\pm$ 0.02	0.05 $\pm$ 0.01	594.00 $\pm$ 151.70
F-value	145.16	62.14	10.89	40.68	40.92	62.09	11.03
p-value	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
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
A v D	<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
B v D	<0.05	<0.05	<0.05	>0.05	<0.05	<0.05	<0.05
C v D	<0.05	>0.05	<0.05	>0.05	<0.05	<0.05	<0.05

The serum Apo A₁, Apo A₂, Apo B, Apo C₂, Apo C₃ and Apo E levels in symptomatic HIV infected subjects before therapy and at 3, 6 and 9 months therapy based on 1.5 mg/ml Troponin diagnostic cut-off for myocardial infarction were significantly different at P<0.05 (F=145.16, 62.14, 10.89, 40.68, 40.92 and 62.09) respectively. Pairwise comparisons showed that the serum Apo A₁ level was significantly lower in symptomatic HIV infected subjects before therapy compared to when on 3, 6 and 9 months therapy at P<0.05 respectively. Similarly, the serum Apo A₁ level was significantly higher in symptomatic HIV infected subjects on 3 months therapy compared to when on 6 and 9 months therapy at P<0.05 respectively. Also, the serum Apo A₁ level in symptomatic HIV infected subjects on 6 months therapy was significantly higher compared to when on 9 months therapy at P<0.05.

Based on 1.5 mg/ml Troponin diagnostic cut-off for myocardial infarction, pairwise comparisons showed that the serum Apo A₂ level was significantly higher in symptomatic HIV

infected subjects before therapy compared to when on 3, 6 and 12 months therapy at $p < 0.05$ respectively. Also the serum Apo A₂ level in symptomatic HIV infected subjects on 3 months therapy was significantly higher compared to when on 6 and 9 months $p < 0.05$ respectively. Similarly, the serum Apo A₂ level in symptomatic HIV infected subjects on 6 months was significantly higher compared to 9 months at $p < 0.05$ respectively.

Based on 1.5 mg/ml Troponin diagnostic cut-off for myocardial infarction, pairwise comparisons showed that the serum Apo B level was significantly lower in symptomatic HIV infected subjects before therapy compared to when on 3 and 6 months $p < 0.05$. But the serum Apo B level was significantly higher in symptomatic HIV infected subjects before therapy compared to when on 9 months at $p < 0.05$. The serum Apo B value was significantly higher in symptomatic HIV infected subjects on 3 month therapy compared to when on 6 and 9 months therapy at $p < 0.05$ respectively. But, the serum Apo B value was significantly higher in symptomatic HIV infected subjects on 6 months therapy compared to when on 9 months therapy at $p < 0.05$.

Based on 1.5 mg/ml Troponin diagnostic cut-off for myocardial infarction, pairwise comparisons showed that the serum Apo C₂ level was significantly higher in symptomatic HIV infected subjects before therapy compared to when on 3, 6 and 9 months therapy at $p < 0.05$ respectively. Also, the serum Apo C₂ level in symptomatic HIV infected subjects on 3 months significantly lower compared to 6 and 9 months $p < 0.05$ respectively. Similarly, the serum Apo C₂ level in symptomatic HIV infected subjects on 6 months therapy is significantly lower compared to when on 9 months therapy $p < 0.05$.

Based on 1.5 mg/ml Troponin diagnostic cut-off for myocardial infarction, pairwise comparisons showed that the serum Apo C₃ level was significantly lower in symptomatic HIV infected subjects before therapy compared to when on 3, 6 and 9 months therapy at $p < 0.05$ respectively. But the serum Apo C₃ level in symptomatic HIV infected subjects on 3 months was significantly higher compared to 6 and 9 months $p < 0.05$ respectively. Similarly, the value in symptomatic HIV infected subjects on 6 months therapy was significantly higher compared to symptomatic HIV infected subjects when on 9 months therapy $p < 0.05$.

Based on 1.5 mg/ml Troponin diagnostic cut-off for myocardial infarction, pairwise comparisons showed that the serum Apo E level was significantly higher in symptomatic HIV infected subjects before therapy compared to when on 3, 6 and 9 months therapy at $p < 0.05$ respectively. But the serum Apo E level in symptomatic HIV infected subjects on 3 months therapy was significantly lower compared to symptomatic HIV infected subjects when on 6 and 9 months $p < 0.05$ respectively. Also, the serum Apo E level in symptomatic HIV infected subjects on 6 months therapy was significantly higher compared to symptomatic HIV infected subjects when on 9 months therapy at $p < 0.05$.

DISCUSSION

In this study, based on 1.5 mg/ml Troponin diagnostic cut-off for myocardial infarction, the serum apolipoproteins concentrations and the blood CD4 counts were significantly different amongst the groups studied. Serum Apo A1 value was significantly lower in participants before ART than when the participants were on 3, 6 and 9 months ART. Serum Apo A1 value was significantly higher at 9 months than at 3 months and 6 months on ART. The decreased

reduction in serum concentration of Apo A1 may affect the structural composition of HDL, since it is the major apolipoprotein in HDL²². Apo A₁ is often used as a biomarker for cardiovascular diseases²³. It has been known that the activity of lecithin cholesterol acyl transferase (LCAT) in the presence of Apo A₁ as a cofactor²⁴, functions in the removal of excess cholesterol from tissue which is incorporated into HDL and transported to the liver for excretion. Low serum Apo A₁ has been reported to increase coronary heart disease CHD as well as in the diagnosis of hyperlipoproteinaemia²⁵. The significant increase in serum levels of Apo A₁ as the months of therapy lengthened, may indicate possible cardio-protective effects of the ART on the heart, which may connote recovery.

In this study, the serum Apo 2 concentration was significantly higher in participants before ART than when the participants were on 3, 6 and 9 months ART. Normal serum Apo A₂ has been observed to increase the rate of hepatic and lipoprotein lipase activity, the effect which tends to increase plasma TG hydrolysis and thus reduce plasma TG²⁶.

The study showed that Apo B concentration was significantly higher in participants before ART than when the participants were on 3 and 6 months ART. But the Apo B value was significantly reduced at 9 months therapy. The reduced value of Apo B as the months of therapy deepened, may indicate possible cardio-protective effects of the ART on the heart, which may connote recovery.

Excess Apo B in individual has been found to be a better predictor of cardiovascular disease²⁷.

Apo C₂ concentration was significantly higher in participants before ART than when the participants were on 3, 6 and 9 months ART. Elevated serum Apo C₂ level has been linked with hypertriglyceridemia, hypercholesterolemia and hyperchylomicronemia²⁸.

This study revealed that serum Apo C₃ and Apo E were significantly higher in participants before ART than when the participants were on 3, 6 and 9 months ART. Apo E protein is found in chylomicrons and intermediate lipoproteins (IDLs) that is essential in the catabolism of triglycerides-rich lipoprotein constituents and it is essential in the transport of cholesterol to neurons via Apo E receptors²⁹. There is evidence that Apo E protects against atherogenesis, hence the reduced value of Apo E observed as the duration of therapy increased may indicate a cardio-protective role on the heart³⁰. Also, there have been reports on the association between Apo E and neurodegenerative conditions such as multiple sclerosis and Alzheimer's disease³¹.

In this study, CD4 counts were significantly increased in the participants who were on 9 months ART, followed by those on 6 months ART but least on subjects before ART. Elevated enzyme activities of LDH, AST and CD4 counts were observed in symptomatic HIV infected subjects not on ART compared to control³².

Serum HDL-cholesterol was s has been reported to be significantly higher in symptomatic HIV infected subjects as length of antiretroviral therapy deepened when compared to value before therapy. Lower CD4 counts have been reported in both HIV and malaria co-infection³². Dyslipidemia –an elevated serum total cholesterol and triglycerides levels have been reported in HIV infected subjects on antiretroviral therapy^{33,34,35}.

Based on 1.5 mg/ml Troponin diagnostic cut-off for myocardial infarction, Ezeugwunne *et al*,³⁶ observed significantly lower enzyme activities in the levels of T-CK, CK-MB, LDH and serum Myoglobin value but higher levels of CD4 counts as length of anti-retroviral therapy deepened. These finding were attributed as a result of possible cardio- protective effect of the drug on the heart, which may connote immune recovery.

Also, Ezeugwunne *et al*,³⁷ reported increased levels of CK-T, CK-MB, total cholesterol, LDL, Apo C2 but lower levels of LDH and AST in male than in female HIV positive subjects studied.

CONCLUSION

The increased values of troponin observed among the groups were higher than the diagnostic cut off for AMI. This may imply that AMI may occur at any group of study. But the significant reduction in the serum levels of Apo A2, Apo B, Apo C3, Apo E and significant increase in serum levels of Apo A1, Apo C2 and blood CD4 counts as length of therapy lengthened, may indicate possible cardio-protective effects of the ART on the heart, which may connote recovery.

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