

## Evaluation of Apolipoprotein and Lipid Profiles in HIV Symptomatic Subjects Before and After 12 Months Antiretroviral Therapy in NAUTH Nnewi, South Eastern Nigeria.

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### ABSTRACT

**Introduction:** Human Immunodeficiency Virus (HIV) is a lentivirus that breaks down the body's immune system, causing Acquired Immune Deficiency Syndrome (AIDS), that affects so many organs, heart inclusive. Information on cardiac status in HIV infected subjects in Nigeria is scanty.

**Aim:** To evaluate the Apolipoprotein and lipid profiles of HIV subjects before and after Antiretroviral therapy (ART) in Nnewi, South East, Nigeria.

**Methods:** A total of 100 symptomatic HIV subjects with mean age of  $40.70 \pm 10.56$  years were randomly recruited, 30 out of the symptomatic HIV positive subjects without malaria co-infection were followed up for this prospective case-controlled study. Serum lipid profile (Tchol, HDL, LDL and TG), apolipoproteins (Apo A1, A2, B, C2, C3, and Apo E) and CD4 counts were measured using standard laboratory methods. Analysis of variance, student t test, and graph pad prism were used for data analyses.

**Results:** paired-wise comparison showed that there were significantly lower levels of CD4 counts, ApoA1, Apo A2, ApoC3, Tchol, LDL, HDL and TG but higher levels of Apo B, Apo C2 and Apo E in symptomatic HIV subjects before ART when compared with after therapy at  $p < 0.05$  respectively.

**Conclusion:** This study showed significant reduction in the serum level of Apo A<sub>2</sub>, Apo B, Apo C<sub>3</sub>, Apo E and significant increase in levels of HDL and CD4 counts respectively in symptomatic HIV subjects as therapy lengthened, thereby suggesting improved cardiac function.

**Keyword:** HIV, apolipoproteins, lipid profile, ART.

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### Author's contributions:

*This work was carried out and approved in collaboration between all authors. EIP, OCC, AJE, and MSC designed the study; EIP, OCC, AJE, and MSC sourced for funding; EIP, OCC, AJE, MSC, IM, ARA, AKA, and OHGO wrote the protocol; OEC, ARA, IM, OHGO, AKA and OCN contributed in literature search; EIP, OCC, AJE and MSC did the experiments; EIP, ARA, IM, and MSC did statistical analysis; OEC, EIP, ARA, OCN drafted the manuscript; OCC, AJE and MSC supervised the study; EIP and OEC Wrote the final manuscript; OEC, AKA, ARA, OHGO, and OCN proof read the manuscript.*

**Received:** March 24, 2019; **Accepted:** April 07, 2019; **Published:** April 25, 2019.

**Citation:** Ezeugwunne IP, Ogbodo EC, Analike RA, Ifeanyichukwu M, Ogah HGO, Amah AK *et al.* Evaluation of Apolipoprotein and Lipid Profiles in HIV Symptomatic Subjects Before and After 12

Months Antiretroviral Therapy in NAUTH Nnewi, South Eastern Nigeria. *J Med Lab Sci*, 2019;29:52-60

## INTRODUCTION

Human Immunodeficiency Virus (HIV) is a member of the genus lentivirus, which lacks DNA but contains RNA thus it does not replicate outside of living host cells. However, HIV enters into host cells, replicates thereby releasing HIV virions from infected cells, depleting CD4 cells and immune system (1). This weakens the immune system, develops AIDS by depleting CD4<sup>T</sup> cells (2) and allows opportunistic infections. HIV targets the immune system mainly CD4 cells (3, 4). Malaria and HIV are the most common infections in sub-Saharan Africa. Both diseases kill millions of people annually (3, 4).

AIDS has been declared a pandemic disease by World Health Organization (WHO), affecting 35million people globally, two- third of them from sub-Saharan Africa. Globally South Africa has the largest population with HIV, followed by Asian, Nigeria and India (5). The HIV pandemic is most severe in Sub-Saharan Africa Global HIV and AIDS Statistics (2017). South Africa has the largest population of HIV individuals globally (19.6 m), followed by Nigeria (3.2 m), and Philippines (2.1 m) (4). 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 (6). Apolipoproteins are proteins that bind lipoproteins (chylomicrons, VLDL, LDL, IDL & HDL), whose main function is to transport lipids. Major classes of apolipoproteins are Apo A, B, C & E. Lipoproteins are transported endogenously and exogenously. Any interference on the Lipid synthesis and transport may elevate parameters such as LDL, IDL, VLDL and TG and they are associated with increased cardiovascular risk (7). Also, the ratio of total cholesterol and HDL is one of the best lipoprotein indices in predicting cardiovascular risk in the general population (8).

It has been suggested that apo B and apo A1 or its ratio be measured so to significantly improve

the assessment of cardiovascular risk (7). There is evidence that the measurement of various forms of apolipoproteins may improve the prediction of the risk of cardiovascular disease (7).

In Nigeria, not much has been researched on the status of apolipoproteins in HIV subjects, hence their evaluation in this study. Antiretroviral drugs (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. Hence it is needful to evaluate the Apolipoprotein and lipid profiles of HIV subjects before and after ART in Nnewi, South East, Nigeria.

## MATERIALS AND METHODS:

This is a prospective study, conducted in NAUTH, Nnewi in Anambra State. Based on 3.1 % prevalence rate of HIV in Nigeria (9) and using the formula of Naing *et al.* (10) for sample size calculation, a total of 100 symptomatic HIV subjects with mean age of 40.70 ±10.56 years were randomly recruited.

Thirty (30) out of the symptomatic HIV positive subjects without malaria co-infection were followed up for 3, 6, 9 and 12 months. Serum lipid profile (Tchol, HDL, LDL and TG), apolipoproteins (Apo A1, A2, B, C2, C3 and ApoE) and CD4 counts were measured using standard laboratory methods. Analysis of variance, student t test, and graph prism were used for data analyses.

They all underwent HIV detection and screening by rapid Immunochromatographic Immunoassay techniques. *Plasmodium falciparum* detection and screening- using Giemsa stained thick and thin blood films and rapid chromatographic immunoassay for qualitative detection in blood.

**Blood sampling:** Six milliliters (6 ml) of fasting blood sample was collected from each of the subject in this study. Two milliliter (2ml) 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 lipid profile, Apo A1, A2, B, C2, C3 and E;

**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 biodata 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 study (11, 12). 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 and malaria falciparum infection were excluded from the study.

**Methods of Assay:**

**Estimation of Apolipoprotein profiles** (Apo A1, A2, C1, C2, C3, E) by the method of Tietz, (13) using the principle of turbidimetry, by kits from Spinreact Laboratories Limited, Spain.

**Lipid profile by colourimetric method using kits supplied by Biosystems, Spain:**

Total cholesterol- Enzymatic method as described by Allain *et al.* (14).

Triglycerides - Enzymatic hydrolysis and oxidation by lipase method as described by Buccolo and David, (15). HDL –Precipitating enzymatic method as described by Assmann *et al.* (16). LDL – Calculated from a formula described by Kaplan *et al.* (17).

**Diagnosis of *Plasmodium falciparum* malaria-** using Giemsa stained thick and thin blood films (18) and rapid chromatographic immunoassay for qualitative detection in blood.

**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.

**Determination of CD4<sup>+</sup>T cells counts-** using Cyflow counting system.

**Statistical Analysis:** The data generated were statistically analyzed. Students<sup>2</sup>-test, one-way analysis of variance (ANOVA), correlation and Graphpad prism 5 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.

**RESULT:**

The result of analysis of variance showed that the mean serum levels of ApoA1, Apo A2, Apo B, Apo C2, ApoC3 and Apo E were significantly different amongst the group (F=346.487, 73.339, 36.280, 99.932, 124.542 and 160.065) (p<0.05), respectively (see table 1).

Also, the serum levels of total cholesterol (Tchol), LDL and HDL as well as the mean CD4 cell count were significantly different amongst the group ( $F=26.268, 7.732, 3.586$  and  $49.459$ ) ( $p<0.05$ ) respectively, whereas, the mean serum level of triglyceride (TG) did not differ significantly amongst the group ( $F=1.072$ ;  $p>0.05$ ), (see table 2).

The mean serum CD4 cell counts, ApoA1, Apo A2, ApoC3, Tchol, LDL, HDL and TG levels

were significantly lower in symptomatic HIV subjects before ART when compared with after therapy at  $p<0.05$  respectively, whereas, the mean serum levels of ApoB, Apo C2 as well as Apo E were significantly higher in symptomatic HIV subjects before ART when compared with after therapy at  $p<0.05$  respectively. (See fig. 1, 2, 3, 5 and 6 respectively).

**Table 1: Serum levels of Apolipoprotein profile in symptomatic HIV infected subjects not on ART (A), 3 months ART (B), 6 months ART (C),9 months ART (D) and 12 months ART (E)**

Groups	Apo A1 (g/L)	Apo A2 (g/L)	Apo B (g/L)	Apo C2 (g/L)	Apo C3 (g/L)	Apo E (g/L)
A (n= 30)	0.04 (0.17)	0.85 (0.19)	2.31 (0.72)	0.05 (0.02)	0.01 (0.01)	0.22 (0.08)
B (n=30)	1.16 (0.16)	0.13 (0.05)	3.31 (0.41)	0.00 (0.00)	0.06 (0.01)	0.01 (0.01)
C (n= 25)	1.34 (0.10)	0.27 (0.19)	2.93 (0.41)	0.01 (0.00)	0.06 (0.01)	0.01 (0.01)
D (n=25)	1.47 (0.09)	0.28 (0.03)	2.62 (0.53)	0.02 (0.01)	0.05 (0.01)	0.02 (0.01)
E (n=20)	0.39 (0.31)	1.59 (0.49)	0.02 (0.01)	0.02 (0.01)	0.04 (0.01)	0.04 (0.01)
F-value	346.487	73.339	36.280	99.932	124.542	160.065
P-value	0.000	0.000	0.000	0.000	0.000	0.000

**Table 2: Serum levels of lipid profile in symptomatic HIV infected subjects not on ART (A), 3 months ART (B), 6 months ART (C),9 months ART (D) and 12 months ART (E)**

Groups	CD4 (μL)	Tchol (mmol/L)	LDL (mmol/L)	HDL (mmol/L)	TG (mmol/L)
A (n= 30)	362.93 (104.56)	3.43 (0.28)	2.37 (0.24)	0.96 (0.04)	0.78 (0.08)
B (n=30)	251.57 (89.50)	3.92 (0.46)	2.85 (0.46)	1.08 (0.96)	0.89 (0.20)
C (n= 25)	354.92 (109.95)	4.55 (0.88)	3.13 (0.98)	1.29 (0.70)	0.95 (0.51)
D (n=25)	522.32 (182.88)	4.82 (0.93)	3.17 (1.06)	1.36 (0.71)	0.94 (0.41)
E (n=20)	754.85 (178.13)	5.57 (1.31)	3.68 (1.34)	1.64 (0.61)	0.85 (0.43)
F-value	49.459	26.268	7.732	3.586	1.072
P-value	0.000	0.000	0.000	0.008	0.373

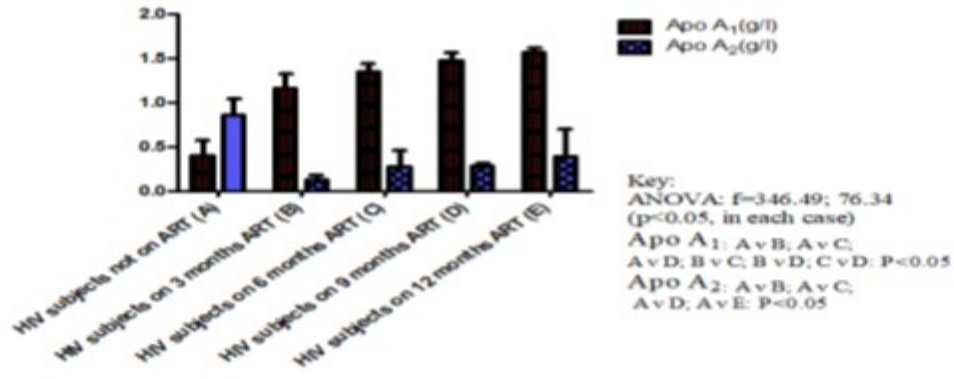


Fig 1: Showing a mean  $\pm$  SD serum levels of Apo A<sub>1</sub> & A<sub>2</sub> in follow-up subjects in pre- and post therapy.

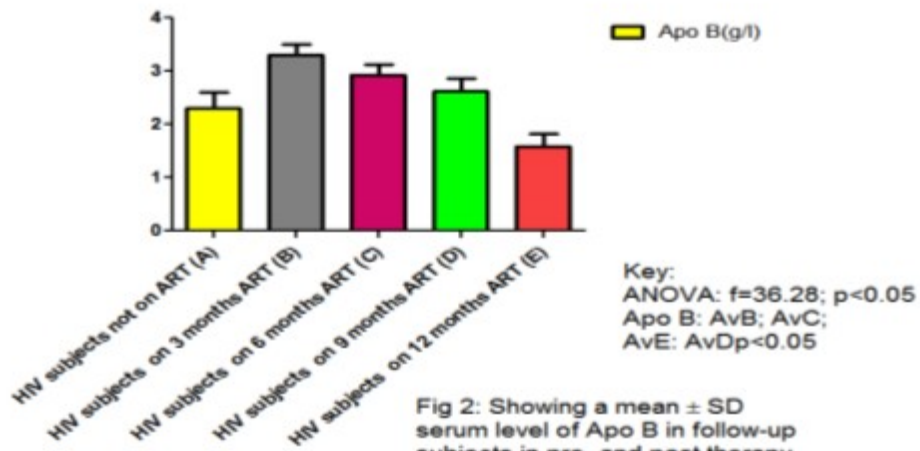
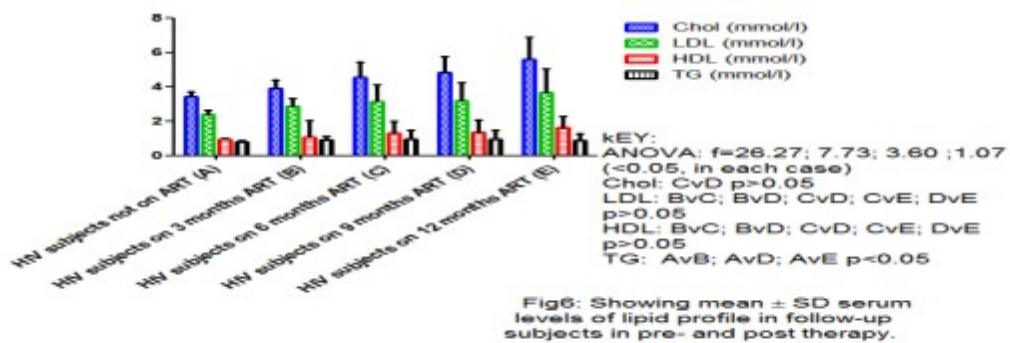
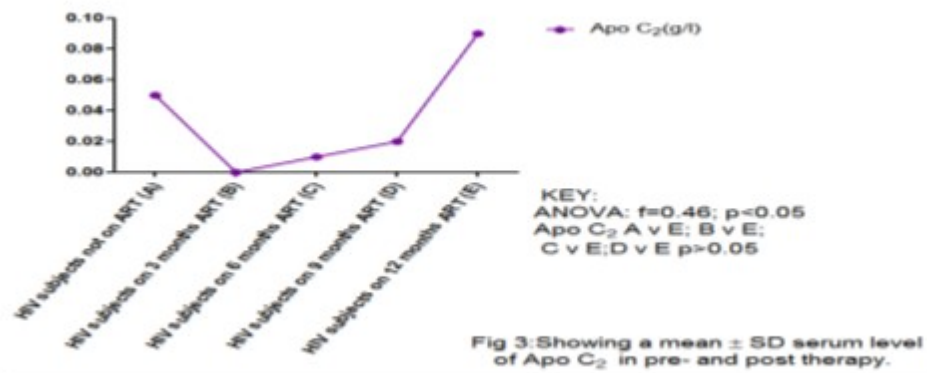
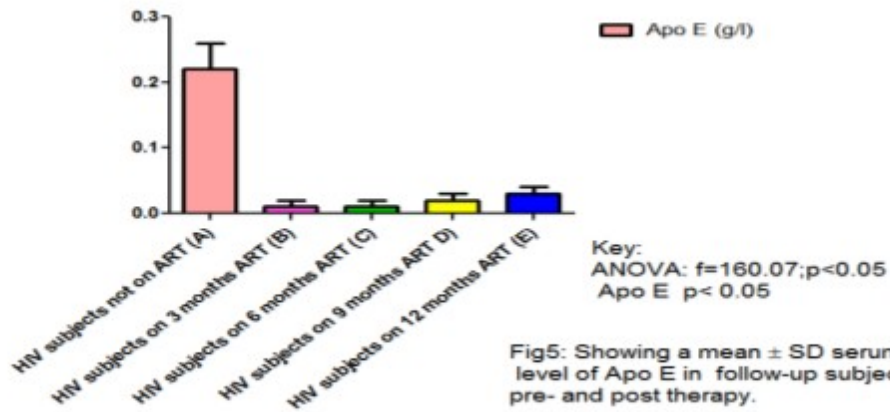


Fig 2: Showing a mean  $\pm$  SD serum level of Apo B in follow-up subjects in pre- and post therapy.





## DISCUSSION

The serum level of Apo A<sub>1</sub> was significantly higher while Apo A<sub>2</sub> was significantly lower in symptomatic HIV subjects on 3, 6, 9, and 12 months therapy when compared with value before therapy. The levels of Apo A<sub>1</sub> & Apo A<sub>2</sub> observed with lengthened therapy might suggest an immune recovery, impaired by HIV infection, hence playing a cardio-protective role. Apo A<sub>1</sub> is often a biomarker for cardiovascular diseases (19, 20). Lower serum Apo A<sub>2</sub> tends to reduce plasma TG and hence increased plasma HDL (21). The serum level of Apo B was significantly lower in symptomatic HIV subjects on 3, 6, 9, and 12 months therapy when compared with value before therapy. Elevated level of Apo B has been found to be a better predictor of cardiovascular diseases (22). There was a significantly sharp reduction of serum Apo C<sub>2</sub> from before therapy to 3 months. The value significantly rises from 3 to 9 months therapy. But, there was a significantly sharp increase of serum Apo C<sub>3</sub> from before therapy to 3 months. The value significantly reduces from 3 to 9 months therapy. The observations indicate immune recovery and cardio-protective roles. Elevated level of serum Apo C<sub>3</sub> has been linked with coronary heart diseases (23).

The serum Apo E level was significantly reduced in symptomatic HIV infected subjects as length of therapy deepened when compared to value before therapy. There is evidence that Apo E protects against atherogenesis, (24). Elevated level of Apo E has been linked with psoriasis (25).

The lipid profile was significantly higher in symptomatic HIV infected subjects as length of therapy deepened when compared to value before therapy. (26, 27, 28). An

increased LDL level is a strong predictor for cardiovascular diseases (29). Although, the elevated level of HDL observed in this subjects as length of therapy elongated may indicate cardio-protectiveness on the host.

High serum total cholesterol and Triglyceride levels (dyslipidemia) have been seen in HIV infected individuals on ART (26, 27). Also, higher serum levels of total cholesterol, LDL, Triglyceride and lower level of HDL have been reported to cause cardiovascular risk in individuals (30, 31). HIV infection has been reported to affects the adipocyte function, causing fat redistribution ) and lipoatrophy (32, 33).

The CD4 count was sharply reduced from before therapy to 3 months therapy in symptomatic HIV infected subjects and significantly increased from 3 months to as length of therapy lengthened.

## CONCLUSION

This study showed significant reduction in the serum level of Apo A<sub>2</sub>, Apo B, Apo C<sub>3</sub>, Apo E and significant increase in levels of HDL and CD4 counts respectively in symptomatic HIV subjects as therapy lengthened, thereby suggesting improved cardiac function

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