Gender Comparison Of Apolipoprotein And Lipid Profiles In HIV Seropositives In Nauth Nnewi, South Eastern Nigeria.


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Abstract
HIV breaks down the body’s immune system and progressively leads to Acquired Immune Deficiency Syndrome, a fatal illness, prevalent more in Africa, Nigeria inclusive. Patients infected with HIV have an increased risk of developing heart disease. Information on cardiac status in HIV infected subjects in Nigeria is scanty. The study assessed the impact of HIV infection on serum Apolipoprotein and lipid profiles. A total of 390 (M = 220, F = 170) subjects were studied. The subjects were grouped based on WHO criteria for staging HIV infection and HIV seronegative controls. Enzyme linked Immuno Assay (ELISA) was used for Apolipoproteins, Myoglobin and Troponin I. Spectrophotometric method was used for lipid profile and enzyme cardiac markers. The results showed significantly elevated serum levels of Apo A1, CK-T, CK-MB, but significantly lower level of total cholesterol (T-Chol), LDL, LDH and AST in male than in femalesymptomatic HIV infected subjects on ART at P<0.05. There were significantly higher serum enzyme activities of CK-T, CK-MB, levels of Apo C2, Apo E, T-Chol, LDL but lower serum activities of LDH, AST and HDL in male than in femalesymptomatic HIV infected subjects on ART at P<0.05. The activities of CK-T, CK-MB; T-Chol, LDL, Apo C2 level were significantly higher but Apo A2, LDL and AST were significantly lower in male than in female asymptomatic HIV positive subjects studied at P<0.05. Conclusively, there were 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.

Key Words: Apolipoprotein, lipid, HIV, CK-T, CK-MB

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I. Introduction:
HIV is the most common infection in sub-Saharan Africa. It has killed millions of people annually (UNAID, 2014, 2017). HIV established infection once the entered the host cells. It replicate in the cells, affecting the immune cells, leading to the depletion of immune cells, causing Acquired Immune Deficiency Syndrome (AIDS) (Martins and Bandres, 1999). This immune depletion leaves the patients susceptible to various opportunistic infections, malignancies, anaemia and death (Okolie et al, 2003).

Cardiac markers are available to assess cardiac diseases such as CK-MB which is myocardic specific (Wendy and Robbert, 2003). AST has been reported to be elevated in cardiac diseases (Vasudevan, 2011). Troponin is also enlisted as a Cardiac marker. Troponin is the most sensitive and specific test for myocardial damage because it has increased specificity compared with creatine kinase (CK-MB) (MANN). LDH
has been found useful in the diagnosis of a myocardial infarction (crook) Myoglobin is a protein released and elevated during myocardial injury (Crook, 2006). It may also be included in assessing heart function (kagan)

AIDS has been declared a pandemic disease by WHO as it affects countries globally (WHO, 2009). It has affected over 30 million people worldwide (Willey et al, 2002; Maplanka, 2007;USAID, 2008). The population affected by HIV in Africa especially sub-Saharan Africa is about 70% (Kumar et al, 2006). It is now the leading cause of death in sub-Saharan Africa and the fourth leading cause of mortality worldwide and over 95% of these deaths have occurred among young adults in the developing world (Guatelli et al, 2002; USAID, 2008). Highly active antiretroviral therapy (HAART) has generally been taken as the gold standard in the management of HIV patients (Odunukwe et al, 2005).

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 (Cohen et al, 2005). Patients infected with HIV have an increased risk of developing heart disease. Amongst the most common heart problem associated with HIV are pericarditis (Sudano et al, 2006), endocarditis (Miro et al, 2003), cancer that affects the heart (Malnick and Goland, 1998), pulmonary hypertension due to inflammation and genetic factors (Pellicelli et al, 2001) and coronary artery disease (CAD) (Iloeje et al, 2005). The pathogenesis of HIV-associated cardiomyopathy includes direct effects of the human virus on the heart (Malnick and Goland, 1998), the inflammatory response of the host myocardium to the virus (Lewis, 2000) and the presence of autoantibodies (Malnick and Goland, 1998) as well as decreased immunity. HIV infection has huge impact on adipocyte during its replication in T cells, affecting FA synthesis, LDL and TG secretions resulting in dyslipidemia and lipodystrophy syndrome (Rasheed et al, 2008).

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, Cardiac proteins and enzymes 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 (NACA, 2011) and using the formula of Naing et al, (2006) for sample size calculation, a total of thirty (400) 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 (WHO, 2006), staging for HIV as a guide, the subjects were grouped as follows:

- Symptomatic HIV on Antiretroviral therapy (ART) (M = 50, F = 50).
- Symptomatic HIV not on ART (M = 64, F = 36).
- Asymptomatic HIV subjects (M = 57, F = 43) and HIV seronegative control subjects (M = 49, F = 51).
- Apolipoprotein and ELISA was used for Apolipoproteins, Myoglobin and Troponin I.
- Spectrophotometric method was used for lipid profile and enzyme cardiac markers. Analysis of variance and student t test were used for data analyses.

**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 Cardiac markers name trogical therapy (ART) (M = 50, F = 50) 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 (WHO, 2006), staging for HIV as a guide, the subjects were grouped as follows:

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- Spectrophotometric method was used for lipid profile and enzyme cardiac markers. Analysis of variance and student t test were used for data analyses.

**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.

**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. 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:

Estimation of cardiac protein markers (Troponin I, myoglobin estimation) by the method of 17,18,19, using the principle of ELISA, by kits Life Sciences Advanced Technologies, Incorporated, USA.

Estimation of cardiac enzymes (CK-total, CK-MB and LDH) by the method of 20,21 and 22,23, using the principle of kinetic determination, by kits from Agappe Diagnostics, Switzerland.

Diagnosis of Plasmodium falciparum malaria- using Giemsa stained thick and thin blood films 24 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.

Estimation of apolipoprotein profile by the method of Tiez, 1983, 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.

II. Results:

Table 1: Comparison of mean ± SD serum levels of Apolipoprotein and Lipid profiles between (a) male and (b) female in (1) symptomatic HIV on ART, (2) symptomatic HIV not on ART, (3) asymptomatic HIV and (4) HIV negative control subjects.

<table>
<thead>
<tr>
<th>Group</th>
<th>Apo A-1 g/L</th>
<th>Apo A-2 g/L</th>
<th>Apo B g/L</th>
<th>Apo C-2 g/L</th>
<th>Apo C-3 g/L</th>
<th>Chol mmol/L</th>
<th>LDL mmol/L</th>
<th>HDL mmol/L</th>
<th>TG mmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) a (n= 50)</td>
<td>1.19 ± 0.21</td>
<td>0.28 ± 0.10</td>
<td>1.47 ± 0.76</td>
<td>0.06 ± 0.05</td>
<td>0.10 ± 0.02</td>
<td>5.86 ± 0.70</td>
<td>3.77 ± 0.44</td>
<td>1.12 ± 0.14</td>
<td>1.75 ± 0.22</td>
</tr>
<tr>
<td>(2) a (n= 64)</td>
<td>0.34 ± 0.19</td>
<td>0.52 ± 0.34</td>
<td>2.49 ± 0.83</td>
<td>0.05 ± 0.02</td>
<td>0.26 ± 0.12</td>
<td>3.56 ± 0.24</td>
<td>1.77 ± 0.05</td>
<td>0.89 ± 0.06</td>
<td>0.08 ± 0.04</td>
</tr>
<tr>
<td>(3) a (n= 57)</td>
<td>0.61 ± 0.25</td>
<td>0.41 ± 0.11</td>
<td>1.43 ± 0.43</td>
<td>0.05 ± 0.01</td>
<td>0.12 ± 0.07</td>
<td>4.30 ± 0.12</td>
<td>2.78 ± 0.07</td>
<td>1.19 ± 0.04</td>
<td>1.19 ± 0.04</td>
</tr>
<tr>
<td>(4) a (n= 49)</td>
<td>1.31 ± 0.04</td>
<td>0.26 ± 0.11</td>
<td>0.80 ± 0.39</td>
<td>0.05 ± 0.01</td>
<td>0.05 ± 0.02</td>
<td>4.68 ± 0.22</td>
<td>2.33 ± 0.14</td>
<td>1.40 ± 0.05</td>
<td>1.45 ± 0.05</td>
</tr>
<tr>
<td>(b) female</td>
<td>1.24 ± 0.05</td>
<td>0.22 ± 0.04</td>
<td>0.56 ± 0.15</td>
<td>0.05 ± 0.02</td>
<td>0.05 ± 0.02</td>
<td>4.56 ± 0.25</td>
<td>2.32 ± 0.12</td>
<td>1.35 ± 0.06</td>
<td>1.43 ± 0.05</td>
</tr>
</tbody>
</table>

P value: 0.000 .030 .030 .653 .821 .141 .011 .786 .000 .013

Key: p value = mean ± SD of parameter compared between group a and b (using t-test).
Gender Comparison Of Apolipoprotein And Lipid Profiles In Hiv Seropositives In Nauth .

Table 2: Comparison of mean ± SD serum levels of Cardiac markers between (a) male and (b) female in (1) symptomatic HIV on ART, (2) symptomatic HIV not on ART, (3) asymptomatic HIV and (4) HIV negative control subjects.

<table>
<thead>
<tr>
<th>Group</th>
<th>Myoglobin ng/mL</th>
<th>Troponin ng/mL</th>
<th>Creatine-kinase total U/L</th>
<th>Creatine-kinase MB U/L</th>
<th>Lactate Dehydrogenase U/L</th>
<th>Aspartate transaminase U/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) a (n= 50)</td>
<td>38.89 ± 37.94</td>
<td>1.37 ± 0.16</td>
<td>100.22 ± 8.15</td>
<td>8.14 ± 2.31</td>
<td>173.48 ± 23.41</td>
<td>20.87 ± 5.41</td>
</tr>
<tr>
<td></td>
<td>42.61 ± 42.23</td>
<td>1.39± 0.14</td>
<td>91.48 ± 4.05</td>
<td>7.01± 1.76</td>
<td>201.68 ± 14.22</td>
<td>25.35 ± 4.55</td>
</tr>
<tr>
<td>P value</td>
<td>.643</td>
<td>.410</td>
<td>.000</td>
<td>.007</td>
<td>.000</td>
<td>.000</td>
</tr>
<tr>
<td>(2) a (n= 64)</td>
<td>63.61 ± 35.83</td>
<td>1.61 ± 0.25</td>
<td>129.64 ± 11.80</td>
<td>15.68 ± 4.00</td>
<td>176.02 ± 23.38</td>
<td>26.31 ± 7.08</td>
</tr>
<tr>
<td></td>
<td>54.72 ± 26.34</td>
<td>1.57± 0.23</td>
<td>109.72 ± 19.06</td>
<td>10.89± 3.12</td>
<td>207.14 ± 13.46</td>
<td>41.09 ± 3.15</td>
</tr>
<tr>
<td>P value</td>
<td>.196</td>
<td>.377</td>
<td>.000</td>
<td>.000</td>
<td>.000</td>
<td>.000</td>
</tr>
<tr>
<td>(3) a (n= 57)</td>
<td>39.36 ± 13.24</td>
<td>0.65 ± 0.15</td>
<td>114.05 ± 9.23</td>
<td>5.92 ± 1.15</td>
<td>143.79 ± 18.86</td>
<td>14.96 ± 5.00</td>
</tr>
<tr>
<td></td>
<td>44.19 ± 14.05</td>
<td>0.69± 0.16</td>
<td>86.67 ± 10.64</td>
<td>3.49± 1.07</td>
<td>174.79 ± 21.62</td>
<td>27.21 ± 7.43</td>
</tr>
<tr>
<td>P value</td>
<td>.082</td>
<td>.507</td>
<td>.000</td>
<td>.000</td>
<td>.000</td>
<td>.000</td>
</tr>
<tr>
<td>(4) a (n= 49)</td>
<td>28.06 ± 13.58</td>
<td>0.26 ± 0.17</td>
<td>85.80 ± 13.48</td>
<td>3.48 ± 1.44</td>
<td>135.90 ± 17.37</td>
<td>7.99 ± 3.08</td>
</tr>
<tr>
<td></td>
<td>32.11 ± 16.21</td>
<td>0.34± 0.13</td>
<td>36.86 ± 14.71</td>
<td>1.57 ± 0.82</td>
<td>173.75 ± 9.00</td>
<td>16.39 ± 4.04</td>
</tr>
<tr>
<td>P value</td>
<td>.179</td>
<td>.009</td>
<td>.000</td>
<td>.000</td>
<td>.000</td>
<td>.179</td>
</tr>
</tbody>
</table>

Key:  p value = mean ± SD of parameter compared between group a and b (using t-test).

Pairwise comparisons showed that the serum activities of CK-T CK-MB, serum total cholesterol were significantly higher in male symptomatic HIV infected subjects on ART when compared to the females subjects at P<0.05 respectively. But the serum activities of LDH, AST and serum apo A1 were significantly lower in male symptomatic HIV infected subjects on ART when compared to the females subjects at P<0.05 respectively.

Also, Pairwise comparisons showed the serum activities of CK-T CK-MB, serum total cholesterol, LDL-cholesterol, HDL-cholesterol, Apo C2, Apo E were significantly higher in male symptomatic HIV infected subjects not on ART when compared to the females subjects at P<0.05 respectively. However, the serum activities of LDH, AST and serum HDL-cholesterol were significantly lower in male symptomatic HIV infected subjects not on ART when compared to the females subjects at P<0.05 respectively. Other parameters remain the same between the sexes at p>0.05 respectively.

The activities of CK-T, CK-MB; T-Chol, LDL, Apo C2 level were significantly higher in male asymptomatic HIV positive subjects than in female subjects studied at P<0.05 respectively but the serum levels of Apo A2, LDL and AST were significantly lower in male asymptomatic HIV positive subjects than in female subjects studied at P<0.05 respectively. Other parameters remain the same between the sexes at p>0.05 respectively.

Finally, there were increased serum activities of CK-T, CK-MB,and serum levels of total cholesterol, LDL- Cholesterol, HDL-cholesterol, triglycerides, Apo A1, Apo A2 and Apo B but lower serum activities of LDH and serum level of myoglobin in male HIV seronegative subjects studied. Other parameters remain the same between the sexes at p>0.05 respectively.

III. Discussion:

The present study showed that the serum concentrations of Apo A1, Apo A2, Apo B, Apo C2, and Apo E were significantly different in HIV positive individuals. Apo A1were significantly lower in in male symptomatic HIV infected subjects not on ART than in the female subjects. Apo A1were significantly higher in in male symptomatic HIV seronegative subjects. The decreased reduction in serumconcentration of Apo A1 may
affect the structural composition of HDL, since it is the major apolipoprotein in HDL (Srivastava and Srivastava, 2000). It has been known that the activity of lecithin cholesterol acyl transferase (LCAT) in the presence of Apo A1 as a cofactor (Philip et al, 1998), as LCAT 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 hyperlipoproteinaemia (Sakurabayashi et al, 2001). Apo A1 is often used as a biomarker for cardiovascular diseases (McQueen et al, 2008).

In this study, serum level of Apo A2 was significantly lower in male than in female asymptomatic HIV positive subjects. But higher serum level of Apo A2 was significantly observed in the male than female HIV seronegatives. Normal serum Apo A3 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 TG (McQueen and Chambaz, 2000).

The present study showed significantly higher serum levels of Apo C2 and Apo E in symptomatic HIV individuals not on ART. Elevated serum level of Apo C2 was observed in male than female asymptomatic HIV positive subjects studied. Elevated serum Apo C2 level has been linked with hypertriglyceridermia, hypercholesterolemia and hyperchylomicronemia (Jackson et al, 1977). Apo E protein is found in chylomicrons and intermediate lipoproteins (IDLs) that is essential in the catabolism of triglyceride-rich lipoprotein constituents and it is essential in the transport of cholesterol to neurons via Apo E receptors (Singh et al, 2002). 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 heart (Larkin et al, 2000). Also, there have been reports on the association between Apo E and neurodegenerative conditions such as multiple sclerosis and Alzheimer’s disease (Fazekes et al, 2011).

Serum Apo B was only observed to be higher in male than in female HIV seronegatives.

Excess Apo B in individual has been found to be a better predictor of cardiovascular disease (Walldius et al, 2001).

The study revealed significantly higher serum activities of CK-T and CK-MB but lower serum activities of LDH and AST in male than female subjects studied in asymptomatic HIV positive subjects on ART, in symptomatic HIV positive subjects not on ART and in asymptomatic HIV seronegatives. Again, significantly higher serum activities of CK-T and CK-MB but lower serum activities of LDH and serum myoglobin were observed in male than in female seropositives. Elevated enzyme activities of LDH, AST and CD4 counts were observed in symptomatic HIV infected subjects not on ART compared to control. Also38

Serum myoglobin was significantly lower only in male than in female control groups. Serum activity of CK-MB is the isoform of CK found in the heart and it is sensitive to myocardial infarction just as where the levels of troponin I and myoglobin.26 Myoglobin is useful for early diagnosis of acute myocardial infarction (AMI). It rises within 2-4 hours after the early diagnoses of acute myocardial infarction. It peaks at 9-12 hours and returns to baseline within 24-36 hours 18,28. Higher serum levels of Myoglobin, Troponin I and serum activities of total CK, CK-MB and LDH have been significantly reported in HIV positive individuals 38 Myoglobin is useful for early diagnosis of acute myocardial infarction. It rises within 2-4 hours after the early diagnoses of acute myocardial infarction. It peaks at 9-12 hours and returns to baseline within 24-36 hours (Kegen, 1978; Silva et al, 1991).

In this present study, the serum total cholesterol levels were significantly increased in male than in female subjects studied in symptomatic HIV positive subjects on ART, in symptomatic HIV positive subjects not on ART and in asymptomatic HIV seronegatives. Again the LDL- cholesterol levels were significantly increased in male than in female subjects studied in symptomatic HIV positive subjects not on ART and in asymptomatic HIV seronegatives. Serum HDL-cholesterol was s has been reported to be significantly higher in asymptomatic HIV infected subjects as length of antiretroviral therapy deepened when compared to value before therapy 28,30, 31. Dyslipidemia—an elevated serum total cholesterol and triglycerides levels have been reported in HIV infected subjects on antiretroviral therapy (Boyles, 2002; Ogundahunsi et al, 2008; Ezeugwunne et al, 2014). An increased LDDL level is a strong predictor for cardiovascular diseases (Riddler et al, 2003). Elevated levels of total Cholesterol, triglycerides and LDL have been reported to cause cardiovascular diseases (Ahanku et al, 2001; Kabiri et al, 2010). This may confirm that that male sex is more prone to cardiovascular diseases (Kabiri et al, 2010).

IV. Conclusion:
There were 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.
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