Pattern of Lipid Profile in Adult HIV Seropositives in Nnewi, Nigeria

Ifeoma Priscilla Ezeugwunne 1*, Charles Chinedu Onyenekwe 2, Joseph Ebere Ahaneku 2
Martins Ifeanyichukwu 3, Gladys Ahaneku 4, Samuel Chukwuemeka Meludu 1
Rebecca Chinyere Chukwuaniukwu 1 Wuraola Serah Nnaemeka 1, Charles Diike 1, Christian Ejike Onah 5
1. Department of Human Biochemistry; College of Health Sciences, Nnamdi Azikiwe University, Nnewi, Nigeria
2. Department of Chemical Pathology; College of Health Sciences, Nnamdi Azikiwe University, Nnewi, Nigeria
3. Department of Immunology, College of Health Sciences, Nnamdi Azikiwe University, Nnewi, Nigeria
4. Department of Medicine, College of Health Sciences, Nnamdi Azikiwe University, Nnewi, Nigeria
5. Department of Chemical Pathology; Nnamdi Azikiwe University Teaching Hospital, Nnewi, Nigeria

* Corresponding author, E-mail: goodnessifeoma007@yahoo.com

The research is financed by Tertiary Educational Trust Fund (TETFUND), Nigeria.

Abstract
To determine the lipid profile level in adult HIV seropositive participants. Blood samples collected from the 300 randomly recruited participants were used for HIV screening, CD4+ T cell count, total Cholesterol, Low Density Lipoprotein (LDL), High Density Lipoprotein (HDL) and Triglyceride. Standard Laboratory methods were used for the analysis. The results showed that the mean serum total Cholesterol, LDL, HDL, Triglyceride and CD4+ T cell levels were significantly different amongst the groups studied. The mean serum total Cholesterol, LDL, HDL and Triglyceride levels were significantly lower in symptomatic HIV participants on antiretroviral therapy (ART) compared with those not on ART (P<0.05) but no significant difference was observed between the groups in CD4+ T cell level (p>0.05). The mean serum total Cholesterol, LDL, HDL, Triglyceride and CD4+ T cell levels were significantly lower in symptomatic HIV participants on ART compared with control group (in each case). Also, the mean serum HDL and CD4+ T cell levels were significantly lower while the mean LDL was significantly higher in symptomatic HIV participants not on ART compared with control subjects (P<0.05) but the values seen in total Cholesterol and Triglycerides were the same in both groups (P>0.05). Hypolipidaemia was seen in HIV positive participants.

Keywords: HIV, lipid profile, participants.

1. Introduction
Human Immunodeficiency virus (HIV) is transmitted as single stranded enveloped RNA virus and upon entry into the host, is converted into double stranded proviral DNA by reverse transcriptase enzyme of the virus. The proviral DNA is then inserted into the host cell genomic DNA, the virus becomes active and replicates within cells (Vandegraaff & Engelman, 2007).

During the asymptomatic state, as the name suggests, the individual is free from major symptoms, although there may be swollen glands (WHO, 2006). But, during the stage of symptomatic stage, there are emergence of opportunistic infection and cancers. At this point, the body immune system has been compromised and severely damaged by HIV and could lead to a disease called Acquired Immunodeficiency syndrome (AIDS) (WHO, 2006).

AIDS has been observed to have effect on lipid profile in HIV seropositive individuals (Grinspoon & Carr, 2005, Oduola et al, 2009). HIV infection has been found to impact on the adipocytes, disabling it from storing most lipids (Broxmeyer, 2004). Even, before the advance of ART, low levels of total Cholesterol, HDL and LDL have been reported in HIV infection (Akiibinu et al, 2008, Madhav et al, 2009). Hence, this study is intended to evaluate the pattern of lipid profile, in adult HIV seropositive individuals.

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 from the voluntary and counseling unit (VCT) of NAUTH for this study. Using the World Health Organization (WHO, 2006), staging for HIV as a guide and questionnaire, the participants were grouped, comprising of 100 symptomatic HIV subjects on ART, 100 symptomatic HIV subjects not on ART and 100 HIV seronegative control subjects. These participants had no history of any disease. Ethical approval was sort 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 total Cholesterol, LDL, HDL and Triglyceride.

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. 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 serum Cholesterol
Procedure
The procedure was as described by the manufacturer (Randox Laboratories, UK). 10 µl of standards, specimens and controls were dispensed into appropriately labeled standards, specimens and controls tubes respectively. 1 ml of cholesterol reagent containing (phenol- 6 mmol/L, pipes buffer- 50 mmol/L, 4-amino antipyrine- 0.3 mmol/L, cholesterol oxidase- 100 U/L, cholesterol estrase- 150 U/L and peroxidase 800 U/L) was added to each of the tube. The reagent blank was prepared similarly with the use of 10 µl of distilled water. These were incubated for 10 minutes at room temperature and absorbance measured at 546 nm against reagent blank and the concentration of serum cholesterol was calculated.

2.4.4 Quantitative determination of serum Triglyceride
Procedure
The procedure was as described by the manufacturer (Randox Laboratories, UK). 10 µl of standards, specimens and controls were dispensed into appropriately labeled standards, specimens and controls tubes respectively. 1000 µl of enzyme reagent was added to each of the tube. The reagent blank was prepared similarly with the use of 10 µl of distilled water. These were incubated for 10 minutes at room temperature and their absorbances measured at 546 nm against reagent blank and the concentration of serum cholesterol was calculated.

2.4.5 Quantitative determination of serum High Density Lipoprotein Cholesterol (HDL-C)
Procedure
The procedure was as described by the manufacturer (Randox Laboratories, UK). 500 µl of serum, control were dispensed into appropriately labeled specimens and controls tubes respectively. 1000 µl of enzyme reagent was added to each of the tube. The reagent blank was prepared similarly with the use of 10 µl of distilled water. These were incubated for 10 minutes at room temperature and their absorbances measured at 546 nm against reagent blank and the concentration of serum cholesterol was calculated.

2.4.6 Quantitative determination of serum Low Density Lipoprotein Cholesterol (LDL-C)
The formula by Kaplan and colleagues (8) was used to calculate the LDL-C level. Initially, the total cholesterol, triglyceride and HDL-C levels of each sample were determined and the LDL level was calculated using this formula: LDL-C = Total cholesterol – (HDL-C + 1/5 x triglyceride). The formula hinges on the postulation that VLDL-C is present in a concentration equal to one fifth of the triglyceride concentration. This postulation is valid for triglyceride concentrations less than 4.56 mmol/L.
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 result of the analysis of variance showed that the mean serum total Cholesterol, LDL, HDL, Triglyceride (mmol/l) levels and CD4+ T cell counts were significant different amongst the groups at P < 0.05 (F = 316.27; 273.30; 134.00, 423.00 and 216.22) respectively.

Between group comparison showed that the mean serum total Cholesterol, LDL, HDL, Triglyceride and CD4+ T-Cell levels were significantly lower in symptomatic HIV infected subjects not on ART compared with asymptomatic HIV infected subjects at p<0.05, in each case.

Again, between group comparison showed that the mean serum total Cholesterol LDL, HDL Triglyceride and CD4+ T-Cell levels were significantly lower in symptomatic HIV infected subjects not on ART compared with HIV seronegative control subjects at p<0.05 respectively.

Also, between group comparison showed that the mean serum total Cholesterol, HDL and CD4+ T-Cell levels were significantly lower in asymptomatic HIV infected subjects compared with HIV seronegative control subjects at p<0.05 respectively. But a significantly higher mean serum levels of LDL and Triglyceride were seen in asymptomatic HIV infected subjects compared with HIV seronegative control subjects at p<0.05 respectively (See table 1).

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

<table>
<thead>
<tr>
<th>Group</th>
<th>Chol (mmol/L)</th>
<th>LDL (mmol/L)</th>
<th>HDL (mmol/L)</th>
<th>TG (mmol/L)</th>
<th>CD4+ (/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A(n=100)</td>
<td>3.51±0.22</td>
<td>1.75±0.06</td>
<td>0.91±0.06</td>
<td>0.79±0.04</td>
<td>374.78 ± 121.59</td>
</tr>
<tr>
<td>B(n=100)</td>
<td>4.27±0.15</td>
<td>2.76±0.07</td>
<td>1.20±0.03</td>
<td>1.35±0.04</td>
<td>437.20 ±129.75</td>
</tr>
<tr>
<td>C(n=100)</td>
<td>4.62±0.24</td>
<td>2.34±0.13</td>
<td>1.37±0.06</td>
<td>1.44±0.05</td>
<td>940.64 ± 148.85</td>
</tr>
</tbody>
</table>

F(p)-value 124.50 (.000) 482.27 (.000) 305.96 (.000) 1300 (.000) 126.37 (.000)
A v B <0.05 <0.05 <0.05 <0.05 >0.05
A v C <0.05 <0.05 <0.05 <0.05 <0.05
B v C >0.05 <0.05 <0.05 <0.05 <0.05

Key: F (p) value = mean ± SD of parameter compared among groups A, B and C (using ANOVA).
A V B p value = mean ± SD of parameter compared between group A and B (using t-test).
B V C p value = mean ± SD of parameter compared between group A and C (using t-test).
B V C p value = mean ± SD of parameter compared between group A and D (using t-test).

4. Discussion
In this study, there were significantly lower levels in serum total cholesterol, LDL, HDL and TG in symptomatic HIV participants with and without Antiretroviral therapy. The finding conformed to the finding by Grunfeld et al (1992) that observed lower levels in serum total cholesterol, LDL, HDL and TG in HIV positive participants. Adewole et al (2010) observed lower levels in LDL and HDL, reaching a dyslipidemic level in HIV positive group when compared with control. The reduced serum lipid profile in symptomatic HIV positives may be suggesting hypolipidaemia in these subjects. Hypolipidaemia is an abnormal lipid distribution in tissues; the supply of cholesterol may be compromised leading to nerve damaged or impairment as well as steroidal hormonal imbalance in the individual.

There were also significantly lower levels of serum total cholesterol, HDL and TG in symptomatic HIV positive individuals on ART compared with control in the present research. These reductions in levels could be the result of slower rate in lipid production due to HIV infection and enhanced lipid catabolic rate associated with HIV infection (Akiibinu et al, 2008, Madhav et al, 2009). Therefore, the significant fall in total cholesterol, HDL and TG status of HIV infected individuals may be a determinant in ascertaining factors that predisposes severity of disease in them.

Some researchers have reported that the presence of abnormal lipid in HIV infected individuals might be due to the effects of viral infection, acute-phase reactant and circulating cytokines (Christeff et al, 2002). Therefore, the significant fall in total cholesterol, HDL and TG status of HIV infected individuals may be a determinant in ascertaining factors that predisposes severity of disease in HIV infected individuals.

Another significant finding in this study was that participants with HIV infection had more depleted CD4+ T cells in symptomatic HIV not on ART. Ifeanyichukwu et al (2011) was able to link rate of CD4+ T cell depletion with HIV disease progression. While Mark et al (2005) reported that the reduced CD4+ T cell counts in HIV
seropositives may be attributed to cell death caused by the HIV infection.

5 Conclusion
In this study, we conclude that the serum levels of total Cholesterol, Low density lipoprotein and the blood level of CD4+ T cell counts were significantly reduced in adult symptomatic HIV positive subjects with or without ART. The reduced serum lipid profile suggests hypolipidaemia as well as dyslipidemia in these subjects. Hence, the study suggests that the prediction of severity and monitoring of disease could be done by evaluating the CD4+ T cell counts and lipid profile in HIV infected individuals. Identification of these biomarkers in these individuals will aid in their early detection, treatment and management.

References

Acknowledgement
Authors are grateful to the staff of Voluntary counseling and testing Unit and Heart to Heart in Nnamdi Azikiwe University Teaching Hospital (NAUTH) and staff of Human Biochemistry Department in Nnamdi Azikiwe University (NAU), Nnewi, Anambra State, Nigeria for their assistance during sample collection and analysis.

Conflict of interest
There is no conflict of interest whatever with anyone or group of persons.
The IISTE is a pioneer in the Open-Access hosting service and academic event management. The aim of the firm is Accelerating Global Knowledge Sharing.

More information about the firm can be found on the homepage: http://www.iiste.org

CALL FOR JOURNAL PAPERS

There are more than 30 peer-reviewed academic journals hosted under the hosting platform.

Prospective authors of journals can find the submission instruction on the following page: http://www.iiste.org/journals/ All the journals articles are available online to the readers all over the world without financial, legal, or technical barriers other than those inseparable from gaining access to the internet itself. Paper version of the journals is also available upon request of readers and authors.

MORE RESOURCES

Book publication information: http://www.iiste.org/book/

IISTE Knowledge Sharing Partners

EBSCO, Index Copernicus, Ulrich's Periodicals Directory, JournalTOCS, PKP Open Archives Harvester, Bielefeld Academic Search Engine, Elektronische Zeitschriftenbibliothek EZB, Open J-Gate, OCLC WorldCat, Universe Digital Library, NewJour, Google Scholar