COMPARATIVE STUDY ON PREVALENCE AND RISK FACTORS FOR DYSLIPIDAEMIA IN HIV-INFECTED AND UNINFECTED CHILDREN SEEN AT THE AMINU KANO TEACHING HOSPITAL, KANO, NIGERIA.

A DISSERTATION SUBMITTED TO THE NATIONAL POSTGRADUATE MEDICAL COLLEGE OF NIGERIA IN PART FULFILLMENT FOR THE AWARD OF THE FELLOWSHIP OF THE FACULTY OF PAEDIATRICS

BY

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DECLARATION

I hereby declare that this work is original unless otherwise acknowledged. The work has neither been presented to any other College for Fellowship award nor submitted elsewhere for publication.

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ATTESTATION

The study reported in this dissertation was done by the candidate under our supervision. We have also supervised the writing of the dissertation.

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DEDICATION

To the HIV-infected children of the World.

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LIST OF ABBREVIATIONS

ACTG	AIDS Clinical Trial Group
ATP	Adenosine Triphosphate
CE	Cholesterol Esterase
CHOD	Cholesterol Oxidase
CRABP	Cytoplasmic Retinoic Acid Binding Protein
ELISA	Enzyme Linked Immunosorbent Assay
GK	Glycerol Kinase
GPO	Glycerol-3- Phosphate Oxidase
HAART	Highly Active Antiretroviral Therapy
HDL	High Density Lipoprotein
LDL	Low Density Lipoprotein
LRP	Lipoprotein Receptor- related Protein
NRTIs	Nucleotide/ Nucleoside Reverse Transcriptase Inhibitors
NNRTIs	Non Nucleotide Reverse Transcriptase Inhibitors
PCR	Polymerase Chain Reaction
PIs	Protease Inhibitors

PIPES	Piperazine- 1,4- bis(2-ethanesulfonic acid)
POD	Peroxidase
$PPAR_{\gamma}$	Peroxidase Proliferator Activated Receptor type $_{\gamma}$
RXR	Retinoid –X- Receptor
TC	Total Cholesterol
TGs	Triglycerides
VLDL	Very Low Density Lipoprotein

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Summary

HIV infection has become a chronic disease in paediatric patients as a result of access to Anti-Retroviral Therapy (ART), which has significantly improved the prognosis and potential for long-term survival. However, prolonged administration of these drugs (ART) is associated with metabolic side effects, especially dyslipidaemia with potential increase in the risk of development of cardiovascular disease as the affected children mature into adults.

The aim of the study was to ascertain the prevalence and risk factors for dyslipidaemia among HIV infected children aged 2-15 years seen at the Aminu Kano Teaching Hospital (AKTH), Kano.

This comparative cross sectional study was conducted on two hundred and forty subjects from August, 2015 to March, 2016. The subjects were made up of three groups of eighty HIV-infected children on Highly Active Antiretroviral Therapy (HAART), eighty HIV-infected HAART naive children and eighty HIV-negative age- and sex-matched apparently healthy children. Their bio-data, socio-demographic characteristics and physical examination findings (anthropometric measurements inclusive) were recorded. Blood samples were also collected from each subject and their lipid concentrations measured using enzymatic methods.

The prevalence of hypertriglyceridaemia was 62.5%, 47.5%, and 40% in HIV-infected children on HAART, HIV-infected HAART naïve and HIV-uninfected children respectively. The difference in prevalence was statistically significant ($\chi 2$ =43.15, p=0.0001). Hypercholesterolaemia was present in 47.5%, 11.3% and 8.7% in HIV-infected children on HAART, HIV-infected HAART naïve children and HIV-uninfected children respectively. The difference in prevalence was statistically significant ($\chi 2$ =8.40, p=0.014). The prevalence of high LDL-cholesterol was 41.3%, 17.5% and

7.5% in HIV-infected children on HAART, HIV-infected HAART naïve and HIV-negative children respectively. These difference was statistically significant ($\chi 2 = 28.92$, $p=0.0001$).

Risk factors associated with hypercholesterolaemia and hypertriglyceridaemia among the HIV infected children on HAART were, age at commencement of HAART less than 2 years, PI- based HAART regimen, age group greater than 5 years, duration of HIV diagnosis greater than 1year and duration of treatment on HAART for more than I year. However on multivariate analysis, PI-based HAART regimen was the only independent predictor of hypercholesterolaemia in the HAART treated group. Duration of diagnosis greater than 1 year was associated with hypercholesterolaemia in HAART naïve HIV-infected children (p value = 0.05).

Dyslipidaemia occurred in both HAART treated and HAART naïve HIV-positive children. It is thus imperative to regularly assess the lipid profile of HIV infected children on regular basis and institute appropriate treatment promptly.

INTRODUCTION

Human immunodeficiency virus (HIV) infection is one of the major global health challenges. According to the World Health Organization (WHO), there are about 36.7 million people living with HIV/AIDS, of which 25.5 million are in Africa.[1] An estimated 1.8 million children were living with HIV at the end of 2015, 90% of them in sub-Saharan Africa.[2] Most of these children acquire HIV from their HIV-infected mothers during pregnancy, delivery or breastfeeding. The number of children globally receiving antiretroviral therapy (ART) according to WHO, has increased from about 456,000 in 2010 to 824,000 in 2015.[2]

Nigeria is estimated to have the highest burden of paediatric HIV in the world.[3] In 2015, about 260,000 children less than 15 years were living with the HIV.[3] Retrospective studies among high risk hospitalized Nigerian children showed seroprevalence rates of paediatric HIV ranging from 5.7- 20%.[4,5] Ogunbosi et al,[6] reported a prevalence rate of paediatric HIV to be 10% in Ibadan. Hassan-Hanga et al,[7] reported a HIV sero-prevalence rate of 6.5% in under-five children with diarrhoea in Kano.

HIV infection has become a chronic disease in paediatric patients as access to ART has significantly improved the prognosis and potential for long-term survival.[8] Highly active antiretroviral therapy (HAART) that consists of protease inhibitors (PIs) or non-nucleoside reverse transcriptase inhibitors (NNRTIs) in combination with nucleoside reverse transcriptase inhibitors (NRTIs) has led to a dramatic improvement in the prognosis of HIV-infected patients.[9] Although these antiretroviral therapies are usually well tolerated, previously unrecognized side effects are becoming more evident with their widespread use and increased duration of treatment.[9,10] Frequent metabolic side effects include dyslipidaemia, altered glucose metabolism, insulin

resistance, elevated free fatty acids , fat redistribution syndrome or lipodystrophy, mitochondrial toxicity including lactic acidosis and bone density abnormalities.[11] These metabolic complications of ART have been well documented in children although paediatric cohort studies are limited.[12] The NRTIs are closely linked to lipodystrophy and lactic acidosis, while the PIs have consistently been associated with increased cholesterol and triglycerides in children which may potentially increase the risk of cardiovascular disease as they mature into adults.[12]

DEFINITION OF THE RESEARCH PROBLEM

Disorders in lipid metabolism have been reported in HIV-infected patients as part of the lipodystrophy syndrome or as isolated abnormalities. Dyslipidaemia consisting of hypertryglyceridaemia with depressed concentration of high-density lipoprotein cholesterol (HDL-c) and elevated low-density lipoprotein cholesterol (LDL-c) has been observed with increasing frequency among HIV-infected patients.[13] This viraemia-associated dyslipidaemia, was recognised years before the widespread use of protease inhibitor (PI) based HAART.[13] Several cross sectional studies in children suggest that hyperlipidaemia, particularly hypercholesterolaemia, is relatively common in HIV- infected children who receive antiretroviral therapy (ART).[14-17] Hypercholesterolaemia, hypertriglyceridaemia and low levels of HDL-c have also been reported in a group of HIV-infected ART-naive Asian children. [18] These group of studies showed that both types of HAART regimens, HAART with PI and HAART without PI or no HAART were associated with an increase in total cholesterol and low-density lipoprotein cholesterol. Prevalence of increased cholesterol in HIV- infected children on ART, has been reported to range from 15% - 66% and increased triglyceride from 13% - 71%.[19-23] Studies on the tolerability and efficacy of PI-containing regimens revealed alterations of serum lipids ranging from dyslipidaemia in 20% to 50% of children on single PI to greater than 90% on dual PI- containing regimens.[11,20,24-26]

Metabolic abnormalities and fat redistribution are more likely to occur in post pubertal children, especially those on protease inhibitor treatment.[27] This disturbance in lipid metabolism predisposes affected patients to premature cardiovascular diseases.[28] It can thus be hypothesized that, HIV-infected children on HAART and treatment naïve HIV-infected children would have an increased prevalence of lipid abnormalities compared to HIV negative controls.

JUSTIFICATION OF THE STUDY

The National guidelines for the treatment of HIV infection recommends initiation of ART early in life as soon as diagnosis of HIV infection is established for improved survival.[29] This prolonged exposure to ART and treatment with multiple drug regimens increases the risk for metabolic complications.[12]

Unfavourable alterations in lipid metabolism have been reported to be risk factors for cardiovascular disease and its complications among children and adolescents with perinatally acquired HIV infection receiving HAART.[30] Dyslipidaemia in particular has been implicated in artheroma formation playing a major role in cardiovascular, cerebrovascular and renal pathologies. An increase in premature coronary artery disease and myocardial infarction, peripheral artherosclerosis, acute pancreatitis and cutaneous xanthomas have been described among young HIV-infected individuals treated with HAART.[31] These complications often results from exposure to risk factors that have their origins in childhood.[30] However, few studies have evaluated lipid profiles of children receiving HAART and its consequences in sub-Saharan Africa and in Nigeria where over 90% of HIV- infected children live. Most of the studies on lipid profiles of HIV-infected patient s on HAART in Nigeria were done in adult populations. As more children receive life-saving ART resulting in longer survival, understanding the adverse effects associated with

exposure to ART is important for paediatricians and other health care workers who cater for these group of children and adolescents. The ART regimen available for treatment are limited, particularly the second line regimens which are Protease Inhibitor (PI) based with only the Lopinavir/Ritonavir combination of the PIs being available for treatment of HIV-infected children and adolescents in Aminu Kano Teaching Hospital. The PI based combinations in the hospital are the Lopinavir/ritonavir/Tenofovir/Emtricitabine and Lopinavir/ritonavir/Zidovudine/Lamivudine combinations for the children who have failed first line HAART and Lopinavir/ritonavir/Abacavir/Lamivudine combination for those infants who failed PMTCT (Prevention of Mother To Child Transmission). These combinations are lipid sparing, having the potential of increasing the lipid concentration of the patients. It is thus imperative to determine these changes early so that timely management can be instituted to prevent complications and improve long term survival of these children, [32] hence the need for this study.

LITERATURE REVIEW

HISTORY OF HIV/AIDS INFECTION

The first case of AIDS appeared in 1981with the occurence of *Pneumocystis jiroveci* (formely *P. carinii*) pneumonia and Kaposi's sarcoma in previously healthy young homosexual men in California and New York.[33] In 1983, Human Immunodeficiency Virus (HIV) was isolated from a patient with lymphadenopathy, and subsequent research studies established the causal relationship between HIV infection and AIDS.[33]

EPIDEMIOLOGY OF HIV/AIDS

In Nigeria, the first AIDS case was reported in 1986 in a 13-year old female hawker.[34] Since then, there has been a gradual increase in the National prevalence of the disease, followed by a decrease. The National Sero-prevalence rate of HIV- infection from 1991 to 2008 rose from 1.8% in 1992, to 4.6% in 2008.[35] According to the National Reproductive Health Survey Plus, the national HIV sero-prevalence rate stood at 3.4% in 2012,[36] with a steady decline in prevalence to 3.1% in 2014 (ANC Survey Report).[29] By 2015,an estimated 3,391,546 people were living with the virus.[3] There were 239,155 new infections by the end of 2013, with 210,031 AIDS related deaths.[3] In 2015, about 260,000 children were living with HIV and only 12% of these children had access to ART.[3]

AETIOLOGY OF HIV/AIDS

The aetiological agents of AIDS have been identified as HIV-1 and HIV-2 viruses, belonging to the Lentivirus group of Retroviridae family. HIV-1 is responsible for the vast majority of infections world-wide. HIV-2 infection is less common and occurs predominantly in West Africa.[34] All the members of the Retroviridae family contain an enzyme called reverse

transcriptase that is used for the synthesis of proviral DNA from the infecting viral RNA. These groups of viruses are associated with many diseases some of which may run a rapid course or have a long latency period.

PATHOGENESIS OF HIV/AIDS

The primary receptor for HIV is the CD4+ molecule on the T-helper cells of humans. Replication of the viral particle begins with attachment of Gp120 to the CD4+ molecule on the surface of a target cell. Following the Gp120-CD4+ binding, a structural change allows for the interaction of the V3 loop region in the Gp120 with a chemokine receptor, including CCR5 and CXCR4. The reaction with the co-receptor results in another conformational change in the viral surface glycoprotein, which exposes a fusion domain contained within the envelope trans-membrane glycoprotein. Exposure of the fusion domain results in the insertion of Gp41 into the cellular membrane. The viral core is released into the cytoplasm of the host cell. Once in the cytoplasm, the viral RNA genome is uncoated and reverse transcribed by the virally encoded reverse transcriptase enzyme to generate a double-stranded viral DNA pre-integration complex. The double stranded DNA virus is then transported into the host cell nucleus where it becomes integrated into the host cell chromosome with the aid of the enzyme integrase.[35] The double stranded DNA virus resides as a provirus and may remain in a 'latent' state for many years or can begin the production of new viral RNA. On activation after latency, the host cell RNA enzyme polymerase II will transcribe the proviral DNA into messenger RNA (mRNA). The mRNA is then translated into viral proteins that undergo extensive post-translational modifications. The viral RNA becomes the genetic material for the next generation of viruses.

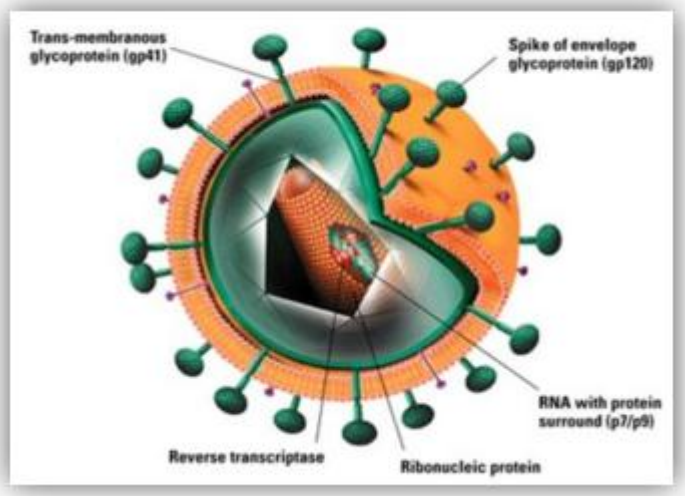

Figure I: Structure of HIV (Culled from the National Guideline for Paediatric HIV Care 2010)[35]

Clinical Course of Illness

The progression of HIV infection in children depends on the integrity of the child's developing immune system. At birth, the viral load is usually very low in HIV infected children but it slowly rises within the first two months of life to well over 1million copies/ml, declining after the age of 4-5 years.[34] CD4+ cell count decreases following primary HIV infection, while HIV RNA rises significantly. With sufficient exposure to viral antigens, cytotoxic T-lymphocyte responses are generated and the HIV viral load typically declines to an equilibrium known as the virologic set-point within 6 - 12 months of infection.[34] Once this viral set-point is reached, the CD4+ cell count rises marginally, but does not often return to baseline values.

Shortly after infection, HIV-infected children may develop flu-like symptoms which can resolve unnoticed, or present as common childhood illnesses in the environment, or progress to illnesses highly specific to HIV-infection. As the illness progresses, the child presents with additional features that indicate severe immune suppression. These include features of opportunistic infections (OIs), recurrent and more severe forms of the common illnesses in the environment as well as malignancies.[35]

HIV DIAGNOSIS.[29]

The diagnosis of HIV infection is by detection of the HIV antibodies in plasma or serum (indirect test) or by the detection of the virus in blood with nucleic acid-based tests (direct test), viral culture and p24 antigen. Antibody detection test is suitable for diagnosis of HIV infection in children 18 months and above, while viral detection tests are used for the diagnosis of HIV infection in children under 18 months of age.

Antibody Tests include:

1. Screening tests: rapid tests or Enzyme-linked Immunosorbent Assays (ELISA).
2. Confirmatory tests: western blot or indirect immunoflorescent assay.

Detection of Virus/Viral Components:

1. Nucleic acid based tests include:
    - DNA Polymerase Chain Reaction (DNA PCR)
    - Reverse Transcriptase Polymerase Chain Reaction (RT- PCR)
2. Viral culture
3. Ultra sensitive p24 antigen (Up24Ag) on plasma or Dried Blood Sample (DBS): This is a specific test that detects the core antigen

TREATMENT

Antiretroviral drugs used in combination for the treatment of HIV infection under optimal conditions should lead to sustained virologic, immunologic, clinical and epidemiological control. Sustained viral suppression results in improved immune function increasing the ability of the body to fight infections.[29] Other supportive therapies like aloe vera gruel gel have also been tried in the management of HIV- infection to a fairly good effect.[37]

Goals of Antiretroviral Therapy [29]

The goals of antiretroviral therapy in children are to:

- Stop and reverse the progression of HIV disease, by sustaining maximal viral suppression and reducing the risk of opportunistic infections.

- Enhance recovery of immune function by progressive increase in CD4+ cell counts at rate of 50 to 100 cells/ul/year
- Promote and restore normal growth and development.
- Improve quality of life.
- Achieve optimal response with minimal drug toxicity.
- Ensure the rational use of ARVs to preserve future therapeutic options.

The National guidelines[29]for the treatment and care of paediatric HIV and AIDS recommends;

- Initiation of HAART in all children living with HIV regardless of WHO clinical stage and at any CD4 cell count
- As a matter of priority, initiate HAART in all children ≤ 2years of age or children younger than 5years of age with WHO clinical stage 3or 4 disease or CD4+ cell counts ≤ 750 cells/mm^3 or CD4 percentage < 25% and for children 5years of age and older with WHO clinical stage 3 or 4 disease or CD4+ cell counts ≤ 350 cell/mm^3.

Table I: Classes of ARV drugs[29]

Anti Retroviral Drug Classes	Approved Drugs
Nucleoside Reverse Transcriptase Inhibitors(NRTIs)	Zidovudine (ZDV, AZT) Lamivudine (3TC) Stavudine (d4T)* Abacavir (ABC) Didanosine(ddl)* Emtricitabine (FTC)
Nucleotide Reverse Transcriptase Inhibitors(NRTIs)	Tenofovir Disoproxil fumarate(TDF) Tenofovir Alafenamide (TAF)
Non-Nucleoside Reverse Transcriptase Inhibitors(NNRTIs)	Nevirapine(NVP) Efavirenz(EFV) Rilpivirine(RPV) Etravirine(ETV)
Protease Inhibitors (PIs)	Lopinavir-ritonavir(LPVr) Ritonavir(RTV) as pharmacoenhancer Nelfinavir(NFV)* Saquinavir(SQV) * Amprenavir(APV) Darunavir(DRV) Tipranavir(TPV) Atazanavir(AZV) Fos-amprenavir(FPV)
Fusion Inhibitors	Enfurvitide(T-20) Anti-GP41Adnectin Combinectin
Integrase Inhibitors	Raltegravid(RAL) Elvitegravir (EVG) Dolutegravir(DTG)
Chemokine Receptor Inhibitors	Maraviroc Vicrviroc Cenicriviroc
*Drugs no longer used for routine ART treatment	

Culled from National guidelines for HIV prevention treatment and care 2016.

Highly Active Antiretroviral Therapy (HAART)

HAART consists of a combination of three or more medications drawn from two - three main classes of ART drugs namely, nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors and protease inhibitors. Typically HAART combines two NRTIs as backbone with either a NNRTI, or a PI. This is the standard treatment of HIV infection that ensures the best possible suppression of viral replication and arrests disease progression.[29] The decision on which dual NRTI combination or 'backbone' to use, and which agent to combine it with, is dependent on numerous factors, including CD4+ T-cell count and percentage, HIV viral load, potential toxicities, drug interactions, pill burden and viral resistance.[38]

Table II: Paediatric ART Combination Regimen in Nigeria.[29]

1st Line	2nd Line
ABC/3TC/EFV	ABC/3TC/LPVr
ABC/3TC/NVP	AZT/3TC/LPVr
AZT/3TC/EFV	AZT or ABC/3TC/RAL
AZT/3TC/NVP	ABC or TDF/3TC/LPVr
TDF/3TC(or FTC)/EFV	
TDF/3TC(or FTC)/NVP	
EFV/TDF/FTC or 3TC	
Children less than 3 years	
ABC/3TC/LPVr	
AZT/3TC/LPVr	
ABC/3TC/NVP	
AZT/3TC/NVP	

BIOCHEMISTRY OF LIPIDS

Lipids are a class of naturally occurring compounds that are soluble in organic solvents such as ether, chloroform, acetone and benzene but nearly insoluble in water.[39] Lipids have important roles in various aspects of life.[40] They:

- serve as hormones and vitamins
- serve as energy source
- aid in digestion
- act as structural components in cell membranes.

Lipids are transported in plasma in macromolecular complexes known as Lipoproteins. Lipoproteins are classified based on their hydrated densities into: [40]

- chylomicrons
- very-low density lipoprotein(VLDL)
- intermediate –density lipoprotein(IDL)
- low-density lipoprotein(LDL)
- high-density lipoprotein (HDL)

Lipids and lipoproteins are involved in the development of atherosclerosis, a pathologic process that is the underlying cause of common cardiovascular disorders like myocardial infarction, cerebrovascular disease and peripheral vascular disease.[40]

The National Cholesterol Education Program expert panel on blood cholesterol levels in children and adolescents recommends that, from one to 19 years of age, the 75th percentile for total cholesterol should be roughly 170 mg/dL, and for LDL cholesterol about 110 mg/dL. The 95th percentile for total cholesterol is roughly 200 mg/dL, and for LDL cholesterol about 130 mg/dL.

HDL-cholesterol should measure 35 mg/dL or higher.[41] Reference values are shown in table III below.

Table III: SERUM LEVELS OF CHOLESTEROL AND TRIGLYCERIDES IN CHILDREN AGED 2-19 YEARS. [41]

	Total cholesterol	LDL cholesterol	Triglycerides
Normal	< 170 mg/dL	< 110 mg/dL	<150 mg/dL
	(<4.4 mmol/L)	**(<2.85 mmol/L)**	**(<1.69mmol/L)**
Borderline	170-199 mg/dL	110-129 mg/dL	150-199 mg/dL
	(4.4-5.2 mmol/L)	**(2.85-3.4 mmol/L)**	**(1.69-2.24 mmol/L)**
High	≥200 mg/dL	≥130 mg/dL	200-499 mg/dL
	(>5.2 mmol/L)	**(>3.4 mmol/L)**	**(2.25-5.63mmol/L)**

HIV AND DYSLIPIDAEMIA

Disorders in lipid metabolism have been reported in HIV-infected patients as part of the lipodystrophy syndrome or as isolated abnormalities.[32] McComsey et al[32] in an extensive review of HIV articles described several patterns of dyslipidaemias among adults and children with HIV infection. The reviews noted that PIs particularly ritonavir and nelfinavir, had the greatest association with dyslipidaemia. The reviews were conducted amongst the European children with HIV infection who had similar PI combinations with ritonavir and nelfinavir. However, African children were excluded from the review.

Even before the availability of ART, low levels of high density lipoprotein cholesterol (HDL) and elevated low density lipoprotein concentrations and triglycerides had been associated with HIV infection.[13] HIV-1 infection causes a specific pattern of dyslipidaemia, resulting from a combination of increased production and decreased clearance of lipoproteins.[42] Adipose tissue hosts multiple immune cell types including monocytes, macrophages, endothelial and vascular smooth muscle cells which are functionally active in the adipose tissue and produce numerous cytokines and other regulatory factors that influence lipid homeostasis, regulation of steroid hormones, prostaglandin, and fat-soluble vitamins. These factors control the storage of excess lipids present in the circulation. HIV-1 has a profound impact on adipocytes which become dysfunctional and unable to store most lipids properly.[42]

Rasheed et al [43] showed that HIV replication alone in human T-cells, without any influence of antiviral drugs or other factors, can stimulate the production of novel cellular enzymes and proteins that enhance fatty acid synthesis, increase the quantity of low density lipoproteins, secrete triglycerides, alter lipid transport and metabolism and oxidize lipids. The study proved that, one of the most essential biological processes involved in dyslipidaemia and lipodystrophy syndrome is

the accumulation of lipids and the disproportionate distribution of tissue-associated fats due to enhanced fatty acid synthesis.[43]

Hellerstein et al [44] measured de novo lipogenesis in three groups of patients (HIV-infected with history of weight loss, asymptomatic HIV-infected, and uninfected males) and found that hepatic lipogenesis was three to four-fold higher in the HIV-infected with weight loss group compared with sero negative controls. Hepatic lipogenesis was also found to be significantly increased in the asymptomatic HIV-infected group. The authors concluded that HIV infection was associated with abnormal fat anabolism.

LABORATORY METHODS FOR THE MEASUREMENT OF LIPIDS

The basic lipids that are measured in the laboratory include; Total cholesterol, HDL cholesterol, LDL cholesterol and Triglycerides.

(i) Cholesterol Assays.

The assays that have been used to measure cholesterol in blood can be divided into three categories.

The first includes the multi-stage method based on the modified Abell-Kendall technique, which involves the Liebermann-Burchardt reaction after hydrolysis and extraction of cholesterol, which is considered the "standard reference method".[45,46] However, application of the standard reference method is more demanding than many laboratory procedures, requiring relatively sophisticated facilities and technical skills. The laboratories of the Lipid Research Clinics and others that use this method have made extensive efforts to standardize and improve the quality of testing. These laboratories are thought to supply the most nearly error-free results in clinical use. The test requires a few milliliters of blood, and laboratories can generally provide results within 24 to 72 hours of receiving the specimen.

The second category of assay are enzymatic methods that can be performed both manually and on automated analyzers.[45] They have been in use since the 1980s, and have become the standard methods for cholesterol measurement in routine Clinical Chemistry laboratories world-wide. They are accurate, precise and easy to use, provided that they are used with care and are calibrated properly. They are convenient because sophisticated equipments are not required for the procedures and a number of measurements in addition to cholesterol can be performed on the same tube of blood. Results can also be made available within minutes.[47]

The third category of assay is a one-step enzymatic method that has recently become available.[45] It is particularly convenient for both patients and providers of care. These tests require only a few drops of blood from a finger prick and give results in 3 to 8 minutes. The equipment can be operated in the Physician's office, Clinic, or Community screening site by personnel without a special training background in Clinical Chemistry. The level of precision and accuracy of results produced by these groups of assays are not acceptable for research purposes.[48]

Isolation of HDL

HDL cholesterol measurements are performed after the other apoprotein B-containing lipoproteins \(i.e., LDL and VLDL) are precipitated from the sample and subsequently the cholesterol content of the remaining HDL-containing fraction is measured. Several precipitation procedures are available for HDL isolation, using one of the following reagents: heparin-Mn++ (hep-Mn++), dextran sulphate-Mg++ (Dxtr), phosphotungstate-Mg++ (PTA) and polyethylene glycol (PEG).[49] However, because no definitive or primary reference methods exist for the separation of HDL, and because differences in the precipitation procedures can alter the population of particles precipitated, not all methods give the same result for HDL cholesterol and therefore the standardisation of HDL cholesterol measurement is difficult.[49,50] A direct method for assessing HDL-cholesterol, which does not require isolation of HDL, has become available.[51] As the new direct HDL method does not require precipitation, its use is essentially simpler than the use of the traditional methods, and therefore it is less error prone. However, direct assay methods for the measurement of HDL cholesterol are only available in highly sophisticated and expensive equipment.

(ii) Triglycerides – History and Principal methods

Early analytical methods for determining triglycerides involve titrimetric procedures of total lipids. After extraction with organic solvents, extracts were saponified and then back-titrated to assess the amount of alkali that was not neutralized by the release of fatty acids. Later methods quantified the glycerol that was formed, as first described by Van Handel and Zilveramit.[52] Kesler and Lederer[52] adapted this method to the Auto analyser (Tecnicon, Tarrytown, NY).

The measurement of glycerol by an enzymatic spectrophotometric procedure was introduced by Wieland[48] after the enzyme glycerol kinase became commercially available. This method is based on the oxidation/reduction of NADH/NAD+ and the corresponding change in absorbance.

A semi-automated enzymatic analysis, introduced by Klotzsch et al[52], added a colorimetric indicator reaction to the Wieland principle and introduced the method to the auto analyser. During the following years, many additional modifications were made to apply the enzymatic methods to the newly developed automated laboratory instrumentation.[52] McGowan et al [53] used the enzyme glycerol-3-phosphate oxidase in conjunction with a Trinder-type reaction.

(iii) Low density lipoprotein cholesterol analysis

In the most widely used indirect method, total cholesterol, HDL cholesterol and triglycerides are measured and LDL cholesterol is calculated from the primary measurements using the empirical formula of Friedwald et al.[54] This approximation works poorly at high triglyceride levels. There are now a number of direct methods for LDL measurement that work well for high blood levels of triglycerides.[49,55] However, direct measurement of the low-density lipoprotein (LDL) level requires specialized equipment and expensive reagents.[56]

The Friedwald formula used for the calculation of the LDL- cholesterol (LDL-c) states:

$$\boldsymbol{LDL - c\ (mmol/L) = Total\ Cholesterol - \left(\left(\frac{Triglyceride}{2.2}\right) + HDL - Cholesterol\right)}$$

Though originally proposed for epidemiological studies, Friedwald formula has become the most widely used indirect method for the estimation of LDL cholesterol. It is based on measuring a number of lipid-related analytes followed by their use in calculating the LDL cholesterol content of a specimen. Total cholesterol, triglycerides and HDL cholesterol are measured and LDL cholesterol is calculated from the preliminary measurements using the empirical equation of Friedwald and colleagues.

CLINICAL SIGNIFICANCE OF DYSLIPIDAEMIA

Dyslipidaemias are disorders of lipoprotein metabolism, characterized by hypertriglyceridaemia, elevated total and LDL cholesterol levels and decreased HDL cholesterol levels. Dyslipidaemia, insulin resistance syndrome, diabetes mellitus (type 1 or type 2) and truncal adiposity are known to increase cardiovascular risk in the HIV-negative population and may similarly predispose HIV-infected subjects to accelerated coronary heart disease due to atherosclerosis.[40] Atherosclerosis has been demonstrated in autopsy studies to have its origins in childhood.[57] Cholesterol concentrations track over time so that those children with high LDL-cholesterol are likely to become adults with high LDL-cholesterol.[58] It has been shown that the beginning of fatty streaks in arteries occurs during childhood, leading to the development of advanced atherosclerotic lesions in adults.[59] Total cholesterol and triglycerides levels are affected by age, sex, dietary habits, life style, infections, alcohol consumption, caffeine intake, contraceptive use, and cigarette smoking.[59] Factors such as obesity, height, dietary changes and changes in exercise routines can influence both pediatric and adult lipid levels.[59] Disease conditions associated with a high risk of developing dyslipidaemia in

children include, chronic renal disease, end stage renal disease, post renal transplant, post orthotopic heart transplant, Kawasaki disease with current aneurysm, juvenile idiopathic arthritis, nephrotic syndrome and hypertension. Some screening studies have reported plasma cholesterol levels in children of different ages and in different populations. In a study by Knuiman et al.[60] the plasma cholesterol levels in children around 8-9 years of age, from different countries, were compared. The results showed variation in the cholesterol levels in various countries. The highest cholesterol mean was in children from Finland 158.5-189.5 mg/dL,(4.1-4.9mmol/L) and the lowest in children from Ghana and the Philippines 127.6- 146.9 mg/dL, (3.3-3.8mmol/L).[61] A similar study compared total cholesterol levels of 13- year-olds from 15 different countries, the highest total cholesterol level, with values greater than 180mg/dL (4.7mmol/L) was found in children from Finland with a prevalence rate of more than 50% and the lowest total cholesterol level with value less than 130mg/dL (3.4mmol/L) was found in children from Nigeria with a cumulative prevalence of 12%.[61] These prevalences suggest a geographic pattern in the variation of total cholesterol at specified ages prior to or during puberty which may be attributed to the diet and the various standards of measurements as some countries measured total serum cholesterol while several other countries measured total plasma cholesterol.

DYSLIPIDAEMIA ASSOCIATED WITH HAART

The advent of PI therapy has made the occurrence of dyslipidaemia to become more prevalent and more pronounced.[62] HAART-associated dyslipidaemia is characterised by hypertriglyceridaemia with depressed plasma concentrations of high-density lipoprotein (HDL) cholesterol and increased total cholesterol, with or without increased low-density lipoprotein (LDL) cholesterol.[42] This profile is linked with the insulin resistance and fat redistribution (HIV

lipoatrophy or lipohypertrophy) [42] However, dyslipidaemia can occur without obvious lipoatrophy and insulin resistance suggesting that ,these features are independent or perhaps dyslipidaemia is a sensitive early marker of disease, related to earlier diagnosis and more careful clinical assessment of HIV-infected patients.[13] These disorders are associated with increased risk of cardiovascular disease and have become an important cause of morbidity and mortality in HIV-infected patients. Clinical features associated with HIV lipoatrophy syndrome (fat atrophy) are; sunken cheeks, hollow temples, sunken eyes, prominent zygomatic arch, prominent veins, and skinny or muscular appearance, loss of contour on buttocks with loose skin folds. HIV lipohypertrophy (fat accumulation) are associated with increased abdominal girth, visceral fat accumulation and dorsocervical or supraclavicular fat pad.

In the largest published series of lipid assessment in HIV-infected children, Farley et al[63] prospectively followed nearly 2000 perinatally acquired HIV-infected children between the ages of 4 and 19 years, and found the prevalence of hypercholesterolaemia to be 13%. From the study, PI therapy and current NNRTI use had the greatest association with dyslipidaemia while receipt of NRTI, was found to be protective against dyslipidaemia.[63] The study however did not measure the other fractions of the lipid profile (LDL, HDL and serum triglycerides) which could influence the result as a high HDL-cholesterol (which offers some protective effect against cardiovascular disease) may have contributed to the hypercholesterolaemia. The European Paediatric Lipodystrophy Group,[21] in a subgroup of 280 children aged 3-18 years attending paediatric HIV clinics in 18 centres, reported the prevalences of hypercholesterolaemia and hypertryglyceridaemia to be 27% and 21% respectively. Out of the 280 children, 10% of them had a cumulative prevalence of dyslipidaemia to be 38%. Hypercholesterolaemia was found to be more common in girls and those on HAART than those on mono or dual ART. In Uganda, Piloya et al[64]

studied a group of 364 HIV-infected children aged 2-18 years, and found 125 of them to have dyslipidaemia, accounting for a prevalence of 34% which is comparable to the European group.[21] Of those children with dyslipidaemia, 16.8% had hypercholesterolaemia and 83.2% had hypertryglyceridaemia. Piloya et al observed that, 63% of the dyslipidaemic children were on NNRTI containing regimen as first line while those on PIs were excluded from the study. This study indicated the contribution of NNRTIs to the occurrence of dyslipidaemia in HIV-infected children on HAART. The observations from the American, European and Ugandan studies are comparable as the children with HIV/AIDS on HAART had dyslipidaemias irrespective of race even though the studies did not evaluate HAART naive children. In Nigeria, studies of patients on HAART in Enugu, Ile-Ife, Abuja, Jos and Kano all revealed elevated levels of total cholesterol, LDL-C and Triglycerides while HDL-C was not significantly decreased.[65-68] These studies were however conducted in the adult population and no data exist for the lipid profile of HIV-infected children in Nigeria.

RISK FACTORS

The occurrence of dyslipidaemia has been found to be associated with certain risk factors such as, age less than six years, current PI use, prolonged PI exposure greater than 3 years, NNRTI use, parent/patient report of excellent adherence and HIV-1 RNA< 400 copies/ml.[63] Farley et al[63] reported a 3.6 times risk of having a total cholesterol greater than the 95th percentile for gender, race, and age for PI-treated children and a 72% increase in the risk of hypercholesterolemia for any additional PI into a child's regimen. Cheseaux et al[69] published the results of a retrospective review of lipid levels in 66 Swiss children before and after the addition of PI therapy to a regimen containing two NRTI drugs: 29 children were treated with ritonavir and 37 with nelfinavir.

Although both groups showed significant increase in their total cholesterol after PI therapy, the ritonavir group had substantially higher cholesterol values than the nelfinavir group. Cheseaux et al, concluded that HIV-infected children treated with HAART have elevations in their cholesterol similar to those seen in patients heterozygous for familial hypercholesterolemia and therefore, have a similar risk for premature atherosclerotic disease. Hypertriglyceridaemia seems more frequent in patients receiving a ritonavir, ritonavir/saquinavir or ritonavir/lopinavir combination therapy, compared with indinavir-, nelfinavir- and amprenavir-based ones. The alterations may sometimes be extreme, reaching a triglyceride plasma concentration >1000 mg/dl in subjects on ritonavir therapy.[70-78] The development of hyperlipidaemia during PI administration appears to be dose- and probably time-related.[31] Serum lipid abnormalities occur shortly after the commencement of therapy, usually between three and twelve months, but their onset may be faster (within weeks) in subjects receiving a ritonavir-containing regimen.[31]

PATHOPHYSIOLOGY OF HAART RELATED DYSLIPIDAEMIA

The pathophysiology of HAART-related dyslipidaemia is complex involving multiple drug-induced lipid metabolism abnormalities, in association with a range of hormonal and immunological imbalances and genetic predisposing factors.[31] Compared with healthy controls, HIV patients already have abnormal lipoprotein concentrations before the initiation of HAART, which worsen after the initiation of therapy. The lipoprotein profile associated with HAART features increased plasma triglyceride, increased total and LDL cholesterol, and decreased HDL cholesterol.[13] These changes are further accompanied by increases in small dense LDL particles, lipoprotein(a) , apolipoproteins B,C-III, E, and H. Increases in plasma triglyceride-very low density lipoprotein results from decreased catabolism of these particles by the HIV- infection.

The most convincing proposed mechanism is based upon the structural similarity between the catalytic region of HIV-1 protease and two homologous human proteins involved in the lipid metabolism which are the cytoplasmic retinoic acid-binding protein type 1 (CRABP-1), and low-density lipoprotein- receptor-related protein (LRP). The amino acid sequence of the C-terminal region of CRABP-1 is 58% homologous to the catalytic region of HIV-1 protease, while LRP shares 63% amino acid homology with the viral protease.[31]

CRABP-1 binds intracellular retinoic acid and presents it to cytochrome P450 (CYP) 3A enzymes, which converts retinoic acid to cis -9-retinoic acid. The cis-9-retinoic acid then binds to a heterodimer (including retinoid X receptor (RXR) and peroxisome proliferator activated receptor type $_{\gamma}$ ($PPAR_{\gamma}$) in adipocyte nuclei. The heterodimer RXR- $PPAR_{\gamma}$ associated with the cis - 9-retinoic acid inhibits adipocyte apoptosis and stimulates adipocyte proliferation and differentiation. PIs probably bind to CRABP-1 which is homologous to the viral protease and erroneously inhibit the formation of cis -9-retinoic acid, leading to a reduced RXR- $PPAR_{\gamma}$ activity, increased apoptosis and diminished proliferation of peripheral adipocytes. These results in peripheral lipoatrophy syndrome and hyperlipidaemia because of adipocyte loss, decreased lipid storage and lipid release into the bloodstream.[70,71,79]

PI-related dyslipidaemia may also involve a genetic predisposition. Experimental research has documented an association between hypertriglyceridaemia (with low serum HDL cholesterol levels) and several polymorphisms found in the apo CIII gene. Variations in the nucleosides 455 and 482 are both associated with increased levels of triglyceride containing lipoproteins (VLDL) and low HDL values.[80]

A report also hypothesizes that mitochondrial alterations found in HIV-infected patients receiving HAART could also play a role in the development of antiretroviral therapy-related lipodystrophy

and dyslipidaemia. It is speculated that HAART when combined with multiple NRTIs, would cause mitochondrial abnormalities by inhibiting the mitochondrial DNA polymerase γ, leading to a mitochondrial DNA depletion, a respiratory chain dysfunction and a reduced cell energy production.[31] Mitochondrial respiratory chain inhibition could be responsible for several abnormalities in different cell types, such as adipocytes, promoting lipodystrophy syndrome and increased plasma lipid levels.[31]

MANAGEMENT OF HAART RELATED DYSLIPIDAEMIA

The Cardiovascular Subcommittee of the AIDS Clinical Trials Group (ACTG)[81] made some recommendations on the management of Dyslipidaemia following the National Cholesterol Education Program (NCEP) guidelines for the evaluation and treatment of dyslipidemia in HIV-infected patients. This NCEP recommends improvements in diet and exercise as the first intervention. The standard lipid-lowering agents, 3-hydroxy-3-methylglutaryl co-enzyme A reductase inhibitors (statins), must be used cautiously because several of them are metabolized by the P-450 enzyme, CYP3A4 and therefore, can affect serum concentrations of PIs leading to myalgias, myopathy and rhabdomyolysis.[33]

The American Academy of Pediatrics[41] (AAP) recommends drug treatment of dyslipidaemia in children, only if older than 10 years and in those with dyslipidaemias with serum LDL ≥ 190 mg/dl or ≥ 160 mg/dl after 6-12 months of dietary modification and positive family history of coronary artery disease. The AAP recommends drug treatment with cholestyramine and colestipol, which are the two acid-binding resins that significantly lower LDL cholesterol. These agents, however, may lead to further increases in serum triglycerides in patients taking a PI based regimen. Another problem with these agents is their potential interference with the absorption of concurrently administered drugs, including antiretroviral drugs, which could lead to treatment failure.[32]

Another strategy for managing dyslipidaemia in HIV-infected children is to switch from a PI to a PI-sparing regimen or a less metabolically active family member, without compromising antiviral efficacy.[13] Among the protease inhibitors, dyslipidaemia appeared to be greatest with ritonavir (particularly the short-term intensive booster doses). Amprenavir and nelfinavir have intermediate effects on plasma lipids, indinavir and saquinavir have fewer dyslipidaemic effects, and lopinavir has the most favourable lipid profile while atazanazvir has negligible effects on serum lipids.[13] Another strategy to control dyslipidaemia, is to discontinue the protease inhibitor within the HAART regimen and switch to an NRTI or NNRTI. Mc Comsey et al [32] conducted a paediatric PI therapy switch, where 17 children were changed from a PI- containing regimen to efavirenz. After 48 weeks, the switch to efavirenz resulted in significant improvements in total cholesterol, LDL-cholesterol and triglycerides levels while maintaining excellent virologic control.[32]

GENERAL AIM

The aim of this study is to compare the prevalence and risk factors for dyslipidaemia among HIV-infected and uninfected children aged 2-15 years attending the Aminu Kano Teaching Hospital (AKTH).

SPECIFIC OBJECTIVES

1. To determine the serum levels of Total-cholesterol, LDL-cholesterol, HDL-cholesterol and Triglycerides, in HIV infected children on HAART aged 2-15 years.
2. To determine the serum levels of Total-cholesterol, LDL-cholesterol, HDL-cholesterol and Triglycerides, in HIV infected HAART naive children and HIV negative children age and sex matched with HIV infected children on HAART.
3. To determine the prevalence of dyslipidaemia in subjects on HAART compared to HAART naive children
4. To determine risk factors for dyslipidaemia in the children on HAART and the HAART naive children.
5. To compare the prevalence of dyslipidaemia between subjects on Protease inhibitors (PI) and those on non- PI based HAART regimens.

SUBJECTS AND METHODS

STUDY LOCATION

The study was carried out in the Paediatric Infectious Diseases Clinic (PIDC) of the Aminu Kano Teaching Hospital (AKTH). The hospital was established in 1998 in Kano, North-West Nigeria. This facility provides tertiary health care to people in Kano and the neighbouring states. Enrollment into the Paediatric Infectious Diseases Clinic (PIDC) commenced in 2003. There were 2,222 children enrolled as at June, 2014 with 852 patients on HAART. At the time of data collection for this study, only children who were less than 18months of age with a positive DNA PCR test, children greater than 18 months of age with a positive rapid test with WHO Clinical stage 3 or 4 disease irrespective of CD4 count, children 24-59months with CD4 count < 750 cells/mm^3 and children greater than 5 years with CD4 count <350cell/mm^3 were commenced on HAART. The other children who were not commenced on HAART were children who did not meet the above criteria. These included children less than 18months of age who had negative DNA PCR test but who were still on follow up at the clinic, other children who were greater than 15 years of age were transferred to the adult section and some were lost to follow up while some had died. The clinic attends to paediatric patients every day of the week from Monday to Friday, with support from AKTH staff, President's Emergency Plan for AIDS Relief (PEPFAR), ACTION AIDS and the Institute of Human Virology, Nigeria. The facility has a functional laboratory where basic investigations such as complete blood count, urea, electrolytes, creatinine, serum glucose, lipid profile and liver function tests are carried out. The Paediatric Infectious Disease Clinic follows the National guidelines for Paediatric HIV and AIDS treatment and care in Nigeria for the initiation of ART in children. The drug regimens available in the clinic are:

First- Line

1. Nevirapine (NVP) + Zidovudine (AZT) + Lamivudine (3TC)
2. Nevirapine (NVP) + Abacavir (ABC) + Lamivudine (3TC)
3. Abacavir (ABC) + Zidovudine (AZT) + Lamivudine (3TC)
4. Nevirapine (NVP) + Tenofovir (TDF) + Emtricitabine (FTC) or Lamivudine (3TC)

Second- Line

1. Lopinavir+ritonavir(LPVr) + Tenofovir(TDF) + Emtricitabine(FTC)
2. Lopinavir+ritonavir(LPVr) + Zidovudine (AZT) + Lamivudine (3TC)

First- Line regimen for Failed PMTCT

1. Lopinavir+ritonavir(LPVr) + Abacavir (ABC) + Lamivudine (3TC)

STUDY DESIGN

The study was a comparative study

STUDY POPULATION

The subjects for the study were children aged 2 - 15 years with HIV infection attending the Paediatric Infectious Diseases Clinic. The comparative group were well nourished apparently healthy children, selected from the Paediatric Out Patient Department amongst children coming for medical check up, minor operations and HIV negative siblings of HIV- infected subjects.

INCLUSION CRITERIA: STUDY SUBJECTS

1. Age between 2 – 15 years.
2. HIV-infected HAART naive children and those on first and second line HAART for at least three months.
3. Parent/care-giver informed consent and assent from children eight years or older.

EXCLUSION CRITERIA: STUDY SUBJECTS

1. Confirmed or known patients with renal disease, diabetes mellitus and cardiac disease
2. Those on corticosteroids for at least one month prior to the commencement of the study.

INCLUSION CRITERIA: HIV NEGATIVE GROUP

1. Age and sex matched HIV negative apparently healthy controls.
2. Parent/care-giver informed consent and assent from children eight years or older.

EXCLUSION CRITERIA: HIV NEGATIVE GROUP

1. Those whose parents/care-givers declined consent to be part of the study.
2. Those on corticosteroids for at least one month prior to the commencement of the study.
3. Confirmed or known patients with renal disease, diabetes mellitus and cardiac disease

ETHICAL CONSIDERATION

An ethical clearance for the study was obtained from the Research and Ethical Clearance Committee of the Aminu Kano Teaching Hospital (Appendix I). For each subject recruited, an informed consent was obtained from the parent(s) or care-giver and assent was also obtained for children greater than eight years. The benefits and possible discomfort was explained to whoever gave the consent as explained in the consent and assent form (Appendix II). The child (if old enough) was opportuned to know his or her lipid status. The discomfort of pain from needle prick for venepuncture was explained to the older children. The children with dyslipidaemia requiring drug treatment were referred to the metabolic clinic.

SAMPLE SIZE DETERMINATION

The minimum number (n) per group of children required for the study was calculated using the formula for comparative study [82]

$$n = \frac{P_1 (1- P_1) + P_2 (1-P_2)}{(P_2- P_1)} \times f(\alpha,\beta)$$

Where; n = no of subject per group

P_1 = prevalence of dyslipidaemia in children with HIV which was taken as 34% (0.34) [64]

P_2 = prevalence of dyslipidaemia in apparently healthy controls which was taken as 12% (0.12) [61]

$f(\alpha,\beta)$ = 10.5 for 90% power with 5% significance (risk of type 1 error)

Therefore, the minimum sample size per group

$$n = \frac{0.34(1- 0.34) + 0.12(1-0.12)}{(0.12-0.34)^2} \times 10.5 = 72$$

From the above calculation, seventy-two (72) was the minimum sample size (n) per group. However, 80 subjects were recruited per group to account for 10% non response. Thus, the study selected 80 HIV positive children on HAART, another 80 HAART naive children (defined as HIV-infected children who are not on HAART) and 80 HIV negative children as controls bringing the total sample size to 240 subjects for the study.

SAMPLING TECHNIQUE

The study subjects that fulfilled inclusion criteria were recruited using systematic sampling. Averages of 53 patients were booked to attend clinic weekly. A list of the patients that have been booked to attend the clinic for one week was obtained and their folders were retrieved from the record office of the clinic. The sampling interval was then calculated based on the required sample size. The patients were grouped into those on first line and those on second line medications. The patients were numbered serially for the two groups and the total number constituted the sampling frame. The selected study participants were identified and recruited for the study on their clinic day. This was continued until the desired sample size was reached. A total of 46 patients on first line HAART and 34 patients on second line HAART were recruited. The HIV positive HAART naive group and the HIV negative group were recruited consecutively using the convenience sampling method after the study subjects were recruited. The study subjects and controls were aged and sex matched. The period of study was from August 2015 to March 2016.

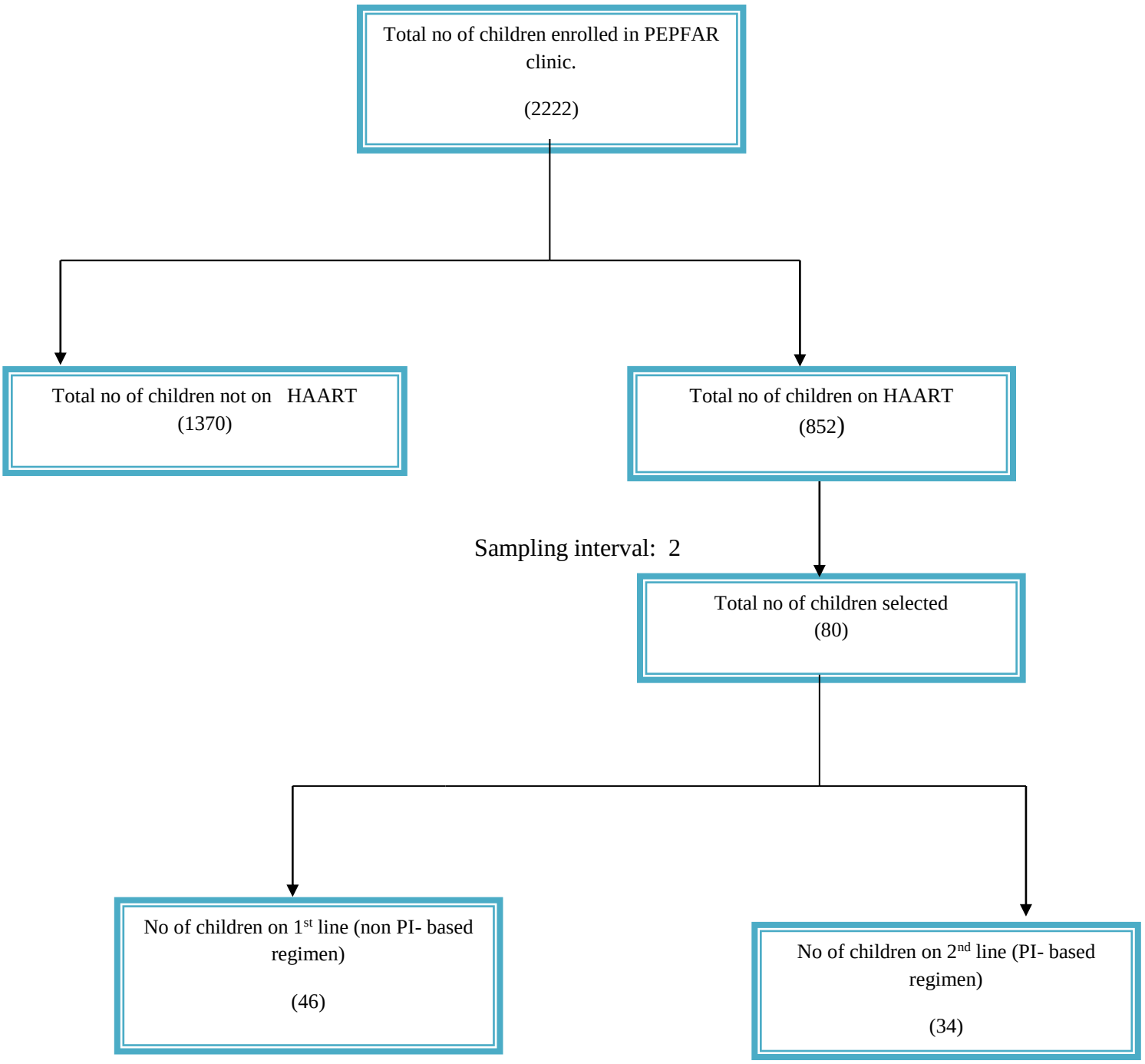

Figure 2: Flow chart showing sampling technique.

SUBJECT SELECTION AND ENROLLMENT

A study proforma (Appendix III) was used to collect information on each of the subjects and controls by the researcher. An informed consent was obtained, they were interviewed, examined and this was followed by collection of blood specimens with the help of an assistant for laboratory analysis.

DATA COLLECTION

At the time of recruitment, information regarding socio-demographic data, including social class of the parents or caregivers were classified using the Oyedeji socio-economic classification scheme (Appendix IV).[83]

The medical history of the patient was obtained from the patient and clinic records including age at diagnosis of HIV infection, duration of diagnosis of HIV-infection, age at commencement of HAART, duration on HAART and type of HAART regimen. Clinical examination including features of lipodystrophy characterized by fat atrophy such as sunken cheeks, hollow temples, sunken eyes, prominent veins, prominent zygoma, skinny appearance or fat accumulation as evidenced by dorsocervical or supraclavicular pad of fat were looked for. Anthropometric measurements: weight, height/length were taken using appropriate instruments. All subjects were weighed with light clothing. The children less than five years or less than 20 kg, were weighed using seca376® digital weighing scale. The weighing scale was standardised to zero, then the weight was measured to the nearest 10 g. Those older than five years or more than 20 kg were weighed using the HealthOMeter 402KLWH® physician balance beam scale with height measurement. The digital weighing scale and beam balance were standardized each morning with

known standard weights before taking the measurements. The Body Mass Index (BMI) was calculated as weight (kg)/ height (meters^2).

The heights of the subjects less than five years were measured using the Harpenden stadiometer. In all subjects, the measurement was taken by the researcher with the help of an assistant (trained nurse). Every subject had the footwear and head covering removed and was positioned with the back against the back-post of the stadiometer ensuring that the heels, buttocks and head touch the back-post with the heels together, legs straight and shoulders relaxed. The head rule was lowered gently on top of the head of the subject while ensuring the head was in the correct position. The child was asked to look straight ahead with the lower margins of the eyes in the same plane as the external auditory meati (Frankfurt plane).

LABORATORY METHODS

Under strict aseptic conditions, after cleaning the blood collection site thoroughly with 70% alcohol, the researcher with the help of an assistant, collected five milliliters of non fasting venous blood by venepuncture using a 21G hypodermic needle into a clot gel activator tube. Samples were separated by centrifugation at 1000 revolutions per minute for 10 minutes. The sera obtained was transferred into appropriately labeled plain containers and frozen at -20°C in a freezer until the time of analysis. Concentrations of serum Total cholesterol (TC), Low density lipoprotein cholesterol (LDL-C), High density lipoprotein cholesterol (HDL-C) and triglycerides (TG) were measured by enzymatic assays[47,53,54] as explained in Appendix V using the machine, Cobas Integra 400 series. Total cholesterol was measured by the enzymatic end point assay method described by Allan et al.[47] High density lipoprotein-cholesterol was also measured by the

enzymatic endpoint method described by Allan et al, after the precipitation of cholesterol in LDL,VLDL and chylomicron fractions by the action of phosphotungistic acid in the presence of magnesium ions.[47] Triglyceride was estimated by the enzymatic colorimetric method of McGowan et al.[53] Low density lipoprotein cholesterol level was calculated from the levels of total cholesterol, HDL-C and triglycerides, using the Friedwald et al's formula.[54]

Data Analysis

Data was analyzed using the Statistical Package for Social Science (SPSS) software for Windows version 16.0 (University of Bristol). Data were presented in tables. Quantitative variables such as age, weight, height, BMI, age at diagnosis of HIV infection, duration of diagnosis of HIV infection, age at commencement of HAART, duration on HAART and serum lipid levels were summarised using mean, standard deviation, median and ranges. Qualitative variables such as gender, socio-economic class, HAART regimen were described using frequencies and percentages. The analysis of variance (ANOVA) was used to compare mean values of variables, while proportion and percentages were compared using Chi-square (χ^2) test. Multivariate analysis was used to analyse the effect of some independent variables such as age, duration of diagnosis of HIV, duration on HAART and dyslipidaemia, type of HAART and dyslipidaemia on the final outcome-presence or absence of dyslipidaemia which is the dependent variable. 'P' values of < 0.05 were accepted as statistically significant.

Operational definitions and interpretation of results

For the purpose of this study, the following terms were defined;

1. HIV-infected HAART naïve children and adolescents were HIV- infected children who had no prior antiretroviral drugs.
2. HIV- infected children on HAART (Non-PI based HAART regimen) was HAART treatment which consisted of either Nevirapine (NVP) + Zidovudine (AZT) + Lamivudine (3TC) or Nevirapine (NVP) +Abacavir (ABC) + Lamivudine (3TC) or Abacavir (ABC) + Zidovudine (AZT) +Lamivudine (3TC).
3. HIV-infected children on Protease Inhibitor based (PI-based) HAART regimen in this study, consisted of a combination of Lopinavir + Ritonavir (LPVr) + Zidovudine (AZT) + Lamivudine(3TC) for the patients who failed first line treatment and Lopinavir+ritonavir (LPVr) + Abacavir (ABC) + Lamivudine (3TC) for those children who failed PMTCT.
4. Abnormal lipid levels known as dyslipidaemia, were defined according to the National cholesterol education programme (NCEP) guidelines,[41] using the values in the Table III, any subject with values for total cholesterol (TC) ≥200 mg/dL (**5.2 mmol/L**), low density lipoprotein (LDL) ≥130 mg/dL (**3.4 mmol/L**), triglyceride (TG) ≥ 150 mg/dL (**1.69 mmol/L**) and high density lipoprotein (HDL) ≤ 35 mg/dL (**0.91 mmol/L**) wereconsidered to have dyslipidaemia.
5. To define undernutrition in children less than 5years, anthropometric computations were conducted for the weight for age z-score (WAZ-score) and height for age z-score (HAZ-score) using the WHO Anthro software which is based on WHO child growth standards of 2006.[85]

6. Underweight was defined as a weight for age z-score (WAZ-score) less than -2 standard deviation (SD) from the WHO reference median values.
7. Stunting was defined as height for age z-score (HAZ-score) less than – 2SD from the reference values.[84]
8. For children 5 years and older, the Body Mass Index (BMI) was calculated as the weight in kilograms divided by the square of the height in metres. Those whose BMI were less than -2SD were considered underweight.[84]

RESULTS

The socio-demographic characteristics of the subjects and control

A total of 240 children aged 2- 15 years were studied. As shown in Table IV, the children were divided into three groups, 80 were HIV positive children on HAART, 80 HIV positive HAART naïve children (HAART NAIVE) and 80 'apparently healthy' HIV negative children who served as the controls (CONTROLS). The children in the HAART group were further sub-divided into 2 groups based on their HAART regimen. 46 patients were on first line HAART-Non PI based regimen and 34 patients were on second line HAART- PI based regimen.

In the HAART group, 47(58.7%) were males and 33(41.3%) were females. The HAART NAIVE group also had 47(58.7%) males and 33(41.3%) females while the control group had 47(58.7%) males and 33(41.3%) females. There was a male preponderance with an overall male : female ratio of 1.4:1. The mean± SD age of the subjects across the study groups was 9.08± 3.80 years for the group on HAART, 9.00±3.66 years for the HAART naive and 8.85±3.86 years for the controls (p=0.92).

The study subjects were mostly of low socio-economic class belonging to social class IV across the three groups.

TABLE IV: The socio-demographic characteristics of subjects on HAART, HAART-NAIVE and HIV-NEGATIVE controls

Variable	HAART (n=80)	HAARTNAIVE (n=80)	CONTROL (n=80)	F test/ χ^2	p value
Age group (years)	**n (%)**	**n (%)**	**n (%)**		
<5	14(17.5)	12(15.0)	16(20.0)		
5-9	27(33.7)	31(38.7)	28(35.0)	0.99*	0.91
10-15	39(48.8)	37(46.3)	36(45.0)		
Mean age ±SD	9.08 ± 3.8	9.00 ± 3.66	8.85 ± 3.86	0.08#	0.92
Gender					
Male	47(58.7)	47(58.7)	47(58.7)		
Female	33(41.3)	33(41.3)	33(41.3)	0.00*	1.00
Socio-Economic class					
I	12(15.0)	2(2.5)	9(11.2)		
II	13(16.2)	3(3.8)	9(11.2)		
III	22(27.5)	23(28.7)	26(32.5)	18.67*	0.02
IV	26(32.5)	40(50.0)	29(36.3)		
V	7(8.8)	12(15.0)	7(8.8)		

χ^{2}* = chi square

F# = ANOVA(Analysis of variance)

Medical history of the HIV-infected subjects. The median age at diagnosis of HIV infection in the HAART group was 30 months (IQR 48.0 months), HAART naive group was 72 months (IQR 58.0 months). The mean duration of diagnosis of HIV infection in the HAART group was 65.38±39.15 months and 37.76±31.61 months in the HAART naive group. The mean age at commencement of HAART for subjects in the HAART group was 49.84± 44.03 months while their mean duration on HAART was 59.61± 40.70 months. Of the 80 HIV positive children on HAART, 34 were on second line and these were children who have failed their first line regimen and now on second line drugs have also been on these PI-based HAART regimen(lopinavir/ritonavir based combination) for a mean duration of 30.83±17.77 months.

Anthropometric Measurements of the HIV-infected children and the controls.

Table V shows the mean anthropometric measurements of the children with HIV infection on HAART, HAART-Naïve and controls. The HIV negative controls had a higher mean weight, height and BMI values compared to the HIV infected children. However, the differences in mean weight ($F = 1.60$, $p = 0.20$), height ($F = 0.71$, $p = 0.49$) and BMI ($F = 1.98$, $p = 0.14$) were not statistically significant across the three groups.

Table V: Anthropometric measurements of the HIV-infected children and the controls

Anthropometry	HAART	HAARTNAIVE	CONTROL	F	p value
Weight (kg)					
Mean± SD	24.8±10.5	24.3±10.8	27.2±11.6	1.60	0.20
Range	10.0-57.0	7.7-57.0	11.0-60.0		
Height (m)					
Mean± SD	1.2±0.2	1.2±0.2	1.3±0.2	0.71	0.49
Range	0.74-1.6	0.72-1.6	0.78-1.6		
BMI (kg/m^2)					
Mean± SD	16.0±2.6	15.5±2.5	16.4±2.9	1.98	0.14
Range	12.3-25.0	9.7-23.4	10.5-27.4		

F = ANOVA (Analysis of Variance)

Table VI shows the frequency distribution of weight for age z-score (WAZ-score), height for age z-score (HAZ-score) for children less than five years and BMI z-score for children and adolescents greater than or equal to five years across the study groups. Among the children less than five years, 7.1% of the children on HAART, 25% of the HAART NAIVE and 13.3% of the controls were underweight. Of the children less than five years, 28.6% of the children on HAART, 33.3% of the HAART NAIVE and 33.3% of the controls were found to be stunted. For the children five years and older, 25.8% of the children on HAART, 20.6% of the HAART NAIVE and 12.3% of the controls were underweight. However, there was no statistically significant difference between the study groups.

Table VI: Frequency distribution of underweight and stunting using WAZ-score, HAZ-score and BMI Z-score of study participants

Variable	HAART	HAART NAIVE	CONTROL	test	p value
WAZ< 5years	**n(%)**	**n(%)**	**n(%)**		
≥ -2SD	13(92.9)	9(75.0)	13(86.7)		
< -2SD	1(7.1)	3(25.0)	2(13.3)		0.35#
Total	14	12	15		
HAZ < 5years					
≥ -2SD	10((71.4)	8(66.7)	10(66.7)		
< -2SD	4(28.6)	4(33.3)	5(33.3)		0.98#
Total	14	12	15		
BMI ≥ 5years					
≥ -2SD	49(74.2)	54(79.4)	57(87.7)		
< -2SD	17(25.8)	14(20.6)	8(12.3)	3.66	0.16*
Total	66	68	65		

 # = Fisher's exact test

χ^{2*}= chi square

TABLE VII: shows the proportion of those with the different clinical features of lipoatrophy in the HIV infected study participants. The presence of sunken cheeks ($p = 0.03$), hollow temples ($p = 0.02$) were features found to be common among the HAART and HAART NAIVE subjects and these features were statistically significant. The prevalence of skinny appearance was highest among the HAART subjects however this feature was not statistically significant among the two groups.

TABLE VII: Clinical features of lipoatrophy syndrome

Variable	HAART n=80	HAART NAIVE n=80	Test Fisher exact test/χ^2	p value
	n(%)	n(%)		
Sunken cheeks				
Yes	8(10.0)	1(1.3)		**0.03**#
No	72(90.0)	79(98.7)		
Hollow temples				
Yes	13(16.3)	3(3.7)		**0.02**#
No	67(83.7)	77(96.3)		
Sunken eyes				
Yes	6(7.5)	5(6.3)		1.00#
No	74(92.5)	75(93.7)		
Prominent zygoma				
Yes	0(0.0)	2(2.5)		
No	80(100.0)	78(97.5)		0.49#
Prominent veins				
Yes	6(7.5)	5(6.3)		
No	74(92.5)	75(93.7)		1.00#
Skinny appearance				
Yes	26(32.5)	22(27.5)		
No	54(67.5)	58(72.5)	χ^2 =0.48	0.49*

 # = Fisher exact test

χ^2 *= Chi square

Table VIII shows the Age Distribution, HAART regimen, mean duration on HAART, proportion of children with hypercholesterolaemia and hypertriglyceridaemia among the HIV infected children on HAART with features of lipoatrophy. Lipoatrophy was commoner in the 10-15 years age group and was statistically significant (p= 0.003). The children with lipoatrophy were mostly on Non PI-based HAART regimen (1st line regimen consisting of Zidovudine, Lamivudine and Nevirapine) with a mean duration on a HAART to be 68.35±41.96 months. However there was no statistical significant difference between those on Non-PI based and PI-based HAART regimen.

Table VIII: Characteristic features of Lipoatrophy among HIV-infected children on HAART

Variable	Lipoatrophy Yes n(%)	No n(%)	Total	Test statistic χ^2/fisher exact test	p value
Age distribution					
< 5	2(14.3)	12(85.7)	14	11.67	**0.003***
5-9	10(37.0)	17(63.0)	27		
10-15	25(64.1)	14(35.9)	39		
HAART Regimen					
Non PI-based	22(47.8)	24(52.2)	46	0.11	0.74*
PI-based	15(44.1)	19(55.9)	34		
Mean duration on HAART±SD (months)	68.35±41.96	52.09±38.48		3.27	0.08#
Total Cholesterol					
High	14(36.8)	24(63.2)	38	2.57	0.11*
Normal	23(54.8)	19(45.2)	42		
Triglyceride					
High	22(44.0)	28(56.0)	50	0.27	0.60*
Normal	15(50.0)	15(50.0)	30		

***= Chi square**

F#=ANOVA(Analysis of Variance)

Table IX: Serum Levels of Total Cholesterol, Triglyceride, LDL-C and HDL-C of all the study participants

Table IX shows the serum lipid levels of the study participants. Dyslipidaemia was found in the HAART group based on a high mean Total Cholesterol, LDL-cholesterol and Triglyceride levels. The difference between the HAART, HAART NAIVE and CONTROL groups were found to be statistically significant. Serum TC (p= 0.0001), LDL-c (p = 0.0001) and TG (p=0.002). On the other hand, dyslipidaemia was also found in the HAART naive group based on low HDL-c levels but this difference was not statistically significant. (p= 0.25).

Table IX: Serum lipids of participants (Mean ±SD) by group

Variable	HAART	HAARTNAIVE	CONTROL	F	p value
TC Mean± SD (mmol/L)	5.34 ± 1.30	4.16±0.94	3.99 ± 1.03	35.46	0.0001
TG Mean± SD (mmol/L)	1.88±0.58	1.67± 0.53	1.59±0.52	6.19	0.002
LDL-cMean±SD (mmol/L)	3.52±1.17	2.51±0.88	2.19 ± 0.92	39.28	0.0001
HDL-cMean±SD (mmol/L)	0.92 ± 0.34	0.86±0.23	0.93 ± 0.33	1.38	0.25

F = ANOVA (Analysis of Variance)

TC = Total Cholesterol

TG = Triglyceride

LDL-c = Low density lipoprotein- cholesterol

HDL-c = High density lipoprotein -cholesterol

Prevalence of Dyslipidaemia in study participants

Table X shows the prevalence of dyslipidaemia in each study group. There were more children with normal TC compared to high TC across all three groups but abnormal TC was found more common in the HAART group. There were almost equal proportions of high and normal TG in all the three groups combined, but the HAART group had a higher frequency compared to other groups. LDL-c mirrored those of TC with relatively more common abnormal LDL-c in the HAART group. Low HDL-c was found to be low across all the three groups.

Table X: Prevalence of Dyslipidaemia among the study participants

Variable	HAART n(%)	HAARTNAIVE n(%)	CONTROL n(%)	PREVALENCE OF DYSLIPIDAEMIA	χ^2	P value
Total Cholesterol						
High	38(47.5)	9(11.3)	7(8.7)	22.5%	43.15	0.0001
Normal	42(52.5)	71(88.7)	73(91.3)			
Triglyceride						
High	50(62.5)	38(47.5)	32(40.0)	50.0%	8.40	0.014
Normal	30(37.5)	42(52.5)	48(60.0)			
LDL-Cholesterol						
High	33(41.3)	14(17.5)	6(7.5%)	22.1%	28.92	0.0001
Normal	47(58.7)	66(82.5)	74(92.5)			
HDL-Cholesterol						
Low	43(53.8)	42(52.5)	43(53.8)	53.3%	0.03	0.98
Normal	37(46.2)	38(47.5)	37(47.5)			

χ^2 = Chi square

Risk factors for Hypercholesterolaemia in children on HAART

Table XI shows the risk factors for hypercholesterolaemia in HIV-infected children on HAART. Commencement of HAART at age less than 2 years and Protease Inhibitor (PI) based HAART regimen were found to be significantly associated with hypercholesterolaemia in the subjects on HAART (p =0.048) and (p = 0.001) respectively.

Table XI: Risk factors for Hypercholesterolaemia in children on HAART

Variable	High TC n(%)	Normal TC n(%)	TOTAL n= 80	Test statistic χ^2	P value
Age					
> 5years	29(43.9)	37(56.1)	66	1.92	0.17
< 5years	9(64.3)	5(35.7)	14		
Gender					
Male	20(42.6)	27(57.4)	47	1.12	0.29
Female	18(54.6)	12(45.4)	33		
Social Class					
I	6(50.0)	6(50.0)	12	0.17	0.97
II	6(46.2)	7(53.8)	13		
III	11(50.0)	11(50.0)	22		
IV	12(46.2)	14(53.8)	26		
V	3(42.9)	4(57.1)	7		
Age at diagnosis					
>2years	22(42.3)	30(57.7)	52	1.61	0.21
<2years	16(57.1)	12(42.9)	28		
Duration of diagnosis					
>1year	38(50.0)	38(50.0)	76		0.12#
<1year	0	4(100.0)	4		
Age at HAART					
> 2 years	21(39.6)	32(60.4)	53		
<2years	17(63.0)	10(37.0)	27	3.91	**0.048**
Duration on HAART					
>1year	34(49.3)	35(50.7)	69	0.63	0.43
<1year	4(36.4)	7(63.4)	11		
HAARTRegimen					
PI based	24 (70.6)	10 (29.4)	34	12.64	**0.001**
Non PI based	14(30.4)	32(69.6)	46		

χ^2 = Chi square # = Fisher's exact test p value

After controlling for confounders, only HAART regimen (PI- based) remained a predictor for hypercholesterolaemia among the HIV infected children on HAART as shown in table XII.(p = 0.001) while non PI-based regimen was protective against hypercholesterolaemia among the HIV-infected children on HAART.

Table XII: Multivariate analyses for risk factors for hypercholesterolaemia in children on HAART

Risk factor for Hypercholesterolaemia	Adjusted Odds Ratio (95%CI)	p value
Age at HAART (years)		
Less than 2 (referent)		
Greater than 2	0.38(0.13-1.08)	0.07
HAART Regimen		
PI based (referent)		
Non-PI based	0.18(0.07-0.49)	0.001

Risk factors for Hypertriglyceridaemia in children on HAART.

Table XIII shows risk factors associated with Hypertriglyceridaemia amongst the HIV-infected children on HAART. The children who were greater than 5 years (p = 0.02), duration of diagnosis of HIV infection greater than 1year (p = 0.02) and duration on HAART greater than 1 year (p = 0.04) were found to be the factors significantly associated with hypertriglyceridaemia statistically. However on multivariate analyses as shown in Table XIV, none of the factors was a predictor of hypertriglyceridaemia.

Table XIII: Risk Factors for Hypertriglyceridaemia in children on HAART

Variable	High Triglyceride n(%)	Normal Triglyceride n(%)	TOTAL n=80	Test statistic χ^2	p value
Age Group					
>5years	45(68.2)	21(31.8)	66	5.20	**0.02**
<5years	5(35.2)	9(64.3)	14		
Gender					
Male	30(63.8)	17(36.2)	47	0.09	0.77
Female	20(60.6)	13(39.4)	33		
Social Class					
I	10(83.3)	2(16.7)	12	7.57	0.10
II	5(38.5)	8(61.5)	13		
III	16(72.7)	6(27.3)	22		
IV	16(61.5)	10(38.5)	26		
V	3(42.9)	4(57.1)	7		
Age at diagnosis					
>2years	33(63.5)	19(36.5)	52	0.06	0.81
<2years	17(60.7)	11(39.30	28		
Duration of diagnosis					
> 1year	50(65.8)	26(34.2)	76		**0.02**[#]
< 1year	0	4(100.0)	4		
Age at HAART					
> 2 years	33(62.3)	20(37.7)	53	0.004	0.95
< 2 years	17(63.0)	10(37.0)	27		
Duration on HAART					
> 1year	49(65.3)	26(34.7)	75	4.11	**0.04**
< 1year	1(20.0)	4(80.0)	5		
HAARTRegimen					
PI based	19(55.9)	15(44.1)	34	1.11	0.29
Non PI based	31(67.4)	15(32.6)	46		

χ^2 = Chi square test

= Fisher's exact test p value

Table XIV: Multivariate analyses of risk factors for hypertriglyceridaemia in children on HAART

Risk factor for Hypertriglyceridaemia	Adjusted Odds Ratio (95%CI)	p value
Age Group (years)		
Less than 5	2.78 (0.76-10.23)	0.12
Greater than 5 (referent)		
Duration on HAART (year)		
Less than 1	2.32 (0.14-38.99)	0.56
Greater than 1		

Table XV shows that duration of diagnosis greater than 1 year was associated with hypercholesterolaemia in HAART naïve HIV-infected children (p value = 0.05).

Table XV: Risk factors for Hypercholesterolaemia in HAART- naïve children

Variable	High TC n(%)	Normal TC n(%)	TOTAL n=80	Test statistic χ^2	P value
Age					
> 5years	8(11.8)	60(88.2)	68	0.12	0.73
< 5years	1(8.3)	11(91.7)	12		
Gender					
Male	6(12.8)	41(87.2)	47	0.26	0.61
Female	3(9.1)	30(90.9)	33		
Social class*					
I&II	1(20.0)	4(80.0)	5	1.74	
III	1(4.3)	22(95.7)	23		
IV&V	7(13.5)	45(86.5)	52		
Age at diagnosis					
>2years	7(9.3)	68(90.7)	75		
<2years	2(40.0)	3(60.0)	5		0.09#
Duration of diagnosis					
>1year	9(16.1)	47(83.9)	56		**0.05#**
<1year	0(0)	24(100.0)	24		

= Fisher's exact test p value

*****Social classes were merged as there were cells with values less than 5

Risk factors for Hypertriglyceridaemia in HAART NAÏVE Children.

Table XVI shows that none of the risk factors considered were significantly associated with hypertriglyceridaemia in HIV-infected HAART naïve children.

Table XVI: Risk factors for Hypertriglyceridaemia in HAART NAÏVE Children

Variable	High Triglyceride (n%)	Normal Triglyceride (n%)	TOTAL n=80	Test statistic χ^2	p value
Age					
> 5years	33(48.5)	35(51.5)	68	0.19	0.66
< 5years	5(41.7)	7(58.3)	12		
Gender					
Male	24(51.1)	23(48.9)	47	0.58	0.47
Female	14(42.4)	19(57.6)	33		
Social Class					
I&II	2(40.0)	3(60.0)	5	0.35	0.83
III	12(52.2)	11(47.8)	23		
IV&V	24(46.2)	28(53.8)	52		
Age at diagnosis					
>2years	37(49.3)	38(50.7)	75	1.62	0.20
<2years	1(20.0)	4(80.0)	5		
Duration of diagnosis					
> 1year	27(48.2)	29(51.8)	56	0.04	0.85
<1year	11(45.8)	13(54.2)	24		

Serum Levels of Total Cholesterol, Triglycerides, LDL-C and HDL-C in study participants on HAART.

Table XVII shows that serum Total cholesterol and LDL-cholesterol levels were higher in the PI-based HAART group than the Non PI- based HAART group and these differences were found to be statistically significant (T test = -2.48; p =0.02 and T test = -3.12; p = 0.003 respectively).

Table XVII: Mean Serum lipids of study HIV infected children

Variable (Mean± SD)	PI-based HAART	Non PI-based HAART	T-value	p value
Total Cholesterol	5.75±1.25	5.04±1.27	-2.48	0.02
Triglycerides	1.82±0.64	1.92±0.55	0.73	0.47
LDL-Cholesterol	3.16±0.92	2.46±1.09	-3.12	0.003
HDL-Cholesterol	0.99±0.38	0.88±0.31	-1.48	0.14

T value = T test

Prevalence of Dyslipidaemia in subjects on PI and Non-PI based HAART.

Table XVIII shows the prevalence of dyslipidaemia in both groups (PI and non-PI based HAART). A significant proportion of children on PI-based HAART had elevated Total cholesterol and LDL-cholesterol than those on non-PI based HAART (χ^2 =12.64; p = 0.0001) and(χ^2 = 10.27; p = 0.001) respectively and this difference was found to be statistically significant. While a significant proportion of those on non –PI based HAART regimen had elevated levels of triglyceride and low levels of HDL-cholesterol than those on PI-based HAART regimen. However, this difference between the two groups was not found to be statistically significant. (χ^2 = 1.11; p =0.29) and (χ^2 = 2.21; p= 0.14) respectively.

Table XVIII: Prevalence of Dyslipidaemia in subjects on PI and non- PI based treatment

Variable	PI-base HAART n(%)	Non PI-based HAART n(%)	χ^2	p value
High Total Cholesterol	24(70.4)	14(30.4)	12.64	0.0001
High Triglycerides	19(55.9)	31(67.4)	1.11	0.29
High LDL-Cholesterol	21(61.8)	12(26.1)	10.27	0.001
Low HDL-Cholesterol	15(44.0)	28(60.9)	2.21	0.14

DISCUSSION

Dyslipidaemia have been reported among HIV-infected patients. Up to 50% of HIV- infected children treated with HAART have been known to develop lipid abnormalities.[85] The characteristic pattern of dyslipidaemia induced by HAART include elevated total cholesterol which ranges from 10-50%, elevated LDL cholesterol and triglyceride levels in 40-80% of HIV infected children on HAART.[86]

In this study, Total cholesterol, LDL-cholesterol and triglycerides levels were found to be significantly higher among the children on HAART compared to HAART naïve and HIV negative groups. However, HDL-cholesterol did not differ between the three groups. These elevations in serum lipids in HIV infection could be due to the replication of HIV in human T-cells which without the influence of antiretroviral drugs, is known to enhance fatty acid synthesis, increase production of low density lipoproteins and triglycerides, dysregulate lipid transport, oxidize lipids and alter cellular lipid metabolism.[43] These elevations are further worsened with the use of HAART.[44,62] Leonard et al [62], described these abnormal elevations in serum lipid levels in HIV-infected children and adolescents on HAART. A similar pattern of dyslipidaemia was reported in a group of HIV-infected adults and seronegative controls in a Nigerian population by Awah et al in Asaba.[97].

The prevalence of hypercholesterolaemia in this study among the HIV-infected children on HAART was 47.5%, hypertryglyceridaemia was 62.5%, high LDL-cholesterol 41.3% and low HDL-cholesterol was 53.8%. The high prevalence of dyslipidaemia noted in this study is attributed to the combination therapy of the HAART regimen, particularly the PI based antiretroviral drugs (lopinavir/ritonavir based combinations) which are known to be highly lipid sparing. Furthermore, the effect of HIV-infection on lipid metabolism even before the commencement of HAART,

contributes to the elevation of serum lipids in HIV infected patients with a longer duration of diagnosis of the HIV infection. This study showed that, the duration of diagnosis greater than 1 year was significantly associated with dyslipidaemia but the association was not significant after multivariate analysis. Also, the high prevalence of dyslipidaemia may be due to the long duration of treatment with HAART as this study showed the mean duration of treatment to be about 5years. Studies have shown that, unfavourable lipid concentrations could begin to manifest within three to twelve months into commencement of PI based therapy or faster in those with ritonavir based combinations.[31] The studies conducted by Vigano et al[21] and Farley et al[63] also reported similar findings of higher prevalence of lipid disorders among HIV-infected children and adolescents on HAART. The prevalence of the various forms of dyslipidaemia noted in this study were similar to the study of Amaya et al[20] in a study conducted on a group of 40 HIV-infected children in the US on PI- and non PI-based HAART regimen. They reported a prevalence of hypercholesterolaemia and hypertriglyceridaemia to be 68% and 28% respectively. Also in Uganda, Piloya et al [64] reported a prevalence of hypercholesterolaemia to be 16.8% and hypertriglyceridaemia to be 83.2%. The differences in prevalence rates could be due to varying drug induced effects of the antiretroviral drugs, particularly the PIs.[13] Various types of diets could also be associated with the pathogenesis of HAART related dyslipidaemia, as the children studied belonged to different regions across the world.[13]

Lower serum lipid levels were found among the HIV-infected HAART naïve children compared to the HAART treated group in this study. Similarly, Chantry et al,[27] in the USA also reported low serum lipid levels in ART naïve children less than 13 years of age, prior to the commencement of HAART. Hypertriglyceridaemia (47.5%) and low HDL-cholesterol (52.5%) were the most common form of lipid abnormalities found in the HAART naive group in this study. Similar

findings of hypertriglyceridaemia (28%) and low HDL-cholesterol (45%) were reported as the most common lipid abnormalities by Kanjanavanit et al[18] in group of 274 HIV-infected HAART naïve Thai and Cambodian children aged 1 to 12 years. The different prevalence rates of lipid abnormalities seen in the HAART-naive children suggest that the severity of dyslipidaemia varies from one country to another.

In the HAART treated group, total cholesterol was found to be significantly elevated in the children on PI-based HAART regimen (which also contains a NRTIs) compared to the children on the non-PI-based HAART regimen (which contains both NRTIs and NNRTI), resulting in a higher prevalence of hypercholesterolaemia in HAART treated HIV infected children. However, there was no significant difference in the elevated triglyceride levels among the two groups studied. This corroborated the findings of Brewinski et al,[88] in a cohort of HIV -infected Latin American children on PI-based HAART regime which showed that the children were at increased risk of hypercholesterolaemia and hypertriglyceridaemia compared with the children that were on NNRTI-containing HAART regimen.

Factors associated with hypercholesterolaemia and hypertriglyceridaemia among the HIV- infected children on HAART noted in this study were, age at commencement of HAART greater than 2 years, PI-based HAART regimen, age group greater than 5 years, duration of HIV diagnosis greater than 1 year and duration of treatment with HAART for more than I year. However, after controlling for confounders on multivariate analysis, PI-based HAART regimen ($p = 0.001$, OR = 0.18, 95% CI: 0.07-0.49) was the only factor found to be independently associated with hypercholesterolaemia in HAART treated group. Farley et al,[63] in a large group of 1812 HIV-infected children in USA, showed that the use of PI-based HAART regimen, age from 4 to < 6 years, HIV-1 RNA < 400 copies/ml, good adherence and the use of NNRTI were found to be

independently associated with hypercholesterolaemia. Triglyceride was not analysed in the study by Farley et al. No significant relationship between gender, hypercholesterolaemia and hypertriglyceridaemia was noted in this study. This was similar to the findings by Brewinski et al,[88] in a group of HIV infected Latin American children. In this study, there was also no significant association between socioeconomic classification, hypercholesterolaemia and hypertriglyceridaemia. This could be due to exposure of the children to the same staple food within the community even though a greater proportion of the children were in social class 4 and 5, almost all the children recruited for the study lived within Kano metropolis which is an urban settlement. This finding is in contrast to the findings of Emmanuel et al,[89] in Markudi who found an association between hypocholesterolaemia and low social class since none of the HIV infected HAART naive children in the study had hypercholesterolaemia. The children were mostly had parents who were peasant farmers in rural agrarian community.

Of the clinical features associated with dyslipidaemia analysed, lipoatrophy was the commonest feature as 37 out of the 80 HIV infected children on HAART (prevalence of 46%) had one form of lipoatrophy or the other none of the children had lipohypertrophy. Lipoatrophy occurred commonly in the older age group 10-15 years who had been on HAART for five and half years, mostly on Non PI-based regimen comprising Lamivudine, Zidovudine and Nevirapine. This age group 10-15 years, was found to be significantly associated with lipoatrophy. This also corroborated the findings of Euden et al[90] who in a group of 80 HIV infected children aged 2-18 years also found lipoatrophy commonly the children who were 12 years and older who were also on non PI-based regimen- Stavudine, Lamivudine and Nevirapine.

Several studies have also reported marked dyslipidaemia in children and adults on HAART, particularly Protease inhibitors, which has been associated with gross metabolic derangements as

well.[11,20,31,62,69,70,74,76,79] Though varying risk factors have so far been identified in different settings across the globe, protease inhibitors have been implicated more widely than any other factor, as a cause of dyslipidaemia in HIV infected children.

CONCLUSION

It could be concluded from the observations made in this study that:

1. The prevalence of dyslipidaemia was higher in the HIV infected HAART treated patients (TC: 47.5%, LDL-cholesterol:41.3%, HDL-cholesterol:53.8%, TG:62.5%) than the HIV infected HAART naive patients.
2. The prevalence of hypercholesterolaemia was higher in patients on PI-based HAART regimen than those on a non PI-based HAART regimen.
3. The risk factor strongly associated with dyslipidaemia was PI-based HAART regimen.
4. Other risk factors for dyslipidaemia identified in the study were age group greater than 5 years, commencement of HAART in children greater than 2 years of age, duration of HIV diagnosis more than 1 year and duration of treatment on HAART for more than 1 year.

RECOMMENDATIONS

1. All HIV positive children should have their base line lipid profile test done prior to commencement of treatment on HAART, with a view to establishing their base line lipid profile status.
2. The treatment regimen with the lowest risk for dyslipidaemia should be offered to patients as the second line treatment option.
3. Patients identified with dyslipidaemia should be commenced immediately on the various treatment options available for children and adolescents.

LIMITATION

Inability to explore dietary cholesterol and triglyceride content of food as it may be a predictor of dyslipidaemia.

LINE OF FUTURE RESEARCH

Determination of the relationship between serum lipid levels and clinical/immunological staging of HIV-infected children and adolescents.

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Appendix I - Ethical Clearance

Appendix II

COMPARATIVE STUDY ON PREVALENCE AND RISK FACTORS FOR DYSLIPIDAEMIA IN HIV-INFECTED AND UNINFECTED CHILDREN SEEN AT THE AMINU KANO TEACHING HOSPITAL, KANO. NIGERIA.

Consent/Assent Form

What Is The Study About

This is aimed at documenting dyslipidaemia in HIV-infected children 2-15yrs on HAART in AKTH, KANO. Dyslipidaemia is an abnormal/elevated amount of cholesterol or fat in the blood which is an important risk factor for coronary heart disease and stroke.

What Is Expected Of You If You Agree To Participate

You will be expected to provide answers to simple questions like your child's age, sex, age at commencement of HAART. Your child will then be examined in detail and certain laboratory investigations shall be performed after collection of blood samples. The cost of the investigations would be paid for by the researcher.

Your Participation Is Voluntary

Your child's participation is voluntary and you may withdraw him or her at any time of the study without any repercussion.

Confidentiality. The information obtained will be treated in absolute confidence. No part or whole of such information shall be divulged to anyone except the investigators. We owe it a duty to keep your child's records absolutely secret.

Benefit From Participation

Your child will benefit by knowing his or her serum lipid levels. In case of any abnormal findings, adequate referral will be made to the appropriate specialist for treatment. For further enquiries, please contact: Dr, Oiza Aliu-Isah , 08037051457.

Name of consenting parent/guardian..----------

Name of assenting child----------

Whose address is ----------

Hereby give consent to carry out research on my child/ward as explained to me. I am aware that the test to be carried out will not harm my child/ward.

I have the right to withdraw at any point during the course of the study if I so wish.

All the terms of this consent have been explained to me in a language that I understand. I am also aware that the information on my child shall be kept in strict confidence.

Sign---------- Sign----------

Child's Parent/ Guardian Interviewer

Date---------- Date.....

Appendix III

Study Profoma

COMPARATIVE STUDY ON PREVALENCE AND RISK FACTORS FOR DYSLIPIDAEMIA IN HIV-INFECTED AND UNINFECTED CHILDREN SEEN AT THE AMINU KANO TEACHING HOSPITAL, KANO. NIGERIA.

Study ID number ……. Serial No……..

A) Socio-demographic data

1. Age(years)……..
2. Sex 1) Male 2) Female
3. Religion……
4. Ethnicity…….
5. Address……
6. Occupation…….
7. Parent/caregiver's Educational Level Father Mother
   a. None ( ) ( )
   b. Primary ( ) ( )
   c. Secondary ( ) ( )
   d. Post secondary ( ) ( )
   e. Tertiary ( ) ( )

8. Parent/caregiver's Occupation | Father | Mother

a. Unemployed	( )	( )
b. Housewife	( )	( )
c. Farming	( )	( )
d. Trading	( )	( )
e. Skilled worker, eg driver, artisan	( )	( )
f. Civil Servants	( )	( )
g. Professionals, Businessmen, managers	( )	( )
h. Others (specify)	( )	( )

9. Social class of child.....

B. MEDICAL HISTORY

1. Age at diagnosis of HIV.....
2. Mode of diagnosis…….
3. Duration of diagnosis…….
4. Age at commencement of HAART…….
5. Duration on HAART……..
6. Type of regimen. 1st line……....2nd line…….

7. Duration on 1st line regimen……..

8. Duration on 2nd line regimen…….

9. History of any illness affecting; heart, liver or kidney: yes( ) no( )

10. If yes, specify disease……

11. Drug history…….....

12. Adherence: yes ( ) no ( )

C. PHYSICAL EXAMINATION

1. Weight (kg)…….

2. Height/length (m)……..

3. Mid Upper Arm Circumference(MUAC) (cm)(2-5 years)……..

4. BMI (kg/m^2)……..

5. BP(mmHg)…….

6.Features of HIV lipodystrophy.

a. Fat atrophy (lipoatrophy)

1. Face: sunken cheeks ( ) hollow temples ( )sunken eyes ( ) prominent zygomatic arch ( )

2. Extremities: prominent veins ( ) skinny or muscular appearance ( )

3. Buttocks: loss of contour ( ) loose skin folds ( )

b. Fat accumulation (lipohypertrophy)

1. Abdomen: abdominal girth in cm ---------- with visceral fat accumulation

2. Dorsocervical fat pad ( ) supraclavicular fat pad ( )

D. LABORATORY PARAMETERS

1. Total Cholesterol (mmol/l)……
2. HDLC (mmol/l)……
3. LDLC (mmol/l)……
4. TGs (mmol/l)…….

Appendix IV

Socio-Economic Classification Scheme By Oyedeji.[83]

For Occupation

Class	**Occupation**
I	Senior Public Servants, Professionals, Managers, large scale traders, businessmen and contractors.
II	Intermediate grade public servants and senior school teachers.
III	Junior school teachers, drivers, artisans, clerks.
IV	Petty traders, labourers, messengers.
V	Unemployed, full-time housewife, students and subsistence farmers.
For Educational Status	
Class	**Educational Attainment**
I	University graduates or equivalents
II	School certificate holders, ordinary level (GCE) who had teaching or other professional training.
III	School certificate or grade II teachers certificate holder
IV	Modern three and primary six certificate holders
V	Can either just read and write or are illiterate.

Socio-Economic Index Score

The social class of the parents or caregivers will be assessed using the Oyedeji socio –economic classification scheme (Appendix v) which will award socio-economic scores to the occupations and educational attainments of their parents or caregivers.[83]

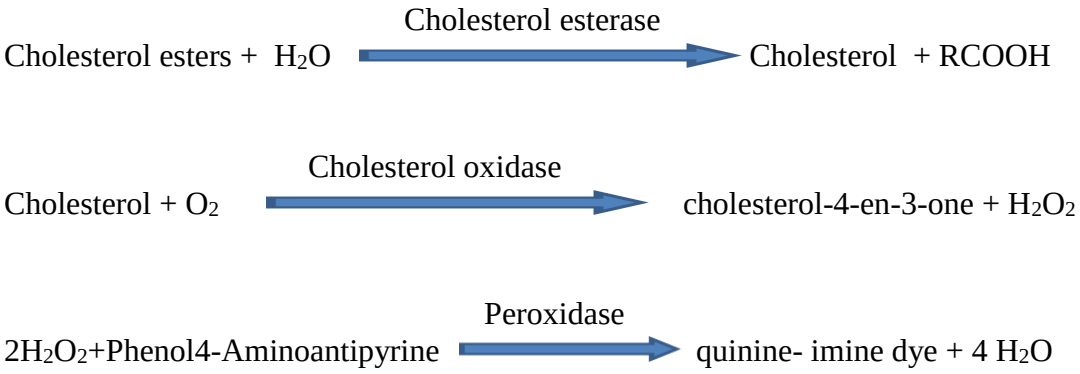
The mean of four scores (two for the father and two for the mother) approximated to the nearest whole number will be the social class assigned to the child as proposed by Oyedeji.[83]

Appendix V

LABORATORY ANALYSIS

TEST PRINCIPLE FOR CHOLESTEROL

Cholesterol esters are cleaved by the action of cholesterol esterase to yield free cholesterol and fatty acids. Cholesterol oxidase then catalyses the oxidation of cholesterol to cholest-4-en-3-one and hydrogen peroxide. In the presence of peroxidase, the hydrogen peroxide formed effects the oxidative coupling of phenol and 4-aminoantipyrine to form a red quinine-imine dye.



The colour intensity of the dye formed is directly proportional to the cholesterol concentration.

It is determined by measuring the increase in absorbance at 500 nm.

REAGENT COMPOSITION

Contents	Initial Concentration of Solution
Reagent	
4-aminoantipyrine	0.30 mmol/L
Phenol	6 mmol/L
Peroxidase	≥ 0.5 U/ml
Cholesterol esterase	≥ 0.15 U/ml
Cholesterol oxidase	≥ 0.1 U/ml
PIPES Buffer	80 mmol/L; PH 6.8
Standard	5.17 mmol/L(200mg/dl)

PROCEDURE

Wavelength:	500nm
Cuvette:	1cm Light Path
Temperature:	20-25˚C
Measurement:	against reagent blank

Pipette into Cuvette:

	Reagent Blank(µl)	Standard (µl)	Sample (µl)
Distilled water	10	-	-
Standard	-	10	-
Sample	-	-	10

Reagent	1000	1000	1000

Mix, incubate for 10 mins at room temperature. Measure the absorbance of the sample (A sample) and the absorbance of the standard (A standard) against the reagent blank within 60minutes.

CALCULATION

Conc. Of Cholesterol in sample=

$$\frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{Conc of standard}$$

TEST PRINCIPLE FOR HDL- CHOLESTEROL

By enzymatic endpoint method, low density lipoproteins (LDL and VLDL) and chylomicron fractions are precipitated quantitatively by the addition of phosphotungstic acid in the presence of magnesium ions. After centrifugation, the cholesterol concentration is the HDL (high density lipoprotein) fraction, which remains in the supernatant, is determined.

REAGENT

Contents	Initial concentration of solution
Phosphotungstic Acid	0.55 mmol/L
Magnesium Chloride	25 mmol/L

PROCEDURE: Precipitation

Pipette into centrifuge tubes:	
Sample	500 µl
Precipitant	1000 µl

Mix and allow to stand for 10 minutes at room temperature. The centrifuge for 10 minutes at 4,000 rpm.

Separate off the clear supernatant within two hours and determine the cholesterol content by Enzymatic endpoint method.

Wavelength:	500nm
Cuvette:	1 cm light path
Temperature:	20-25^{o} C
Measurement:	against reagent blank

Pipette into test tubes

	Reagent Blank	Standard	Sample
Distilled water	100µl	-	-
Supernatant	-	-	100µl
Standard	-	100µl	-
Reagent	1000µl	1000µl	1000µl

Mix and incubate for 10 minutes at room temperature. Then measure absorbance of the sample (A sample) and standard (A standard) against the reagent blank within 60 minutes.

CALCULATION

Concentration of HDL-Cholesterol in supernatant=

$$\frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{Conc. of standard}$$

LDL- CHOLESTEROL

LDL- Cholesterol concentration will be obtained using the Friedwald formula.[55] The Friedwald formula:-

$$\text{LDL-Cholesterol (mmol/L)} = \text{Total Cholesterol} - \left(\frac{\text{(Triglyceride)}}{2.2} + \text{HDL} - \text{Cholesterol} \right)$$

TEST PRINCIPLE FOR TRIGLYCERIDES

The triglycerides will be determined after enzymatic hydrolysis with lipase. The indicator is a quinoneimine formed from hydrogen peroxide, 4 – aminoantipyrine and 4 – chlorophenol under the catalytic influence of peroxidase.

REACTION:

$$\text{Triglycerides} + H_2O \xrightarrow{\text{Lipase}} \text{glycerol} + \text{Free fatty acid}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{Glycerol kinase}} \text{Glycerol} - 3\text{- phosphate} + \text{ADP}$$

$$\text{Glycerol - 3- phosphate} + O_2 \xrightarrow{\text{Glycerol phosphate oxidase}} \text{dihydroxy acetone phosphate} + H_2O_2$$

$$2H_2O_2 + \text{4-aminoantipyrine} + \text{4-chlorophenol} \xrightarrow{\text{Peroxidase}} \text{quinoneimine} + \text{HCL} + 4H_2O$$

REAGENTS

Vial R1	BUFFER	Concentration in the test
4 – chlorophenol		3.5 mmol/L
PIPES		100 mmol/L
Magnesium chloride		6 mmol/L

Vial R2	ENZYMES	
Lipase		≥1500 U/L
Peroxidase (POD)		≥ 1800 U/L
Glycerol – 3- phosphate oxidase (GPO)		≥ 400U/L
Glycerol Kinase (GK)		≥ 1000U/L
4 – amino antipyrine		0.30 mmo/L
Adenosine triphosphate Na		1.72 mmol/L

Vial R3	STANDARD	Initial concentration of solution
Glycerol		2.28 mmol/L

Corresponding to 200mg/dl of trioleine and to 2.28 mmol/L of triglyceride

PROCEDURE

Pipette into test tube	Sample	Standard	Blank
Sample	10µL		
Standard		10µL	
Reagent	1ml	1ml	1ml

Mix and incubate for 5 minutes at room temperature. Measure absorbance of sample and standard against reagent blank at 500 nm.

Calculation: $\frac{\text{Absorbance Sample}}{\text{A bsorbance Standard}}$ X Standard concentration