THE PREVALENCE OF DYSLIPIDEMIA IN HIV INFECTED CHILDREN RECEIVING HIGHLY ACTIVE ANTIRETROVIRAL THERAPY AT THE KENYATTA NATIONAL HOSPITAL

BY

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DECLARATION

I certify that this dissertation is my own original work.

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DEDICATION

I dedicate this work to my loving and caring wife Nancy and daughter Cindy for their understanding, patience and support.
# TABLE OF CONTENTS

Title ......................................................................................................................................... i  
Declaration ........................................................................................................................... ii  
Supervisors ........................................................................................................................... iii  
Dedication ............................................................................................................................ iv  
Table of contents ................................................................................................................ v  
List of tables ....................................................................................................................... vii  
List of figures ....................................................................................................................... viii  
List of abbreviations ........................................................................................................... ix  
Acknowledgements ............................................................................................................. x  
Abstract ................................................................................................................................. xi  
Introduction and Literature review .................................................................................... 1  
Study Justification .............................................................................................................. 9  
Objectives ............................................................................................................................ 10  
Materials and methods ....................................................................................................... 10  
Study setting ........................................................................................................................ 10  
Study population ............................................................................................................... 11  
Patient selection ................................................................................................................. 11  
Clinical methods ............................................................................................................... 12  
Laboratory methods .......................................................................................................... 12  
Sample size ......................................................................................................................... 13  
Study definitions ............................................................................................................... 14  
Data analysis ....................................................................................................................... 16  
Ethical considerations ....................................................................................................... 16  
Results ................................................................................................................................. 17  
Discussion ........................................................................................................................... 31  
Conclusions .......................................................................................................................... 36  
Recommendations .............................................................................................................. 36  
References ............................................................................................................................ 37  
Appendix 1 Study questionnaire ....................................................................................... 42
Appendix 2 Consent explanation form.................................................................46
Appendix 3 Consent form........................................................................................48
Appendix 4 Revised WHO clinical staging of HIV/AIDS for infants and children....49
Appendix 5 Laboratory reagents and methods.........................................................52
Appendix 6 Kenyatta National Hospital Ethics Committee approval letter............55
LIST OF TABLES

Table 1: Characteristics of study population ........................................................................18
Table 2: Current HAART regimens of study population ....................................................22
Table 3: Current HAART regimens in patients with alterations in their treatment ........23
Table 4: Reasons for change in HAART regimens ..............................................................23
Table 5: Prevalence of dyslipidemia in the study population ..............................................24
Table 6: Association between dyslipidemia and patient factors for the study population ..................................................................................................................25
Table 7: Multivariate analysis ................................................................................................26
Table 8: Association between dyslipidemia and patient factors among patients with no alterations in HAART regimens .................................................................27
Table 9: Association between dyslipidemia and patient factors among patients with alterations in regimens ........................................................................29
LIST OF FIGURES

Figure 1: Flow chart for selection of study subjects.....................................................17
Figure 2: Age by sex distribution of the study population...........................................19
Figure 3: WHO stage at initiation of HAART..............................................................20
Figure 4: Duration of HAART in months.................................................................20
Figure 5: CD4 percentage at initiation of HAART and at time of study......................21
ABBREVIATIONS

ART: Antiretroviral therapy
ABC: Abacavir
AIDS: Acquired Immune Deficiency Syndrome
AZT: Zidovudine
CCC: Comprehensive Care Centre
CDC: Centre for Disease Control
DDI: Didanosine
d4T: Stavudine
EFV: Efavirenz
HAART: Highly active antiretroviral therapy
HAZ: Height for age z-score
HDL: High density lipoprotein
HIV: Human Immunodeficiency Virus
KNH: Kenyatta National Hospital
LDL: Low density lipoprotein
NCEP: National Cholesterol Education Program
NNRTIs: Non-nucleoside reverse transcriptase inhibitors
NRTIs: Nucleoside reverse transcriptase inhibitors
NVP: Nevirapine
LPV/r: Lopinavir/ritonavir
PIs: Protease inhibitors
TG: Triglycerides
TC: Total cholesterol
USA: United States of America
VLDL: Very low density lipoprotein
WAZ: Weight for age z-score
WHO: World Health Organization
WHZ: Weight for height z-score
3TC: Lamivudine
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ABSTRACT

Background: Implementation of highly active antiretroviral therapy (HAART) has resulted in decline in morbidity and mortality in HIV-infected children, with more children living into adulthood. However, the long term adverse effects, including dyslipidemia, have raised concern on increased cardiovascular risk in this population.

Objective: To determine the prevalence of dyslipidemia in HIV-infected children receiving HAART at the Kenyatta National Hospital.

Study design: Hospital based Cross sectional study

Study methods: HIV-infected children on HAART aged between 18 months and 15 years were recruited. Demographic, clinical and immunologic data were recorded. The United States National Cholesterol Education Programme III guidelines in children were used to define dyslipidemia (Total cholesterol ≥ 5.17 mmol/l, triglycerides ≥ 1.69 mmol/l, low density lipoprotein cholesterol ≥ 3.36 mmol/l and high density lipoprotein cholesterol < 0.9 mmol/l). The prevalence of dyslipidemia was determined and associated factors were explored.

Results: For a total of 170 patients analyzed, the prevalence of dyslipidemia was 40% (95% Cl, 18-36). The prevalence of hypercholesterolemia was 27.1% and prevalence of hypertriglyceridemia 11.8%. High low density lipoprotein (LDL) cholesterol was observed in 19.4% of the patients and low high density lipoprotein (HDL) cholesterol in 5.3%. The prevalence of dyslipidemia among patients on non-nucleoside reverse transcriptase inhibitor based regimens was 37% compared to 90% in patients on protease inhibitor based therapy. Factors found to be associated with the presence of dyslipidemia were age 10 years and below (OR 3.2; 95% CI: 1.3 – 7.7, p = 0.009) and protease inhibitor therapy (OR 7.5; 95% CI: 1.5 – 38.5, p = 0.015).

Conclusion: There is a high prevalence of dyslipidemia in HIV-infected children taking HAART at the Kenyatta National Hospital Comprehensive Care Centre. There is need to perform baseline lipid profiles in patients starting HAART and there after reassessment at least every six months.
1. LITERATURE REVIEW

1.1 INTRODUCTION

The implementation of highly active antiretroviral therapy (HAART) has profoundly decreased morbidity and mortality in human immunodeficiency virus (HIV) infected patients (1,2). Suppression of viral replication and reconstitution of immunologic competence are associated with increased life expectancy (3). Consequently for patients with access to HAART, HIV has become a chronic disease with need for life long therapy.

Highly active antiretroviral therapy is associated with toxic effects that can significantly compromise quality of life and in some cases, jeopardize survival. Well known acute complications include pancreatitis, hepatitis and hypersensitivity reactions. There is increasing attention to the long term complications of HAART which may compromise the gains derived from this treatment. Recognized long term complications include changes in lipid metabolism resulting in potentially atherogenic dyslipidemia, changes in body fat distribution (lipodystrophy), insulin resistance and lactic acidosis. Insulin resistance and/or diabetes have been reported in HIV-infected adults on HAART. However, in the paediatric age group, insulin resistance may be found along with the body habitus changes, but disturbances in glucose homeostasis are rare (4,5,6).

Dyslipidemia describes abnormal serum concentrations of cholesterol and lipoproteins which is potentially atherogenic. According to United States of America National Education and Nutrition Program (NCEP) report of the Expert Panel on Blood Cholesterol Levels in Children and Adolescents, dyslipidemia is defined as serum levels of total cholesterol, triglycerides or low density lipoprotein (LDL) cholesterol above the 95th percentile or high density lipoprotein (HDL) cholesterol below the 5th percentile for age and sex (7). This roughly corresponds to total cholesterol, triglycerides, LDL cholesterol and HDL cholesterol levels of 200 mg/dl (5.17 mmol/l), 150 mg/dl (1.69
mmol/l), 130 mg/dl (3.36 mmol/l) and 35 mg/dl (0.9 mmol/l) respectively for those aged one to 19 years.

The reported prevalence of dyslipidemia in normal HIV uninfected children have differed from one region to another. The prevalence of elevated total cholesterol and elevated LDL cholesterol among children aged six to 17 years in the United States of America (USA) using data from the National Health and Nutrition Survey (NHANES) of 1999 to 2006 was 10.7% and 6.6%, respectively (8). In a community based cross sectional study in Northern Mexico involving 439 healthy non-obese children aged 10 to 13 years, the prevalence of elevated total cholesterol, high LDL cholesterol, high triglycerides and low HDL cholesterol was 15.9%, 14.6%, 9.3% and 6.2%, respectively (9). Else where, the prevalence was found to be 3%, 4.1%, 5.4% and 14.1% respectively, for elevated total cholesterol, high LDL cholesterol, high triglycerides and low HDL cholesterol among 1326 children aged seven to 12 years from urban and rural areas of Eastern Iran (10).

Although the clinical sequelae of atherosclerosis generally occur in middle-aged and older adults, the arterial lesions of atherosclerosis have their origin in childhood (11). Fatty streaks are the earliest grossly visible arterial lesions of the atherosclerotic process and serum lipid levels have been related to the extent of early arterial lesions of atherosclerosis in children in the general population. In the Bolagusa Heart Study, an epidemiologic study of cardiovascular risk factors in children in the USA, early atherosclerotic lesions in coronary arteries of diseased subjects 7-24 years of age were correlated with antemortem cholesterol levels. Aortic streaks were related to total cholesterol, triglycerides and LDL cholesterol independent of race, age and sex (12).

The relationship between serum cholesterol levels and clinical disease is not easily evaluated in children because clinically significant coronary heart disease is rare in children except in those with genetic dyslipidemias such as familial hypercholesterolemia. Children with familial hypercholesterolemia have markedly elevated levels of total cholesterol and LDL-cholesterol. They develop coronary artery
disease early in life, as early as six years of age in those with homozygous familial hypercholesterolemia.

1.2. Dyslipidemia AND HAART

While exposure to HAART has been associated with development of significant dyslipidemia, changes in lipid levels have been observed in HIV-infected patients not on HAART (17). These changes comprise modest elevations in triglycerides and low density lipoproteins and decreases in high density lipoprotein levels.

The derangements in lipid metabolism have become more severe and prevalent with the introduction of HAART. This was first detected in HIV-infected adults (18,19). The reported abnormalities include elevated total serum cholesterol, elevated low density lipoproteins, increased triglycerides and low or high levels of high density lipoproteins. Later, similar abnormalities in serum lipids were reported in children and adolescents on combination antiretroviral therapy (6,13,20,21). Protease inhibitor (PI) therapy has had the greatest association with dyslipidemia in the paediatric population as well as in adults (5,20,22,23). However, non-nucleoside reverse transcriptase inhibitors (NNRTIs) and nucleoside reverse transcriptase inhibitors (NRTIs) have also been implicated (24,25,26). The lipid derangements in NNRTI based regimens have been reported to be less severe than in PI containing regimens. Fontas et al found high levels of low density lipoproteins and triglycerides in patients receiving NNRTIs. This was associated with marked increase in high density lipoprotein levels which is proposed to be cardioprotective.

The reported prevalence of dyslipidemia in HIV infected children on HAART have ranged between 13% and 75% in different patient populations (6,13,14,20). The European Paediatric Lipodystrophy group, in a cross-sectional study evaluated total cholesterol and triglyceride levels among 280 children on antiretroviral therapy across Europe. Elevated total cholesterol was observed in 27% of the children and elevated triglycerides in 21%. Ten percent of the children had elevation of both total cholesterol
and triglycerides. The overall prevalence of dyslipidemia (i.e elevated total cholesterol and/or elevated triglycerides) in this patient population was 38% (21). Carter et al in a longitudinal study in the USA reported a prevalence of elevated total cholesterol of 67% and elevated triglycerides of 47% among 178 HIV infected children. In this study, 99 of the children (55%) were on PI based therapy (23).

In a multi-centre cross-sectional study of patients aged 7 to 24 years in the USA, the prevalence of elevated total cholesterol, LDL-cholesterol, triglycerides and low HDL-cholesterol among HIV infected children and HIV negative controls was compared. There were 161 patients on PI based therapy, 79 non-PI based therapy and 146 HIV negative controls. The prevalence of elevated total cholesterol, LDL-cholesterol, triglycerides and low HDL-cholesterol was 29%, 19%, 52% and 10%, respectively among the patients on PIs compared with 10%, 6%, 13% and 4%, respectively among the HIV negative controls. This study reported similar rates of abnormal lipid values in HIV negative controls and patients on non-PI based HAART except for HDL cholesterol which was higher in the HIV negative controls (27).

Dyslipidemia has been shown to develop relatively early in patients on HAART. In a prospective study evaluating short term lipid changes associated with HAART in naïve HIV-infected adults, there was a significant increase in total cholesterol (146.1 ± 45.5 to 208.7 ± 59.9 mg/dl, p = 0.0001), HDL (28.6 ± 11.5 to 56.5 ± 31.4mg/dl, p = 0.001), LDL (87.9 ± 35.9 to 117.3 ± 52.1mg/dl, p = 0.001) after 24 weeks of HAART (28). Carter J et al in the follow-up of Perinatally HIV-infected Children Study demonstrated an increasing linear relationship between median cholesterol levels and duration of treatment in the first six months on a PI inclusive regimen (23). Median cholesterol levels continued to increase up to 24 months duration after which there was minimal change on the same regimen. Analysis from a retrospective longitudinal study examining the significance, extent and frequency of raised metabolite levels in paediatric HIV patients before and during treatment with HAART showed an apparent stabilization of total cholesterol, LDL-cholesterol and HDL-cholesterol after two to three years of HAART (29). These results suggest that HAART is associated with
increases in cholesterol levels above baseline levels, an increase that occurs as early as the first six months and continuing over several years of exposure to HAART then leveling off.

Several factors have been shown to be associated with dyslipidemia. In a prospective cohort study involving nearly 2000 perinatally HIV-infected children between the ages of 4 and 19 years by Farley et al, the following were the risk factors associated with increased total cholesterol levels; current PI use (OR = 5.3, 95% CI: 3.1-9.2), age less than 6 years (OR = 2.9, 95% CI: 1.7-4.9), HIV RNA less than 400 copies/ml (OR = 2.3, 95% CI: 1.7-3.2), good adherence as measured by self report of no missed doses in past 3 days (OR = 2.2, 95% CI: 1.3-3.8), white race (OR = 2.2, 95% CI: 1.4-3.3) and current NNRTI use (OR = 1.7, 95% CI: 1.2-2.3). There was a positive trend between CD4 percentage (p = 0.005) and CD4 count (p = 0.001) categories and prevalence of hypercholesterolemia. Duration of PI use did not affect the prevalence, with prevalences of 16% for those with less than 1 year, 16% for 1-3 years and 17% for 3 or more years of PI treatment (20).

Tossiopoulos et al evaluated factors associated with dyslipidemia among 2122 HIV infected children in a prospective cohort study [27]. Boosted PI therapy was associated with more risk of dyslipidemia (OR 13.9, 95% CI: 6.73-28.6) compared to non boosted PI (OR 8.65, 95% CI: 4.19-17.9) and NNRTI use (OR 1.33, 95% CI: 1.04-17.9). Other factors associated with dyslipidemia in this study were younger age, optimal viral suppression and self reported perfect adherence to therapy during the previous three days. Gender, race, body mass index (BMI) and CD4% at baseline were not associated with dyslipidemia (30).

Pathogenesis
Several mechanisms have been implicated in the pathogenesis of dyslipidemia in HIV-infected patients. Dysregulation of fatty acid metabolism occurs with enhanced lipolysis in the peripheral tissues with net release of free fatty acids (FFAs) into the plasma compartment (31). The cause of the dysregulation is not well known but could include
the effects of specific HAART drugs or effects of components of the HIV virus. In-vitro studies have demonstrated that various protease inhibitors induce lipolysis (32). This increased lipolysis is not matched by a proportionate increase in the rate of fatty acid oxidation. In the liver, increased uptake of fatty acids enhances the synthesis of triglycerides and apolipoprotein B, reduces degradation of apolipoprotein B and increases production of very low density lipoproteins (VLDL) thereby contributing to hypertriglyceridemia and increased low density lipoproteins.

Apart from HAART mediated increase in VLDL, impaired clearance of VLDL in HAART naïve HIV-positive patients has been documented and this persists in those on HAART. Circulating cytokines, especially interferon alpha may also play a role (33,34,35).

NRTIs, especially stavudine increase total cholesterol and triglyceride levels. They have multiple adverse effects on the functioning of the mitochondrial organelle, provoking mitochondrial toxicity by inhibition of mitochondrial deoxyribonucleic acid (DNA) polymerase gamma, incorporation of the NRTI into the mitochondrial DNA causing depletion, enzyme impairment and uncoupling of oxidative phosphorylation, which results in apoptosis including peripheral adipocytes. This leads to generalized lipoatrophy and dyslipidemia (36,37,38). Stavudine, didanosine and possibly zidovudine have been associated most often with these side effects.

Protease inhibitors have been associated with increases in total cholesterol, LDL cholesterol and triglycerides (14,19,20,23,39). The effect on HDL cholesterol remains unclear.

NNRTIs have been associated with high total cholesterol, increased LDL and increased HDL cholesterol levels. The effect of nevirapine on HDL cholesterol levels appears to be more profound compared to efavirenz (25,40).
Monitoring and Management

For HIV-infected adolescents and adults, the adult AIDS Clinical Trials Group (ACTG) guidelines outline recommendations for evaluating and monitoring patients who are initiating or currently on HAART (41). A fasting lipid profile, including total cholesterol, HDL cholesterol and triglycerides, with calculation of LDL cholesterol is recommended before initiating antiretroviral therapy and should be repeated every 3 to 6 months (41,42). In certain circumstances, monitoring of random (non-fasting) lipid profiles may be useful as a screening mechanism when obtaining fasting specimens is difficult such as in infants or young children. If non-fasting total or LDL cholesterol or triglycerides are elevated, then fasting levels should be determined on a schedule similar to adolescents and adults.

Management of lipid derangements in children is difficult due to limited drug options compared to adults and lack of clear guidelines in case of HIV-infected children. The American Academy of Paediatrics suggests following the National Cholesterol Education Program guidelines as in the HIV negative population (7). These guidelines emphasize dietary changes and exercise as the cornerstones in the management of dyslipidemia in children. Lifestyle changes may be difficult to achieve in paediatric patients with HIV. An adequate trial period of 6 to 12 months should be given to these management strategies, except in patients with triglycerides $\geq 500$ mg/dl ($\geq 11.2$ mmol/l), which puts them at high risk for pancreatitis. These guidelines recommend drug treatment in children only if older than 10 years and the presence after 6-12 months of lifestyle modification of LDL-cholesterol $\geq 190$mg/dl ($\geq 4.9$ mmol/l), or $\geq 160$mg/dl ($\geq 4.1$ mmol/l) with a positive family history of premature coronary heart disease (before 55 years of age) or in the presence of two or more cardiac risk factors such as hypertension, obesity, diabetes and physical inactivity. Recommended drugs are the bile acid sequestrants (cholestyramine and colestipol), which act by binding bile acids in the intestinal lumen. However, these drugs may interfere with the absorption of concurrently administered drugs, including antiretroviral drugs, which may potentially lead to virological failure.
Fibric acid derivatives such as fenofibrate and gemfibrozil are not routinely recommended but may be used in patients with triglyceride levels \( \geq 500\text{mg/dl} \) due to risk of pancreatitis. The 3-hydroxyl-3-methyl glutaryl coenzyme A reductase inhibitors (statins) are not recommended for children less than 10 years because they are metabolized by the cytochrome-P-450 enzyme CYP3A4, which is inhibited by PIs leading to increased serum concentrations hence myalgias, myopathy and rhabdomyolysis. The preferred statin that can be recommended for use in paediatric patients taking antiretroviral drugs is pravastatin, which has been approved for children above 8 years with familial hypercholesterolemia (43).

Modification of ART regimen is another strategy for managing dyslipidemia. McComsey et al published the first paediatric switch study (44). Seventeen children were changed from a PI containing regimen to EFV. After 48 weeks, there was significant improvement in total cholesterol, LDL cholesterol and triglycerides with virological control remaining excellent.

1.3 HAART and cardiovascular disease

The correlation of dyslipidemia with HAART raises the question of the extent to which HAART increases the risk of premature atherosclerotic disease in the paediatric population starting therapy at very young ages. The effects of elevated cholesterol and other unfavourable lipid profiles on cardiovascular events in the general population is well established, with accelerated atherosclerosis resulting in hypertension, acute coronary artery syndromes (unstable angina and myocardial infarction) and stroke (45). Lipodystrophy, dyslipidemia and insulin resistance, all major components of the metabolic syndrome may synergize to predispose to premature atherosclerosis.

Moreover, endothelial dysfunction, arterial stiffness and increased intima-media thickness (IMT), all well documented markers of atherosclerosis have been found in HIV-infected children on HAART (46,47). HIV has been demonstrated to invade the linings of the brain and coronary arteries and induces an inflammatory response. It is
hypothesized that chronic endothelial inflammation due to HIV infection and several lipid markers due to HIV or antiretroviral therapy may increase atherosclerotic disease in HIV-infected patients.

This study aimed at elucidating the lipid profiles associated with HAART in paediatric age group for commonly used antiretroviral drug combinations in a developing country which would be useful in planning on cardiovascular risk reduction.

2. STUDY JUSTIFICATION

Current estimates indicate that there are more than 150,000 HIV infected children in Kenya. With the launch of the national antiretroviral therapy program in 2004, approximately 25,000 children have accessed treatment with exponential increase in numbers enrolled expected each year (48). The impact of this program is increased survival of children on HAART. Whereas this is desirable, these improved survival rates also expose these children to the long term complications of HAART including dyslipidemia. This together with traditional risk factors for cardiovascular disease acquired with age may predispose to early atherosclerosis and cardiac events at relatively young adult age.

The Kenyatta National Hospital (KNH) treatment program had enrolled over 1485 children by March 2009 and approximately 861 of these were on treatment. Lipid profiles in these patients are not routinely done, with the exception of patients on protease inhibitor inclusive regimens. Therefore the prevalence of dyslipidemia in children in the treatment program remained unquantified. This study aimed at establishing the prevalence of dyslipidemia in this group of children. Such data would be useful in guiding on the necessity of monitoring lipid profiles and management of dyslipidemia in HIV-infected children at the unit.
3. OBJECTIVES

3.1 Primary objective

To determine the prevalence of dyslipidemia as measured by total cholesterol, LDL cholesterol, HDL cholesterol and triglyceride levels in HIV-infected children on HAART for at least four months at the KNH Comprehensive Care Center (CCC).

3.2 Secondary objective

To define correlates of dyslipidemia in HIV-infected children on HAART for at least four months at the KNH CCC. Specific correlates include age, gender, baseline WHO clinical HIV stage, duration of therapy, HAART regimen, immunological status and nutritional status.

4. MATERIALS AND METHODS

4.1 Study design

Hospital based cross-sectional study.

4.2 Study site

The study was carried out at the paediatric CCC at the Kenyatta National Hospital, a national referral and teaching hospital in Nairobi, Kenya. The clinic is dedicated to out patient care of HIV infected children and is open daily from 8 am to 5 pm, Monday to Friday. Majority of the patients attending the clinic are of low to middle socioeconomic status and are resident in the city of Nairobi with a few drawn from the surrounding areas. On first visit, a patient’s anthropometric measurements and vital signs are taken and the patient is then seen by the clinician who takes a full medical history, examination and orders base line investigations including CD4 count and percent, full blood count, liver function tests and kidney function tests. Adherence counseling is
started, any opportunistic infections treated and cotrimoxazole prophylaxis initiated. The patients are seen a week later with above results for consideration of initiation of HAART based on the CD4 percent and/or World Health Organization (WHO) staging criteria. Patients on follow up are given regular appointments to review clinical progress, adherence, drug adverse effects and refill of their prescriptions. CD4 counts and percent are monitored every six months after initiation of HAART.

4.3 Study population
HIV-infected children age 18 months to 15 years on HAART for at least four months and on follow up at the CCC.

4.4 Patient selection
4.41 Inclusion criteria
1) HIV-infected children on HAART for at least four months
2) Age 18 months to 15 years.
3) Written informed consent by parent or guardian and verbal assent from children older than 7 years.

4.42 Exclusion criteria
1) Children who had missing data in their charts, for example baseline or current CD4 counts.
2) Children in whom no blood sample for lipid profile determination was collected.

4.5 Patient recruitment
The principal investigator assisted by a trained assistant visited the CCC during working hours from Monday to Friday. Consecutive patients’ files were reviewed and for those who met the inclusion criteria, the parent or guardian was introduced to the study and requested to give a signed informed consent (appendix 2 and 3). The children of parents/guardians who consented were then enrolled into the study and assigned a study number. Verbal assent was also sought from children older than 7 years.
4.6 Sampling technique
Consecutive sampling was used till the desired sample size was attained.

4.7 Clinical methods
Data on socio-demographic characteristics was recorded on a standardized questionnaire (appendix 1). Clinical and immunological characteristics, including current and previous antiretroviral therapy regimens, baseline WHO clinical stage, baseline and current CD4 count and percent were abstracted from patient charts. Weight and height were measured by a trained assistant and only one measurement was taken. Height was measured using a standard height board to the nearest 0.5 cm. Weight was determined using a standard beam scale to the nearest 0.1 kg. A full physical examination was done and findings recorded. Staging of HIV disease was done using WHO staging criteria (appendix 4).

4.8 Laboratory methods
The parent was instructed to ensure the child fasted for at least 4 hours after which 2 ml of venous blood was drawn by a trained laboratory technologist into a plain vacutainer using a gauge 22 needle and a 5ml syringe at the University of Nairobi Paediatric Department laboratory. The vacutainer was labeled with the patient’s name and study number. Serum was separated and stored on the bench at room temperature until analysis of lipid profiles was done on the same day (less than 8 hours). Extra serum was stored at 4°C till the next day in case a repeat was needed. Children who were unable to provide a fasting specimen on same day were given a re-visit date for blood sample collection.

Determination of lipid profiles was done at the University of Nairobi Paediatric Department laboratory using an automated chemistry analyzer, Humastar 180® and Human test kits (Human Gesellschaft for Biochemica and Diagnostica mbH, Germany) for total cholesterol, HDL cholesterol, and triglycerides. Total cholesterol and triglycerides were determined by enzymatic methods while HDL cholesterol was determined after precipitation of chylomicrons, VLDL and LDL cholesterol (appendix...
5). Low density lipoprotein cholesterol was calculated from total cholesterol (TC), HDL and triglycerides (TG) by the Friedewald formula as shown below.

\[ \text{LDL (mmol/l)} = \text{TC (mmol/l)} - \left[ \frac{\text{HDL (mmol/l)} + \text{TG (mmol/l)}}{2.2} \right] \]

CD4 counts abstracted from patient records were done in the same laboratory using a FACSCount machine. CD4 counts were measured and recorded as number of cells per microlitre and CD4 percent calculated as shown below.

\[
\text{CD4 \%} = \frac{\text{Absolute CD4 count per microlitre}}{\text{Total lymphocyte count per microlitre}} \times 100
\]

4.9 Quality control

Each day, an internal quality control specimen was analyzed for total cholesterol, triglycerides and HDL cholesterol of known value. The obtained value was compared to the known value and Standard Deviation Index (SDI) analyzed. Only assays whose values were within ±2 SDI were included. Trained and competent laboratory technologists were involved in running the tests. The Laboratory is also on external quality assurance scheme with Human Quality Assessment Services (HUQAS) affiliated to Digital PT of Canada.

4.10 Sample size estimation

Fischer’s formula for prevalence studies was used to calculate the sample size (49):

\[
n = \frac{Z^2 \cdot p(1-p)}{d^2}
\]

\( n \) is the minimum sample size.

\( Z \) value, 1.96, is the standard normal deviate corresponding to \( \alpha = 0.05 \)

\( P \) is the presumed prevalence. A prevalence of hypercholesterolemia of 13% from published data by Farley et al was used (20).
d is the precision, 0.05 with which the prevalence was determined.
Therefore n = 174 patients.

5. STUDY DEFINITIONS

5.1 Dyslipidemia
Dyslipidemia for the purpose of this study was defined according to the United States National Cholesterol Education Programme (NCEP) report of the Expert Panel on Blood Cholesterol Levels in Children and Adolescents (7). Presence of any of the following was considered to be dyslipidemia:

5.11 Total cholesterol \( \geq 5.17 \text{mmol/l} \)
5.12 Low Density Lipoproteins \( \geq 3.36 \text{mmol/l} \)
5.13 High Density Lipoproteins < 0.9mmol/l
5.14 Triglycerides \( \geq 1.69 \text{mmol/l} \)

5.2 HIV-infected child
This was defined as positive rapid ELISA test in children aged more than 18 months and positive DNA PCR test in children aged less than 18 months at initial diagnosis as documented in patient clinical records.

5.3 Age
This was the age of the study subjects from the date of birth to the date of recruitment into the study. This was recorded to the nearest month as given by the parent or guardian.

5.4 HAART regimens
5.41 First line regimens
These were combinations of three drugs used in the study site according to the national guidelines (50). These were as follows:
Zidovudine + Lamivudine + Nevirapine or Efavirenz (AZT + 3TC + NVP or EFV)
Stavudine + Lamivudine + Nevirapine or Efavirenz (d4T + 3TC + NVP or EFV)

For patients on treatment for tuberculosis at time of initiation of HAART, the preferred regimens were Zidovudine + Lamivudine + Abacavir (AZT + 3TC + ABC) for children less than 3 years and Zidovudine + Lamivudine + Efavirenz (AZT + 3TC + EFV) for patients older than 3 years. Zidovudine + Lamivudine + Kaletra (AZT + 3TC + LPV/r) was the first line regimen in perinatally nevirapine exposed children.

5.42 Second line regimens
The standard national second line regimen used at the study site was Abacavir + Didanosine + Kaletra (ABC + DDI + LPV/r).

5.5 Duration of HAART
This was the total duration in months on HAART since its initiation.

5.6 CD4 counts, CD4 percent
5.61 Base line CD4 count/percent
This was the CD4 count/percent at initiation of therapy

5.62 Current CD4 count/percent
This was the CD4 count/percent within 6 months of the period of study
6. DATA ANALYSIS

Data collected was entered into and analyzed using SPSS version 11.5. The nutrition software of Epi Info 3.2 was used to compute height for age z-scores (HAZ), weight for age z-scores (WAZ) and weight for height z-scores (WHZ). The z-scores represent the number of standard deviations of the measured parameters (height for age, weight for age and weight for height) for each child from the mean of a normal population. BMI was calculated as weight/height$^2$ and expressed in kilograms per squared meter. The BMI percentiles for age and sex were computed based on the CDC 2000 charts. Data was summarized into medians, inter-quartile ranges, and percentages and results presented in tables and bar graphs.

Prevalence of dyslipidemia was calculated as number of patients with dyslipidemia divided by the total number of patients and expressed as a percentage. The Chi-square test was used to compare categorical data. Odds ratios and their corresponding 95% confidence intervals were calculated. Associations were considered significant when p-value was equal to or less than 0.05. Fisher’s exact was used where the frequency of a variable was less than five.

7. ETHICAL CONSIDERATIONS

The study was conducted after approval by the KNH Ethical Committee and informed consent was obtained from parents or guardians accompanying the child. Verbal assent was also obtained for Children older than 7 years. Confidentiality was maintained by using coded anonymized questionnaires and observing patient-clinician confidentiality. Results of this study will be availed to the CCC clinical care team and management for appropriate action.
8. RESULTS

Between April 2008 and September 2008, a total of 205 patients who met the inclusion criteria were recruited into the study. Thirty one patients (15.1%) were omitted due to failure to turn up for blood sample collection, while four patients (1.9%) had some data missing in their files. Therefore, a total 170 patients were included in the analysis.

**Figure 1: Flow chart for selection of study subjects**

210 - Parents/guardians of children on HAART for > 4 months approached

205 – Questionnaire administered and clinical examination done

201 – Abstraction of clinical and laboratory data

170 children– Blood for lipid profile drawn after at least 4 hours of fasting

5 - Consent withheld

4 – Missing data
- 1 No baseline CD4 counts
- 3 No current CD4 counts

31 – No blood sample obtained
The demographic and clinical characteristics of the study population were as shown in table 1.

### Table 1: Demographic and clinical characteristics of study population (N = 170).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Number</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>92</td>
<td>54%</td>
</tr>
<tr>
<td>Female</td>
<td>78</td>
<td>46%</td>
</tr>
<tr>
<td>WHO HIV stage at initiation of HAART</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>7</td>
<td>4%</td>
</tr>
<tr>
<td>II</td>
<td>45</td>
<td>26%</td>
</tr>
<tr>
<td>III</td>
<td>95</td>
<td>56%</td>
</tr>
<tr>
<td>IV</td>
<td>23</td>
<td>14%</td>
</tr>
<tr>
<td>Median</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inter-quartile range (IQR)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>6.5</td>
<td>3.2 - 9.8</td>
</tr>
<tr>
<td>Duration of HAART (months)</td>
<td>18</td>
<td>11 - 27.3</td>
</tr>
<tr>
<td>Baseline CD4 percent (%)</td>
<td>9</td>
<td>4 - 14</td>
</tr>
<tr>
<td>Current CD4 percent (%)</td>
<td>23</td>
<td>19 - 29</td>
</tr>
<tr>
<td>Baseline CD4 count (cells/ul)</td>
<td>340</td>
<td>141-743</td>
</tr>
<tr>
<td>Current CD4 count (cells/ul)</td>
<td>894</td>
<td>583 - 1321</td>
</tr>
<tr>
<td>Current height for age z-score</td>
<td>-1.29</td>
<td>-2.24 to -0.48</td>
</tr>
<tr>
<td>Current weight for age z-score</td>
<td>-0.86</td>
<td>-1.59 to -0.108</td>
</tr>
<tr>
<td>Current weight for height z-score</td>
<td>+0.19</td>
<td>-0.598 to +0.71</td>
</tr>
<tr>
<td>Current Body mass index (BMI)</td>
<td>16.1</td>
<td>15.1 - 17.3</td>
</tr>
</tbody>
</table>
Gender and age of the study population.

There were 92 males (54%), therefore a male to female ratio of 1.1:1. The median age of the study subjects was 6.5 years with an inter-quartile range of 3.2 to 9.8 years. Sixty nine of the children (40.6%) were in the age group five to 10 years while less than five years were 63 (37.1%) and over 10 years to 15 years were 38 (22.3%). Females were more likely to be older than males but the difference was statistically insignificant (p=0.092). (Figure 2).

![Figure 2: Age by sex distribution of the study population](image)

W.H.O clinical HIV stage

Ninety five patients (56%) were in WHO stage III at initiation of therapy while only seven patients (4%) were in WHO stage I. Of the remaining patients, forty five (26%) were in stage II while twenty three (14%) were in stage IV. (Figure 3)
Figure 3: WHO stage at initiation of HAART.

Duration of HAART

The median duration of HAART was 18 months with an inter-quartile range of 11 to 27 months. 107 patients (63%) had been on HAART for a period of between 12 and 36 months. Forty five patients (26.5%) had been on HAART for a period of less than 12 months while only eighteen (10.5%) had been on treatment for more than 36 months (Figure 4). Among 34 patients who had alterations in HAART regimens during their period of treatment, the median duration of their new regimens was 13.5 months with an inter-quartile range of 8.7 to 17 months.

Figure 4: Duration of HAART in months
CD4 count and percent

The median baseline CD4 count was 340 cells/ul with an inter-quartile range of 141 to 743 cells/ul, while the median CD4 percent at initiation of HAART was 9% with an inter-quartile range of 4% to 14%. The median current CD4 counts and CD4 percent showed an increase to 894 cells/ul (inter-quartile range 583 to 1321) and 23% (inter-quartile range 19% to 29%), respectively. At initiation of HAART, most patients (76.9%) had CD4 percent less than 15 while only 11.9% had CD4 percent less than 15 at the time of the study (Figure 5)

![Figure 5: CD4 percentage at initiation of HAART and at time of study](image)

Nutritional status

The median current height for age, weight for age and weight for height z-scores were -1.29 SD (inter-quartile range -2.24 to -0.48), -0.86 SD (inter-quartile range -1.59 to -0.108) and +0.19 SD (inter-quartile range -0.598 to +0.71), respectively. A total of 10 patients (5.9%) had height for age ≤ -3 SD, while six patients (3.5%) had weight for height z-score ≤ -3 SD. Only one patient had weight for age z-score ≤ -3 SD.
HAART regimens

Majority of patients (93.5%) were on NNRTI-based first line regimens at the time of the study. Seventy five (44.1%) were on a combination of zidovudine, lamivudine and efavirenz (AZT+3TC+EFV) and 59 (34.7%) were receiving zidovudine, lamivudine and nevirapine (AZT+3TC+NVP). Twenty four patients were on stavudine containing regimens, either stavudine, lamivudine and efavirenz (d4T+3TC+EFV, 8.2%) or stavudine, lamivudine and nevirapine (d4T+3TC+NVP, 5.9%). One patient was on abacavir, lamivudine and efavirenz (ABC+3TC+EFV). Ten patients (5.9%) were on PI based regimens (ritonavir boosted lopinavir). The remaining one patient (0.6%) was on zidovudine, lamivudine and abacavir (AZT+3TC+ABC). (Table 2)

Table 2: Current HAART regimens of the study population (N = 170)

<table>
<thead>
<tr>
<th>Regimens</th>
<th>Number</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. NNRTI based</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AZT+3TC+EFV</td>
<td>75</td>
<td>44.1</td>
</tr>
<tr>
<td>AZT+3TC+NVP</td>
<td>59</td>
<td>34.7</td>
</tr>
<tr>
<td>d4T+3TC+EFV</td>
<td>14</td>
<td>8.2</td>
</tr>
<tr>
<td>d4T+3TC+NVP</td>
<td>10</td>
<td>5.9</td>
</tr>
<tr>
<td>ABC+3TC+EFV</td>
<td>1</td>
<td>0.6</td>
</tr>
<tr>
<td>2. PI based</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ABC+DDI+LPV/r</td>
<td>7</td>
<td>4.1</td>
</tr>
<tr>
<td>DDI+3TC+LPV/r</td>
<td>1</td>
<td>0.6</td>
</tr>
<tr>
<td>AZT+3TC+LPV/r</td>
<td>2</td>
<td>1.2</td>
</tr>
<tr>
<td>3. Other</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AZT+3TC+ABC</td>
<td>1</td>
<td>0.6</td>
</tr>
<tr>
<td>Total</td>
<td>170</td>
<td>100.0</td>
</tr>
</tbody>
</table>

A total of 34 patients (20%) had a change in their HAART regimens in the course of therapy. In all of them, change occurred only once and involved single drug substitution or a change in all three drugs. Table 3 below summarizes the HAART regimens at the time of the study in patients who had alterations in their regimens.
Table 3: Current HAART regimens in patients with alterations in their treatment (N = 34).

<table>
<thead>
<tr>
<th>Current regimen</th>
<th>Number</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. NNRTI based</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AZT+3TC+NVP</td>
<td>11</td>
<td>32.4</td>
</tr>
<tr>
<td>AZT+3TC+EFV</td>
<td>6</td>
<td>17.7</td>
</tr>
<tr>
<td>d4T+3TC+EFV</td>
<td>3</td>
<td>8.8</td>
</tr>
<tr>
<td>d4T+3TC+NVP</td>
<td>2</td>
<td>5.9</td>
</tr>
<tr>
<td>ABC+3TC+EFV</td>
<td>1</td>
<td>2.9</td>
</tr>
<tr>
<td>2. PI based</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ABC+DDI+LPV/r</td>
<td>7</td>
<td>20.6</td>
</tr>
<tr>
<td>DDI+3TC+LPV/r</td>
<td>1</td>
<td>2.9</td>
</tr>
<tr>
<td>AZT+3TC+LPV/r</td>
<td>2</td>
<td>5.9</td>
</tr>
<tr>
<td>3. Other</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AZT+3TC+ABC</td>
<td>1</td>
<td>2.9</td>
</tr>
<tr>
<td>Total</td>
<td>34</td>
<td>100.0</td>
</tr>
</tbody>
</table>

The reasons for the changes in drug regimens were mainly toxicity or treatment failure as shown in table 4 below. Seven patients (21%) had single drug substitutions due to toxicity while 17 patients (50%) had single drug substitutions due to tuberculosis treatment. Ten patients had whole regimen change due to treatment failure as measured by CD4 percent.

Table 4: Reasons for change in HAART regimens (N = 34)

<table>
<thead>
<tr>
<th>Change</th>
<th>Reason for change</th>
<th>Number</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single drug substitution</td>
<td>Toxicity</td>
<td>7</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>Tuberculosis treatment</td>
<td>17</td>
<td>50</td>
</tr>
<tr>
<td>Whole regimen change</td>
<td>Treatment failure</td>
<td>10</td>
<td>29</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>34</td>
<td>100</td>
</tr>
</tbody>
</table>
PREVALENCE OF DYSLIPIDEMIA.

A total of sixty eight (68) children in the study population had dyslipidemia, therefore an overall prevalence of 40% (95% CI 31% – 49%). The prevalence of raised total cholesterol, high triglycerides, high LDL-cholesterol and low HDL-cholesterol was 27.1%, 11.8%, 19.4% and 5.3%, respectively as shown in table 5. Eight patients (4.7%) had a raised total cholesterol to HDL ratio (>5).

Table 5: Prevalence of dyslipidemia in the study population (N = 170)

<table>
<thead>
<tr>
<th>Category</th>
<th>Number</th>
<th>Prevalence</th>
<th>(95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elevated total cholesterol</td>
<td>46</td>
<td>27.1 %</td>
<td>(18 – 36)</td>
</tr>
<tr>
<td>Elevated triglycerides</td>
<td>20</td>
<td>11.8%</td>
<td>(5 – 18)</td>
</tr>
<tr>
<td>Elevated LDL-cholesterol</td>
<td>33</td>
<td>19.4%</td>
<td>(12 – 27)</td>
</tr>
<tr>
<td>Low HDL-cholesterol</td>
<td>9</td>
<td>5.3%</td>
<td>(0.7 – 10)</td>
</tr>
<tr>
<td>Elevated Total cholesterol : HDL ratio</td>
<td>8</td>
<td>4.7%</td>
<td>(0.6 – 9)</td>
</tr>
<tr>
<td>Any lipid derangement (single, two or more combinations)</td>
<td>68</td>
<td>40.0%</td>
<td>(31 – 49)</td>
</tr>
</tbody>
</table>

Separate analysis was then done for the subset of 159 patients who were on NNRTI-based regimens and the ten patients on PI-based regimens at the time of the study. Among the patients on NNRTI-based regimens, a total of fifty nine (59) patients had dyslipidemia, therefore a prevalence of 37% (95% CI 27% – 45%). The prevalence of elevated total cholesterol, triglycerides, LDL-cholesterol and low HDL-cholesterol was 26.3% (n = 42), 10% (n = 16), 18% (n = 29) and 3.8% (n = 6), respectively.

For the subset of ten patients on PI-based regimens, nine had dyslipidemia, therefore a prevalence of 90%. The prevalence of elevated total cholesterol, high triglycerides, elevated LDL-cholesterol and low HDL-cholesterol levels was 40% (n = 4), 40% (n = 4), 40% (n = 4) and 30% (n = 3), respectively.
CORRELATES OF DYSLIPIDEMIA

Exploration for associations between patient characteristics and dyslipidemia was done using the total number of children in the study population. Total duration of HAART was used, including for patients with alterations in HAART regimens and regimens categorized into PI and non PI based.

Table 6: Association between dyslipidemia and patient characteristics (N = 170).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Number of children (N = 170)</th>
<th>Dyslipidemia Number (%)</th>
<th>OR (95% CI)</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>92</td>
<td>37 (40%)</td>
<td>1.0 (0.5 -1.9)</td>
<td>0.950</td>
</tr>
<tr>
<td>Male</td>
<td>78</td>
<td>31 (40%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age(in years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤10</td>
<td>131</td>
<td>59 (45%)</td>
<td>2.7 (1.1-6.8)</td>
<td>0.014</td>
</tr>
<tr>
<td>&gt;10</td>
<td>39</td>
<td>9 (23%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial WHO stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I &amp; II</td>
<td>52</td>
<td>16 (31%)</td>
<td>1.77 (0.9-3.8)</td>
<td>0.104</td>
</tr>
<tr>
<td>III &amp; IV</td>
<td>118</td>
<td>52 (44%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration of HAART</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;12</td>
<td>45</td>
<td>12 (27%)</td>
<td>2.2 (1.0-5.1)</td>
<td>0.033</td>
</tr>
<tr>
<td>≥12</td>
<td>125</td>
<td>56 (45%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial CD4%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;15%</td>
<td>131</td>
<td>55 (42%)</td>
<td>1.5 (0.6-3.3)</td>
<td>0.334</td>
</tr>
<tr>
<td>≥15%</td>
<td>39</td>
<td>13 (33%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current CD4%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;15%</td>
<td>22</td>
<td>12 (55%)</td>
<td>1.9 (0.7-5.3)</td>
<td>0.136</td>
</tr>
<tr>
<td>≥15%</td>
<td>148</td>
<td>56 (38%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HAZ ≤ -3 SD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;-3 SD</td>
<td>10</td>
<td>2 (20%)</td>
<td>0.4 (0.1-2.3)</td>
<td>0.330 †</td>
</tr>
<tr>
<td>WHZ ≤ -3 SD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;-3 SD</td>
<td>6</td>
<td>2 (33%)</td>
<td>0.9 (0.1-5.9)</td>
<td>1.000 †</td>
</tr>
<tr>
<td>&gt;90th percentile</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI &lt;90th percentile</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HAART regimens</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PI present</td>
<td>10</td>
<td>9 (90%)</td>
<td>15.4 (1.9 -332.7)</td>
<td>0.007 †</td>
</tr>
<tr>
<td>No PI</td>
<td>160</td>
<td>59 (37%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* P-values were calculated using x² test
† Fisher exact test
59 of 131 (45%) children aged less than 10 years had dyslipidemia compared to 9 of 39 (23%) children aged more than 10 years (OR 2.7; 95% CI: 1.1 – 6.8, \( p = 0.014 \)).

56 of 125 (45%) children on HAART for more than 12 months had dyslipidemia compared to 12 of 45 (27%) children on HAART for less than 12 months (OR 2.2; 95% CI: 1.0 – 5.1, \( p = 0.033 \)).

9 of 10 (90%) children on PI-based regimens had dyslipidemia compared to 59 of 160 (37%) on non-PI regimens (OR 15.4; 95% CI: 1.9 – 332.7, \( p = 0.007 \)).

There was no association between dyslipidemia and gender, baseline WHO clinical stage, CD4 percent or nutritional status as shown by the respective \( p \) values in table 6 above.

**Multivariate analysis**

Factors found to be significantly associated with presence of dyslipidemia in the univariate analysis were entered into a multiple regression analysis.

**Table 7: Multivariate analysis**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Parameter Estimate.</th>
<th>Std. Error</th>
<th>p-value</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (in years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \leq 10 )</td>
<td>1.1</td>
<td>0.4</td>
<td>0.009</td>
<td>3.2</td>
<td>1.3 – 7.7</td>
</tr>
<tr>
<td>( &gt; 10 )</td>
<td>Reference</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \geq 12 )</td>
<td>0.7</td>
<td>0.4</td>
<td>0.068</td>
<td>2.1</td>
<td>0.9 – 4.5</td>
</tr>
<tr>
<td>( &lt; 12 )</td>
<td>Reference</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HAART regimens</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PI Present</td>
<td>-2.0</td>
<td>0.8</td>
<td>0.015</td>
<td>7.5</td>
<td>1.5 – 38.5</td>
</tr>
<tr>
<td>No PI</td>
<td>Reference</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

In the multivariate analysis, patients less than 10 years were 3.2 times more likely to have dyslipidemia compared to those more than 10 years (\( p = 0.009 \)). Children on PI inclusive regimens were 7.5 times more likely to have dyslipidemia compared to children on non PI based HAART (\( p = 0.015 \)). Although children on treatment for more than 12 months were 2.1 more likely to have dyslipidemia compared to those treated for less than 12 months, this did not attain statistical significance (\( p = 0.068 \)). (Table 7)
Correlates of dyslipidemia among subset of patients with no alterations in HAART regimens

Further analysis was done using the sub-set of 136 patients who had no changes in their HAART regimens and therefore had been on first line regimens since initiation of treatment. HAART regimens were categorized into stavudine and zidovudine-based.

Table 8: Association between dyslipidemia and patient factors in patients with no alterations in HAART regimens (N = 136).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Number of children (N = 136)</th>
<th>Dyslipidemia Number (%)</th>
<th>OR (95% CI)</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>61</td>
<td>23 (38%)</td>
<td>1.2 (0.6-2.5)</td>
<td>0.714</td>
</tr>
<tr>
<td>Male</td>
<td>75</td>
<td>26 (35%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤10</td>
<td>105</td>
<td>44 (42%)</td>
<td>3.8 (1.3-12.2)</td>
<td>0.009</td>
</tr>
<tr>
<td>&gt;10</td>
<td>31</td>
<td>5 (16%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial WHO stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I &amp; II</td>
<td>46</td>
<td>15 (33%)</td>
<td>1.3 (0.6-2.9)</td>
<td>0.554</td>
</tr>
<tr>
<td>III &amp; IV</td>
<td>90</td>
<td>34 (38%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration of HAART (months)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;12</td>
<td>43</td>
<td>12 (28%)</td>
<td>1.7 (0.8-4.1)</td>
<td>0.181</td>
</tr>
<tr>
<td>≥12</td>
<td>93</td>
<td>37 (40%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial CD4%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;15%</td>
<td>106</td>
<td>42 (40%)</td>
<td>2.2 (0.8-6.1)</td>
<td>0.102</td>
</tr>
<tr>
<td>≥15%</td>
<td>30</td>
<td>7 (23%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current CD4%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;15%</td>
<td>18</td>
<td>10 (56%)</td>
<td>2.6 (0.9-7.8)</td>
<td>0.065</td>
</tr>
<tr>
<td>≥15%</td>
<td>118</td>
<td>39 (33%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HAZ ≤ -3 SD</td>
<td>10</td>
<td>2 (20%)</td>
<td>0.4 (0.1-2.3)</td>
<td>0.330 †</td>
</tr>
<tr>
<td>HAZ &gt; -3 SD</td>
<td>126</td>
<td>47 (37%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WHZ ≤ -3 SD</td>
<td>6</td>
<td>2 (33%)</td>
<td>0.9 (0.1-5.9)</td>
<td>1.000 †</td>
</tr>
<tr>
<td>WHZ &gt; -3 SD</td>
<td>130</td>
<td>47 (36%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI ≥ 90th percentile</td>
<td>14</td>
<td>5 (36%)</td>
<td>1.0 (0.3-3.4)</td>
<td>0.980</td>
</tr>
<tr>
<td>BMI &lt; 90th percentile</td>
<td>122</td>
<td>44 (36%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HAART regimens</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stavudine inclusive</td>
<td>19</td>
<td>4 (21%)</td>
<td>0.4 (0.1-1.5)</td>
<td>0.144 †</td>
</tr>
<tr>
<td>Zidovudine inclusive</td>
<td>117</td>
<td>45 (38%)</td>
<td></td>
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</tr>
</tbody>
</table>

* P-values were calculated using x² test, † Fisher exact test
44 of 105 (42%) children aged less than 10 years had dyslipidemia compared to 5 of 31 (16%) children aged more than 10 years (OR 3.8, 95% CI: 1.3 – 12.2, p = 0.009). There was a trend for children with current CD4 percent less than 15% to have dyslipidemia (56%) compared to those with current CD4 percent more than 15% (33%), OR 2.6, 95% CI: 0.9 - 7.8, p = 0.065. There was no association between dyslipidemia and gender, baseline WHO clinical stage, duration of therapy, initial CD4 percent, nutritional status or HAART regimens. (Table 8)
Correlates of dyslipidemia among subset of patients with alterations in HAART regimens

Exploration for associations between dyslipidemia and patient characteristics for those who had alterations in HAART regimens was done. Duration of treatment of the new regimen was used and regimens divided in to PI and non PI based.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Number of children (N = 34)</th>
<th>Dyslipidemia Number (%)</th>
<th>OR (95% CI)</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>17</td>
<td>11 (65%)</td>
<td>2.1 (0.4 - 10.4)</td>
<td>0.307</td>
</tr>
<tr>
<td>Male</td>
<td>17</td>
<td>8 (47%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤10</td>
<td>27</td>
<td>15 (56%)</td>
<td>1.0 (0.2 - 6.5)</td>
<td>1.000†</td>
</tr>
<tr>
<td>&gt;10</td>
<td>7</td>
<td>4 (47%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial WHO stage I &amp; II</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>III &amp; IV</td>
<td>6</td>
<td>3 (50%)</td>
<td>1.4 (0.2 - 10.5)</td>
<td>1.000†</td>
</tr>
<tr>
<td></td>
<td>28</td>
<td>16 (57%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration of HAART (months)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;12</td>
<td>15</td>
<td>7 (47%)</td>
<td>2.0 (0.4 - 9.9)</td>
<td>0.343</td>
</tr>
<tr>
<td>≥12</td>
<td>19</td>
<td>12 (63%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial CD4%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;15%</td>
<td>25</td>
<td>13 (52%)</td>
<td>0.6 (0.1 - 3.4)</td>
<td>0.697†</td>
</tr>
<tr>
<td>≥15%</td>
<td>9</td>
<td>6 (67%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current CD4%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;15%</td>
<td>3</td>
<td>1 (33%)</td>
<td>0.4 (0.1 - 6.0)</td>
<td>0.571†</td>
</tr>
<tr>
<td>≥15%</td>
<td>31</td>
<td>18 (58%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI ≥ 90th percentile</td>
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<td></td>
</tr>
<tr>
<td>BMI &lt;90th percentile</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>HAART regimens</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PI-based</td>
<td>10</td>
<td>9 (90%)</td>
<td>12.6 (1.2-310.8)</td>
<td>0.020</td>
</tr>
<tr>
<td>Non PI based</td>
<td>24</td>
<td>10 (42%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* P-values were calculated using x² test
† Fisher exact test
9 of 10 (90%) children on PI-inclusive regimens had dyslipidemia compared to 10 of 24 (42%) on non PI regimens (OR 12.6; 95% CI: 1.2 - 310.8, p = 0.02). There was no association between dyslipidemia and gender, age, baseline WHO clinical stage, duration of therapy, CD4 percentage or BMI percentile as shown by the respective p-values in table 9 above.
9. DISCUSSION

In this cross sectional study conducted at the Kenyatta National Hospital Comprehensive Care Centre, male children comprised 54% of the study subjects. Seventy eight percent of the children were less than 10 years old, reflecting on the patient population at the CCC.

There was marked improvement in the surrogate markers of HIV disease severity between baseline and at time of this study. This is as shown by increase in the median CD4 count and CD4 percent. At baseline, the median CD4 count and CD4 percent for the study population were 9% and 340 cells per microlitre, respectively, with an increase to 23% and 894 cells per microlitre, respectively. This is attributed to the efficacy of HAART as demonstrated in previous studies which showed a reduction in morbidity and mortality in HIV-infected children on therapy (1, 2).

The prevalence of severe malnutrition in this group of children as measured by current weight for height z-scores was low (3.5%). This could be attributed to the effect of HAART because the study population comprised of children on antiretroviral therapy for at least 4 months. With improved immunological status, the vicious cycle of opportunistic infections and other diseases, poor health and malnutrition in HIV-infected patients is reversed.

Majority of the patients (93.5%) were on regimens containing NRTIs comprising zidovudine and lamivudine or stavudine and lamivudine together with a NNRTI of either nevirapine or efavirenz. This is as recommended by the national guidelines for antiretroviral therapy (50). Eighty percent of the patients had never had a change in their HAART regimens since initiation of therapy.

The overall prevalence of dyslipidemia in the study population was 40% (95% CI: 31 - 49), with a prevalence of elevated total cholesterol of 27.1%. Prior studies on the prevalence of dyslipidemia among HIV-infected children have ranged between 13% and
75% (6, 13, 14, 20). The finding of a prevalence of 40% though within the range of observations reported previously, there were a number of methodologic differences. Most of these studies were based on measurement of total cholesterol only while in our study, the definition of dyslipidemia was derangement in one or more of the four lipid metabolites, that is total cholesterol, triglycerides, LDL cholesterol and HDL cholesterol.

In a cross sectional study in Europe involving 280 children with a definition of dyslipidemia as elevated total cholesterol ≥ 5.17 mmol/l and/or elevated triglycerides ≥ 1.69 mmol/l, the prevalence of dyslipidemia was 38% (95% CI: 32.1 – 44.2) (21). The prevalence of elevated total cholesterol and elevated triglycerides in this study was 27% and 21%, respectively. This patient population consisted mainly of Caucasians and patients were predominantly on protease inhibitor based combination antiretroviral therapy. Though the prevalence of dyslipidemia and hypercholesterolemia in this study is comparable to our findings, the prevalence of elevated triglycerides was higher in this study probably because no fasting was required before blood collection for lipid profiles. No local data found on dyslipidemia in either HIV-infected or non HIV infected children for comparison with our study.

The low prevalence of low HDL cholesterol (5.3%) and raised total cholesterol to HDL ratio (4.7%) in this study could be because most of the patients were on NNRTI based HAART. Nevirapine and efavirenz have been shown to increase HDL cholesterol levels, therefore lowering the total cholesterol to HDL ratio (24,28). Increased HDL cholesterol has been shown to be cardioprotective. Estimations in adults are that, for every 0.025 mmol/l increase in HDL, the risk of coronary artery disease is reduced by two to five percent.

There was a difference in the prevalence of dyslipidemia among patients who were on NNRTI-based therapy (37%) compared with patients who were on PI-based therapy (90%). Protease inhibitors have strongly been associated with dyslipidemia in HIV-infected persons on treatment in previous studies (22, 24).
Several factors have been associated with dyslipidemia in HIV-infected children. In this study, we found age less than 10 years to be a significant risk factor for dyslipidemia compared to age more than 10 years (OR 3.2, 95% CI: 1.3 – 7.7; p = 0.009). This has been reported in a study by Farley et al where children less than 11 years were more likely to have raised total cholesterol compared with older children (20). The reason why younger HIV-infected children seem to be at an increased risk of dyslipidemia is not well known.

In multivariate analysis, there was a trend for children on treatment for more than 12 months to have dyslipidemia compared to those treated for less than 12 months (OR 2.1; 95% CI: 0.9 – 4.5, p = 0.068). In a study by Carter et al, median cholesterol levels were found to increase from pre-treatment levels to median values six months after initiating therapy. This increase continued through 24 months of therapy after which the median cholesterol levels changed minimally after (23). Such evaluation of increase in cholesterol levels with time was not possible in our study given that the study design was cross sectional. Among the subset of 34 patients with alterations in HAART regimens, duration of treatment was not found to be associated with dyslipidemia (p = 0.343).

Protease inhibitor inclusive therapy was associated with presence of dyslipidemia in the study population (OR 7.5; 95% CI: 1.5 – 38.5, p = 0.015). This concurs with other studies which have found an association between dyslipidemia and protease inhibitor therapy (20,27,30). Among patients who did not have alterations in HAART regimens, stavudine based regimens were not found to be associated with dyslipidemia compared with zidovudine based regimens. Stavudine has been associated with dyslipidemia in other studies (38).

Children who were more immunosuppressed at initiation of HAART as depicted by the baseline WHO clinical stage (III and IV) were 1.7 times more likely to have dyslipidemia compared to patients who were in stage I and II. In addition, children who had baseline CD4 percent less than 15% were 1.5 times more likely to have
dyslipidemia compared to children with baseline CD4 percent more than 15%. Although these findings were not statistically significant, dyslipidemia has previously been noted in antiretroviral-naive HIV positive individuals but not as high as in patients on treatment. There are suggestions of a possible role of circulating cytokines and the virus itself in the pathogenesis of dyslipidemia (33,34,35).

In our study, children with current CD4 percent less than 15% were more likely to have dyslipidemia compared with patients with values more than 15% but this did not reach statistical significance (OR 1.9, 95% CI: 0.7 - 5.3; p = 0.136). Other studies in HIV-infected children reported that children who were most immunosuppressed were less likely to have dyslipidemia (23,29).

There was no relationship between height for age or weight for height z-scores and dyslipidemia. Similarly BMI ≥ 90th percentile for gender and sex was not found to be a risk factor for dyslipidemia in this study. BMI has been associated with increased risk of high triglycerides in HIV infected children receiving HAART especially PI based therapy and in studies of cardiovascular disease risk in general pediatric population (23,51).

Limitations of this study include the cross sectional design. With this study design, data collection was limited to a single time point for each subject and therefore changes over time could not be assessed making it difficult to make causal inference. There was also lack of baseline lipid profiles of children studied and lack of lipid profiles of normal African children for comparison. In addition, some of the data was abstracted from the patient charts and there was difficult in verifying some of the information, for example the WHO stage assigned to a patient at initiation of HAART. There could have been over estimation or under estimation of the WHO stage for some patients. Finally, no objective measurement of adherence to antiretroviral drugs, for example pill count or pharmacy refill was made.
In conclusion, this study demonstrated the presence of a high prevalence of dyslipidemia (40%) in HIV-infected children on HAART. Age less than 10 years, and treatment with protease inhibitor inclusive regimens were found to be significantly associated with dyslipidemia. The risk of atherosclerotic disease among HIV-infected children receiving HAART, especially those on protease inhibitors, is unknown. However, persistent dyslipidemia in children is likely to lead to atherosclerotic disease with premature cardiovascular disease in early adult life as evident in children with heterozygous familial hypercholesterolemia. Although the benefits of HAART outweigh these risks, children on combination antiretroviral therapy should have serum lipids monitored at baseline, before introduction of protease inhibitors and at least every six months there after. Dietary changes and exercise are the recommended first strategies in children with HAART-associated dyslipidemia. For patients with inadequate response to these strategies, other management options may be necessary, including changes in antiretroviral regimens and use of lipid-lowering drugs.
10. CONCLUSIONS

1. There is a high prevalence of dyslipidemia in HIV infected children on HAART at the Kenyatta National Hospital with an overall prevalence of 40% for the entire study population. Patients on PI-based regimens had a higher prevalence of dyslipidemia (90%) compared to those on NNRTI-based regimens (37%).

2. Dyslipidemia was associated with age less than 10 years (OR 3.2; 95% CI: 1.3 – 7.7, p = 0.009), and protease inhibitor inclusive therapy (OR 7.5; 95% CI: 1.5 – 38.5, p = 0.015).

11. RECOMMENDATIONS

1. We recommend baseline lipid profile in patients starting antiretroviral therapy and then six monthly assessments after initiation of HAART.

2. We recommend interventional measures in children receiving HAART who develop dyslipidemia. Dietary modification and exercise should be the initial step in management of dyslipidemia and for cases where these measures are ineffective, lipid lowering drugs can be used.

3. There is need for further studies to evaluate the possible target organ effects of dyslipidemia in HIV infected children.
12. REFERENCES


37. Brickman K et al. Mitochondrial toxicity induced by nucleoside-analogue reverse transcriptase inhibitors is a key factor in the pathogenesis of antiretroviral therapy related lipodystrophy. Lancet 1999; 354: 1112-1115.
Appendix 1. QUESTIONNAIRE

Study title: Prevalence of dyslipidaemia in HIV-infected children receiving HAART at the Kenyatta National Hospital Comprehensive Care Centre.

Patient study number ________ Initials ________ Hospital No.__________

Demographic data

1. Age (yrs) ______


3. Age at diagnosis of HIV in years ___________ Date of diagnosis ______________

4. Residence ___________

Medical history

5. Has the child had any serious illness requiring hospitalization in the last 3 months?
   Yes [1] No [2]

If yes to above what illness ______________


7. Does the child suffer from any of the following conditions:
   a) renal failure Yes [1] No [2]
   b) nephrotic syndrome Yes [1] No [2]
   d) other chronic disease (specify) ______________

Drug history


If yes to above, when was HAART started? (Date) ________________

9. WHO stage at initiation of HAART ________________
10. What HAART combination is the child on?

a) AZT + 3TC + NVP [1]
b) AZT + 3TC + EFV [2]
c) d4T + 3TC + NVP [3]
d) d4T + 3TC + EFV [4]
e) AZT + ABC + NVP [5]
f) AZT + ABC + EFV [6]
g) OTHER [7] (specify regimen)
h) AZT + 3TC + ABC [8]
i) ABC + DDI + LPV/r [9]
j) DDI + 3TC + LPV/r [10]
k) ABC + 3TC + EFV [11]
l) AZT + 3TC + LPV/r [12]

11. Complete HAART history.

<table>
<thead>
<tr>
<th>No</th>
<th>Regimen</th>
<th>Date started</th>
<th>Date stopped</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Examination

12. Weight (kg) _______ Weight for age Z-score ________________

13. Height (cm) _______ Height for age Z-score ________________

Weight for height Z-score ________________

13. Calculated BMI ________________ kg/m²

14. Systemic examination

General examination

<table>
<thead>
<tr>
<th></th>
<th>Present</th>
<th>absent</th>
</tr>
</thead>
<tbody>
<tr>
<td>a) Palor</td>
<td>[ 1 ]</td>
<td>[ 2 ]</td>
</tr>
<tr>
<td>b) Fever</td>
<td>[ 1 ]</td>
<td>[ 2 ]</td>
</tr>
<tr>
<td>c) Wasting</td>
<td>[ 1 ]</td>
<td>[ 2 ]</td>
</tr>
<tr>
<td>d) Cyanosis</td>
<td>[ 1 ]</td>
<td>[ 2 ]</td>
</tr>
<tr>
<td>e) Dehydration</td>
<td>[ 1 ]</td>
<td>[ 2 ]</td>
</tr>
<tr>
<td>f) Lymphadenopathy</td>
<td>[ 1 ]</td>
<td>[ 2 ]</td>
</tr>
<tr>
<td>g) Oral thrush</td>
<td>[ 1 ]</td>
<td>[ 2 ]</td>
</tr>
<tr>
<td>h) Skin rash</td>
<td>[ 1 ]</td>
<td>[ 2 ]</td>
</tr>
</tbody>
</table>
i) Oedema

Respiratory system

a) Tachypnea
b) Chest indrawing
c) Crepitations
d) Rhonchi

Cardiovascular system

a) Tachycardia
b) Displaced cardiac apex
c) Murmur

Abdomen

a) Splenomegally
b) Hepatomegally

Central nervous system

a) Neck stiffness
b) Normal muscle tone
c) Normal muscle power

15. WHO stage (current)

Laboratory results

16. Initial CD4 count Date done
17. Current CD4 count Date done
18. Initial CD4 % Date done
19. Current CD4 % Date done
21. Serum lipid profiles (mmol/l)  

a. Total cholesterol  

b. Triglycerides  

c. LDL cholesterol  

d. HDL cholesterol  

1 Normal | 2. High  

1 Normal | 2. Low
Appendix 2

CONSENT EXPLANATION FORM

I am Dr Sammy Kilonzo, a post graduate student doctor at the college of Health Sciences, University of Nairobi carrying out a study on blood lipid levels in HIV-infected children on anti-HIV drugs.

The study
The study is titled: “Prevalence of dyslipidemia in HIV infected children on HAART in Kenyatta National hospital”. The study is about changes that occur in blood cholesterol in patients taking drugs for treatment of HIV. As children grow in to young adults, these changes in blood cholesterol may increase the risk of heart disease. I would like to establish the extent of this problem in children with HIV in our set up.

What the study will involve:
If you agree your child to take part in this study, I shall take a history of the child from you then examine the child including weight, height and waist circumference measurements. I will also ask you to fast the child for a minimum of 4 hours then I shall take 2 ml of blood from the child for measurement of blood cholesterol levels. The laboratory test will be free of charge and the results will be available as part of management of the child. All information obtained will be kept confidential.

Benefits:
Your child will have a free assessment of cholesterol levels and this will be available to enable us to advice on any measures to take. The results of this study may be of benefit to other children with HIV.

Any dangers?
The child will experience some pain during withdrawal of blood.
Child's rights:
Participation in this study is voluntary and you are free to decline involvement of your child in it or withdraw without suffering any loss or affecting the care or treatment given to your child.

My contacts are:   Dr Sammy Kilonzo
P.O Box 8134-00200 Nrb
Tel. 0723 476835

If you have any questions later, you are free to contact me or you can get in touch with the chairperson of the ethics and research committee of Kenyatta National Hospital, Prof Bhatt on telephone no. 2726300 ext 44102.
Appendix 3

CONSENT FORM

I ........................................................................................................ the parent/guardian to agree/give consent for my child to participate in this study on the assessment of blood lipid levels in HIV disease. The study will entail taking the child’s relevant history, physical examination, weight, height and waist circumference. Venous blood (2 ml) will be drawn after a fasting period of at least 4 hours for measurement of blood lipid levels. This will not put the child at any risk and the information given and results shall remain confidential.

Sign ........................................ Date ..............................................
APPENDIX 4

REVISED WHO CLINICAL STAGING OF HIV/AIDS FOR INFANTS AND CHILDREN

Clinical Stage 1
Asymptomatic
Persistent generalized lymphadenopathy (PGL)

Clinical Stage 2
Hepatosplenomegaly
Papular pruritic eruptions
Seborrhoeic dermatitis
Extensive human papilloma virus infection (> 5% body area)
Extensive molluscum contagiosum (>5% body area)
Fungal nail infections
Recurrent oral ulcerations (2 or more episodes in 6 months)
Lineal gingival erythema (LGE)
Angular cheilitis
Parotid enlargement
Herpes zoster
Recurrent or chronic upper respiratory tract infections (otitis media, otorhoea, sinusitis, 2 or more episodes in a 6 month period)

Clinical Stage 3
Unexplained moderate malnutrition not adequately responding to standard therapy
Unexplained persistent diarrhoea (>14 days)
Unexplained persistent fever (intermittent or constant, for longer than one month)
Oral candidiasis (outside neonatal period)
Oral hairy leukoplakia
Acute necrotizing ulcerative gingivitis/periodontitis
Pulmonary tuberculosis
Severe recurrent presumed bacterial pneumonia (2 or more episodes in 6 months)
Chronic HIV-associated lung disease including bronchiectasis
Lymphoid interstitial pneumonitis (LIP)
Unexplained anaemia (<8g/dl), and or neutropenia (<1000/mm3) and or thrombocytopenia (<50 000/ mm3) for more than one month

Clinical Stage 4
Unexplained severe wasting or severe malnutrition not responding to standard therapy
Pneumocystis pneumonia
Recurrent severe presumed bacterial infections (e.g. empyema, pyomyositis, bone or joint infection, meningitis, but excluding pneumonia, 2 or more episodes within one year)
Chronic herpes simplex infection; (orolabial or cutaneous of more than one month’s duration)
Disseminated or extrapulmonary tuberculosis
Kaposi’s sarcoma
Oesophageal candidiasis
CNS toxoplasmosis
HIV encephalopathy
CMV infection (CMV retinitis or infection of organs other than liver, spleen or lymph nodes, onset at age one month or more)
Extrapulmonary cryptococcosis including meningitis
Any disseminated endemic mycosis (e.g. extrapulmonary histoplasmosis, coccidiomycosis, penicilliosis)
Cryptosporidiosis
Isosporiasis
Disseminated non-tuberculous mycobacteria infection
Candida of trachea, bronchi or lungs
Visceral herpes simplex infection
Acquired HIV associated rectal fistula
Cerebral or B cell non-Hodgkin lymphoma
Progressive multifocal leukoencephalopathy (PML)
HIV-associated cardiomyopathy
HIV-associated nephropathy
Symptomatic HIV seropositive (ELISA or rapid test) infant <18 months with 2 or more of the following: oral thrush, severe pneumonia, severe wasting/malnutrition, severe sepsis.
**CHOLESTEROL liquicolor**

**CHOD-PAP-Method**
Enzymatic Colorimetric Test for Cholesterol with Lipid Clearing Factor (LCF)

**Package Sizes**
- **REF** 10017: 4 x 30 ml Complete test kit
- **10019**: 3 x 250 ml Complete test kit
- **10028**: 4 x 100 ml Complete test kit
- **10015**: 9 x 3 ml Standard

**Method**
The cholesterol is determined after enzymatic hydrolysis and oxidation. The indicator quinoneimine is formed from hydrogen peroxide and 4-aminophenazone in the presence of phenol and peroxidase.

**Reaction Principle**
- Cholesterol + H₂O → cholesterol + fatty acid
- Cholesterol + O₂ → cholestene-3-one + H₂O₂
- 2 H₂O₂ + 4-aminophenazone + phenol → quinoneimine + 4 H₂O

**Contents**
- **RG1**: 4 x 30 ml, 3 x 250 ml or 4 x 100 ml Enzyme reagent
- **STO**: 3 ml Standard Cholesterol
- Phosphate buffer (pH 6.5) 100 mmol/l
- 4-Aminophenazone 0.3 mmol/l
- Phenol 5 mmol/l
- Peroxidase > 5 KU/l
- Cholesterolester > 150 U/l
- Cholesteroloxidase > 100 U/l
- Sodium azide 0.05 %

**Reagent Preparation**
The **RG1** and the **STO** are ready for use.

**Reagent Stability**
The reagents are stable up to the given expiry date, even after opening, when stored at 2,..8°C. The opened reagent is stable for 2 weeks at 15...25°C. Contamination must be avoided.

**Specimen**
Serum, heparmised or EDTA-plasma

**Note**: Lipemic specimens usually generate turbidity of the sample/reagent mixture which leads to falsely elevated results. The CHOLESTEROL liquicolor test avoids these falsely elevated results through its built-in Lipid Clearing Factor (LCF). The LCF clears up totally a turbidity caused by lipemic specimens.

**Assay**
- **Wavelength**: 500 nm, Hg 546 nm
- **Optical path**: 1 cm
- **Temperature**: 20...25°C or 37°C
- **Measurement**: Against reagent blank. Only one reagent blank per series is required.

**Pipetting Scheme**
Pipe into cuvettes

<table>
<thead>
<tr>
<th>Pipette into cuvettes</th>
<th>Reagent blank</th>
<th>Sample or [STO]</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sample/STO</strong></td>
<td>1000 µl</td>
<td>1000 µl</td>
</tr>
<tr>
<td><strong>RG1</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mix, incubate 10 min. at 20...25°C or 5 min. at 37°C. Measure the absorbance of the sample/STO against the reagent blank (∆A) within 60 min.

**Calculation of the Cholesterol Concentration**
1. With Factor

<table>
<thead>
<tr>
<th>Wavelength</th>
<th>C [mg/dl]</th>
<th>C [mmol/l]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hg 546 nm</td>
<td>840 x ∆A</td>
<td>21.7 x ∆A</td>
</tr>
<tr>
<td>500 nm</td>
<td>553 x ∆A</td>
<td>14.3 x ∆A</td>
</tr>
</tbody>
</table>

2. With Standard

Only the standard recommended by HUMAN (enclosed in kit or separately available, **REF** 10015) should be used.

\[
C = 200 \times \frac{\Delta A_{\text{sample}}}{\Delta A_{\text{STD}}} \quad \text{[mg/dl]} \\
C = 5.17 \times \frac{\Delta A_{\text{sample}}}{\Delta A_{\text{STD}}} \quad \text{[mmol/l]} 
\]

**Performance Characteristics**

- **Linearity**: The test is linear up to a cholesterol concentration of 750 mg/dl (19.3 mmol/l). Dilute samples with a higher cholesterol concentration 1 + 2 with physiological saline (0.9%) and repeat the determination. Multiply the result by 3.

**Clinical Interpretation**
- Suspect over 220 mg/dl or 5.7 mmol/l
- Elevated over 260 mg/dl or 6.7 mmol/l

**The European Atherosclerosis Society recommends to decrease the cholesterol level to approximately 189 mg/dl for adults up to 30 years and to approximately 200 mg/dl for adults over 30 years.**

**Quality Control**
All control sera with values determined by this method may be employed. We recommend to use our animal serum based HUMATROL or our human serum based SERODOS quality control sera.

**Automation**
Proposals to apply the reagents on analysers are available on request. Each laboratory has to validate the application in its own responsibility.

**Notes**
1. The test is not influenced by hemoglobin values up to 200 mg/dl or by bilirubin values up to 5 mg/dl.
2. The reagents contain sodium azide as preservative (0.05%). Do not swallow. Avoid contact with skin and mucous membranes.

**References**
2. Richmond, W., Clin. Chem. 19, 1350 (1973)
TRIGLYCERIDES liquicolor mono

GPO-PAP Method

Enzymatic Colorimetric Test for Triglycerides with Lipid Clearing Factor (LCF)

**Method**

The triglycerides are determined after enzymatic hydrolysis with lipases. Indicator is quinoneimine formed from hydrogen peroxide, 4-aminoantipyrine and 4-chlorophenol under the catalytic influence of peroxidase.

**Reaction Principle**

\[ \text{Triglycerides} \xrightarrow{\text{lipases}} \text{glycerol + fatty acids} \]

\[ \text{Glycerol + ATP} \xrightarrow{\text{Glycerol kinase}} \text{glycerol-3-phosphate + ADP} \]

\[ \text{Glycerol-3-phosphate + } O_2 \xrightarrow{\text{Peroxidase}} \text{dihydroxyacetone phosphate + H}_2\text{O} \]

\[ \text{H}_2\text{O} + 4\text{-aminoantipyrine} \xrightarrow{\text{POD}} \text{quinoneimine + HCl + H}_2\text{O} + \text{4-chlorophenol} \]

**Contents**

<table>
<thead>
<tr>
<th>RGT</th>
<th>3 ml Standard</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Triglycerides</td>
</tr>
<tr>
<td></td>
<td>200 mg/dl or 2.28 mmol/l</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>RGT</th>
<th>15 ml: 100 ml or 250 ml Monoreagent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PIPES buffer (pH 7.5)</td>
</tr>
<tr>
<td></td>
<td>50 mmol/l</td>
</tr>
<tr>
<td></td>
<td>4-chlorophenol</td>
</tr>
<tr>
<td></td>
<td>5 mmol/l</td>
</tr>
<tr>
<td></td>
<td>4-aminoantipyrine</td>
</tr>
<tr>
<td></td>
<td>0.25 mmol/l</td>
</tr>
<tr>
<td></td>
<td>Magnesium ions</td>
</tr>
<tr>
<td></td>
<td>4.5 mmol/l</td>
</tr>
<tr>
<td></td>
<td>ATP</td>
</tr>
<tr>
<td></td>
<td>2 mmol/l</td>
</tr>
<tr>
<td></td>
<td>Lipoxygenases</td>
</tr>
<tr>
<td></td>
<td>1.3 U/ml</td>
</tr>
<tr>
<td></td>
<td>Peroxidase</td>
</tr>
<tr>
<td></td>
<td>0.5 U/ml</td>
</tr>
<tr>
<td></td>
<td>Glycerol kinase</td>
</tr>
<tr>
<td></td>
<td>0.4 U/ml</td>
</tr>
<tr>
<td></td>
<td>Glycerol-3-phosphate oxidase</td>
</tr>
<tr>
<td></td>
<td>1.5 U/ml</td>
</tr>
</tbody>
</table>

**Pipetting Scheme**

<table>
<thead>
<tr>
<th>Pipette into cuvettes</th>
<th>Rb</th>
<th>Sample or STD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample / STD</td>
<td>...</td>
<td>10 µl</td>
</tr>
<tr>
<td>RGT</td>
<td>1000 µl</td>
<td>... 1000 µl</td>
</tr>
</tbody>
</table>

| Mix and incubate for 10 min. at 20...25°C or for 5 min. at 37°C |
| Measure the absorbance of the sample (ΔA sample) and the Standard (ΔA STD) against the reagent blank within 60 min. |

**Calculation of the Triglycerides Concentration**

C = 200 x \[ \frac{ΔA \text{ sample}}{ΔA \text{ STD}} \] [mg/dl] or C = 2.28 x \[ \frac{ΔA \text{ sample}}{ΔA \text{ STD}} \] [mmol/l]

**Performance Characteristics**

**Linearity**

The test is linear up to a triglycerides concentration of 1000 mg/dl or 11.4 mmol/l. Samples with a higher concentration have to be diluted 1 + 4 with physiological saline (0.9%) and retested. Multiply the result by 5.

**Typical performance data can be found in the Verification Report, accessible via www.human.de/data/gb/vr/SU-TRIMR.pdf or www.human-de.com/data/gb/vr/SU-TRIMR.pdf**

**Clinical Interpretation for Atherosclerotic Risk**

- **Suspect:** over 150 mg/dl or 1.71 mmol/l
- **Increased:** over 200 mg/dl or 2.28 mmol/l

**Quality Control**

All control sera with triglycerides values determined by this method can be employed. We recommend to use our animal sera HUMANATROL or our human serum based SERODOS quality control sera.

**Automation**

Proposals to apply the reagents on analysers are available on request. Each laboratory has to validate the application in its own responsibility.

**Notes**

1. To correct for free glyceroi subtract 10 mg/dl (0.11 mmol/l) from the triglycerides value calculated.
2. The test is not influenced by hemoglobin values up to 150 mg/dl or by bilirubin values up to 40 mg/dl. Ascorbate may give falsely low values at > 4 mg/dl.
3. The reagents contain sodium azide (0.05%) as preservative. Do not swallow. Avoid contact with skin and mucous membranes.

**References**

5. ISO 15223 Medical devices-Symbols to be used with medical device labels, labelling and information to be supplied

**Assay**

- **Wavelength:** 500 nm, Hg 546 nm
- **Optical path:** 1 cm
- **Temperature:** 20...25°C or 37°C
- **Measurement:** against reagent blank (Rb). Only one reagent blank per series is required.

**Specimen**

- Serum, heparinised plasma or EDTA plasma
- **Stability:** 3 days at 2...8°C
- 4 months at -20°C
- **Note:** Lipemic specimens usually generate turbidity of the sample reagent mixture which leads to falsely elevated results. The TRIGLYCERIDES liquicolor mono test avoids these falsely elevated results through its built-in Lipid-Clearing Factor (LCF). The LCF clears up totally a turbidity caused by lipemic specimens.

**Reagent Preparation and Stability**

<table>
<thead>
<tr>
<th>RGT</th>
<th>and STD are ready for use.</th>
</tr>
</thead>
</table>

The reagents are stable, even after opening, up to the stated expiry date when stored at 2...8°C. At 20...25°C the RGT is stable for 4 weeks. **Contamination must be avoided.**

Protect from light.

**Human Gesellschaft für Biochemica und Diagnostica mbH**

Max-Planck-Ring 21 • D-65205 Wiesbaden - Germany
HDL CHOLESTEROL liquicolor
direct Homogeneous Test for the
determination of HDL-Cholesterol
Enzymatic Colorimetric Test

Intended Use
HDL CHOLESTEROL liquicolor is a homogeneous enzymatic assay for the
quantitative determination of HDL cholesterol (HDL). HDL is regarded as a
protecting lipid component against coronary heart disease (CHD). Together with LDL cholesterol it is of diagnostic importance to estimate
the individual risk for CHD.

Method
The assay combines two specific steps: 1st step chylomicrons, VLDL and
LDL cholesterol are specifically eliminated and destroyed by enzymatic reactions. In the 2nd step remaining cholesterol from the HDL fraction is determined by well established specific enzymatic reactions in the
presence of specific surfactants for HDL.

Reactions Principle
1st step: LDL, VLDL and Chylomicrons
2nd step: HDL + lipoproteins

Contents

<table>
<thead>
<tr>
<th>Item</th>
<th>Complete Test Kit</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDL</td>
<td>10284</td>
</tr>
<tr>
<td>VLDL</td>
<td>10284</td>
</tr>
<tr>
<td>Cal</td>
<td>10284</td>
</tr>
<tr>
<td>Sub</td>
<td>10284</td>
</tr>
<tr>
<td>Enzymes (white cap)</td>
<td></td>
</tr>
<tr>
<td>Good's buffer, pH 6 (25°C)</td>
<td>200 ml</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>100 ml</td>
</tr>
<tr>
<td>Cholesterol esterase</td>
<td>100 ml</td>
</tr>
<tr>
<td>Cholesterol oxidase</td>
<td>100 ml</td>
</tr>
<tr>
<td>Catalase</td>
<td>100 ml</td>
</tr>
<tr>
<td>Ascorbate oxidase</td>
<td>100 ml</td>
</tr>
<tr>
<td>N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline (HDAOS)</td>
<td>100 ml</td>
</tr>
<tr>
<td>Preservative</td>
<td>100 ml</td>
</tr>
</tbody>
</table>

Substrate (green cap)

<table>
<thead>
<tr>
<th>Item</th>
<th>Complete Test Kit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peroxidase</td>
<td>100 mU/ml</td>
</tr>
<tr>
<td>4-Aminoantipyrin (4-AA)</td>
<td>100 mU/ml</td>
</tr>
<tr>
<td>Good's buffer, pH 7 (25°C)</td>
<td>100 mU/ml</td>
</tr>
<tr>
<td>Preservative</td>
<td>100 mU/ml</td>
</tr>
<tr>
<td>Detergents</td>
<td>100 mU/ml</td>
</tr>
<tr>
<td>Sodium azide</td>
<td>100 mU/ml</td>
</tr>
</tbody>
</table>

CAL 1 x 4 ml Calibrator

Reagent Preparation and Stability

Enzymes and SUB are ready for use.

Stability: After opening the reagents are stable up to 2 months when stored at 2-8°C. Avoid contamination. Do not freeze. Do not mix caps. Protect from light.

CAL Reconstitute the content of the vial with exactly 4 ml distilled water.
Avoid foaming. Let stand for at least 30 minutes before use.

Stability: 10 days at 2-8°C. If required, freshly prepared calibrator can be divided into portions and kept frozen at -20°C for maximum 30 days.

Specimen

Serum, plasma

Stability: We recommend to test directly after sampling, otherwise store the serum at -20°C (up to several weeks, avoid repeated freezing and thawing).

In plasma following concentrations of the anticoagulant should not be exceeded: EDTA-2Na < 1000 mg/l; Na-citrate < 5000 mg/l; heparin < 750 mg/l; NaF < 2000 mg/l; Na-oxal. < 3000 mg/l

Assay

Wavelength: Hg 578 nm, 593 nm. (570 to 610 nm)

Optical path: 1 cm

Temperature: 37°C

Measurement: Against reagent blank, one blank per series is sufficient

Procedure (manual procedure)

Warm the reagents and the cuvette to 37°C. Temperature must be kept
constant (± 0.5°C) for the duration of the test.

Pipette into cuvettes

Water

Cal/Sample

Mix gently and incubate for exactly 5 min at 37°C

Sub

Mix gently, incubate at 37°C and read the absorbance AA of [CAL]

Calculation

Calculate the concentration of the sample as follows:

AA

Conv factor

Concentration = Conv factor x (AA x 73.54 x 0.1131) (mmol/l)

Performance Characteristics

Linearity: < 120 mg/dl HDL

Linear limit depends on the analyzer specific application. If the sample
concentration of HDL exceed the measuring range, dilute the sample 1:1 with saline (0.85%) and repeat the test. Multiply the result by 2.

Interference: Dilute samples with triglycerides exceeding 1200 mg/dl
with physiologic saline (0.9%) 1:1 and multiply the result by 2.

Typical performance date can be found in the Verification Report.

References


Hypothesis of atherosclerosis is able to be treated by measuring HDL cholesterol.
Dear Dr. Kilonzo,

RESEARCH PROPOSAL: "THE PREVALENCE OF DYSLIPIDEMIA IN HIV INFECTED CHILDREN RECEIVING HAART AT THE KENYATTA NATIONAL HOSPITAL"

This is to inform you that the Kenyatta National Hospital Ethics and Research Committee has reviewed and approved your above cited research proposal for the period 18th February 2008 – 17th February 2009.

You will be required to request for a renewal of the approval if you intend to continue with the study beyond the deadline given. Clearance for export of biological specimen must also be obtained from KNH-ERC for each batch.

On behalf of the Committee, I wish you fruitful research and look forward to receiving a summary of the research findings upon completion of the study.

This information will form part of database that will be consulted in future when processing related research study so as to minimize chances of study duplication.

Yours sincerely,

PROF AN GUANTAI
SECRETARY, KNH-ERC

cc. Prof. K.M. Bhatt, Chairperson, KNH-ERC
The Deputy Director CS, KNH
The Dean, School of Medicine, UON
The Chairman, Dept. of Paediatrics & Child Health, UON
Supervisors: Prof. C. Kigondu, Dept. of Clinical Chemistry, UON
Dr. C. Jowi, Dept.of Paediatrics & Child Health, UON
Dr. D. Wamalwa, Dept.of Paediatrics & Child Health, UON