BIOCHEMICAL PROFILES IN CHILDREN WITH HIV AT GERTRUDE’S CHILDREN’S HOSPITAL COMPREHENSIVE CARE CENTRE, NAIROBI, KENYA

By

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SEPTEMBER 2011
DECLARATION

This thesis is my original work and has not been presented for a degree in any other University or for any other award.

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DEDICATION

This thesis is dedicated to my husband Jimmy, children Joan and Steve for their patience, perseverance and endurance during the preparation of this thesis. To my parents Mr. Gideon Gatuthu and Mrs. Ruth Gatuthu whose academia, prayers and support inspired me.

Your encouragement was outstanding
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<th>Abbreviation</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>ABC</td>
<td>Abacavir</td>
</tr>
<tr>
<td>AIDS</td>
<td>Acquired Immunodeficiency Syndrome</td>
</tr>
<tr>
<td>ALP</td>
<td>Alkaline Phosphatase</td>
</tr>
<tr>
<td>ALT</td>
<td>Alanine Aminotransferase</td>
</tr>
<tr>
<td>ART</td>
<td>Antiretroviral therapy</td>
</tr>
<tr>
<td>ARV</td>
<td>Antiretroviral Drugs</td>
</tr>
<tr>
<td>AST</td>
<td>Aspartate Aminotransferase</td>
</tr>
<tr>
<td>BALP</td>
<td>Blood Alkaline Phosphatase</td>
</tr>
<tr>
<td>BMD</td>
<td>Bone Mineral Density</td>
</tr>
<tr>
<td>Ca++</td>
<td>Calcium</td>
</tr>
<tr>
<td>CBC</td>
<td>Complete Blood Count</td>
</tr>
<tr>
<td>CCC</td>
<td>Comprehensive Care Clinic</td>
</tr>
<tr>
<td>CDC</td>
<td>Centre for Disease Control and Prevention</td>
</tr>
<tr>
<td>CD4</td>
<td>Cluster of Differentiation 4</td>
</tr>
<tr>
<td>CD8</td>
<td>Cluster of Differentiation 8</td>
</tr>
<tr>
<td>CPK</td>
<td>Creatinine phosphokinase</td>
</tr>
<tr>
<td>CYP450</td>
<td>Cytochrome P450</td>
</tr>
<tr>
<td>Ddi</td>
<td>Didanosine</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic Acid</td>
</tr>
<tr>
<td>D4T</td>
<td>Stavudine</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme-Linked Immunosorbent Assay</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<td>--------------</td>
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</tr>
<tr>
<td>FDA</td>
<td>Food and Drug Administration</td>
</tr>
<tr>
<td>GCH</td>
<td>Gertrude Children’s Hospital</td>
</tr>
<tr>
<td>HIV</td>
<td>Human Immunodeficiency Virus</td>
</tr>
<tr>
<td>HAART</td>
<td>Highly Active Antiretroviral Therapy</td>
</tr>
<tr>
<td>KNH</td>
<td>Kenyatta National Hospital</td>
</tr>
<tr>
<td>LADME</td>
<td>Liberation Absorption Distribution Metabolism Elimination</td>
</tr>
<tr>
<td>LDH</td>
<td>Lactate Dehydrogenase</td>
</tr>
<tr>
<td>MOH</td>
<td>Ministry of Health</td>
</tr>
<tr>
<td>MTCT</td>
<td>Mother-to-Child Transmission</td>
</tr>
<tr>
<td>Mths</td>
<td>Months</td>
</tr>
<tr>
<td>NA</td>
<td>Nucleic Acid</td>
</tr>
<tr>
<td>NAD</td>
<td>Nicotinamide Adenine Dinucleotide</td>
</tr>
<tr>
<td>NADH</td>
<td>Nicotinamide Adenine Dinucleotide Dehydrogenase</td>
</tr>
<tr>
<td>NASCOP</td>
<td>National AIDS/STD Control Programme</td>
</tr>
<tr>
<td>NIDDM</td>
<td>Non Insulin Dependent Diabetes Mellitus</td>
</tr>
<tr>
<td>NFV</td>
<td>Nelfinavir</td>
</tr>
<tr>
<td>NNRTI</td>
<td>Non nucleoside reverse Transcriptase Inhibitors</td>
</tr>
<tr>
<td>NtRTI</td>
<td>Nucleotide Reverse Transcriptase inhibitors</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase Chain Reaction</td>
</tr>
<tr>
<td>PHOS</td>
<td>Phosphorous</td>
</tr>
<tr>
<td>PI</td>
<td>Protease Inhibitors</td>
</tr>
<tr>
<td>PLHA</td>
<td>People Living With HIV/AIDS</td>
</tr>
<tr>
<td>PMTCT</td>
<td>Prevention of Mother-to-Child Transmission</td>
</tr>
<tr>
<td>PQD</td>
<td>Peroxidase</td>
</tr>
<tr>
<td>PVL</td>
<td>Plasma Viral Load</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>RNA</td>
<td>Ribonucleic Acid</td>
</tr>
<tr>
<td>RT</td>
<td>Reverse Transcriptase</td>
</tr>
<tr>
<td>TB</td>
<td>Tuberculosis</td>
</tr>
<tr>
<td>3TC</td>
<td>Lamivudine</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
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<td>ZDV</td>
<td>Zidovudine</td>
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ABSTRACT

Human Immunodeficiency Virus infection and antiretroviral therapy are associated with complex metabolic alterations. In patients on highly active antiretroviral therapy (HAART), the risk of significant metabolic derangements increases with duration of treatment. Individual drugs or classes of antiretroviral drugs are associated with specific toxicities. Because suppression of viral replication requires long term or even lifelong use of HAART, HIV infected children will have the longest exposure with the attendant risk of experiencing adverse side effects. Nucleoside Reverse Transcriptase Inhibitors including didanosine and lamivudine are implicated in mitochondrial toxicity, leading to lactic acidosis. They may also cause elevation of liver enzymes, hyperglycemia and lipodystrophy. Non- Nucleoside Reverse Transcriptase Inhibitors including nevirapine and efavirenz have also been found to cause acute hepatitis. Protease Inhibitors may cause elevation of the liver function tests, abnormal lipids and hyperglycemia. There is very little local data on metabolic derangements in treated Human Immunodeficiency Virus infected children. This study has described some biochemical profiles in Human Immunodeficiency Virus infected children who are either treatment naïve or on Highly Active Antiretroviral Therapy (HAART). Non fasting plasma specimens were taken. Biochemical markers of bone metabolism (alkaline phosphates, calcium, phosphorous); hepatocyte damage (Aspartate aminotransaminases, Alanine aminotransaminases); mitochondrial toxicity (lactic acid) and carbohydrate intolerance (glucose) were measured. Approval for the study was obtained from Gertrude Children’s Hospital Ethics and Research Committee. An auto analyzer using commercial reagents was used. Of the 133 children studied 81 (60.9%) were on HAART while 52 (39.1%) were not on treatment. Age ranged from 2 months to 15 years (median 6 years). The male to female ratio was 1.5:1. Their CD4 counts ranged from 3 to 2070 cells/mm3 while the viral load ranged from <40 to 4,194,860 copies/ml (median 1,231,772). ARV treatment duration ranged from 0 to 121 months (median 12). Most children had biochemical parameters within the reference ranges. Transaminases elevation was the most common biochemical derangement being found in 15% and 7.4% of treatment naïve and HAART patients respectively. All patients had normocalcaemia, mean values 2.17 mmol/l and 2.22 mmol/l in HAART treatment and naïve patient’s respectively. Mild lactic elevation was found in treatment naïve (n=5) and patients on treatment (n=5). All the affected children were between the ages of 1-5 years. No correlation was found between the biochemical profiles and the CD4 counts or viral load. Guidelines for initiating ARV therapy recommend estimation of transaminases. This study has found that plasma glucose, lactate, calcium, phosphorous, ALP, ALT and AST in young children with HIV were not elevated due to treatment with HAART. The initiation of HAART in the children was associated with increased T- cell count. There was a reduction in viral load and increased CD4+ cell count in patients on HAART treatment. HIV and HAART are not associated with changes in serum biochemical parameters in children. A longitudinal study may unmask such derangements and is recommended with increased use of ART.
CHAPTER ONE
INTRODUCTION

1.1 Background Information
Human immunodeficiency virus (HIV) is a common problem in Kenya affecting both children and adult population. Since 2002 there has been a general decline in infection rates both in rural and urban areas. Infection rates also declining among age group 15-24yrs. National prevalence dropped from 13.4% in 2000 to 7.4% in 2005.

HIV/AIDS disease has been in our midst for about two decades, with the first documented case in 1983. The virus which causes acquired immune deficiency syndrome (AIDS) was first recognized in homosexual men in the United States in 1980. The virus was isolated from the serum of a hemophiliac patient in France in 1983 (Aggleton et al., 2000), and since then the virus has spread widely to all parts of the world.

Antiretroviral (ARV) drugs are used to manage HIV/AIDS, which following recent price reduction has become commonly available to more patients suffering from AIDS. There are different types of ARVs used for a reduction in viral load. They are classified by Food and Drug Administration (FDA) into five categories: (1) Nucleoside and nucleotide reverse transcriptase inhibitors (NRTI, NtRTI), (2) Non-nucleoside reverse transcriptase inhibitors (NNRTI), (3) Protease inhibitors (PI) and (4) Fusion inhibitors and Integrase Inhibitors.

The introduction of ARVs as part of HIV clinical care has made AIDS a manageable chronic illness with restored economic productivity and social functioning. However, these effects are
seen only in settings where resources are available to buy the drugs and there are health service capacities to optimize their sustained, safe and effective use. There are multiple requirements for such an effect that are grouped into three: (a) the drugs (b) the client and (c) the health system. Persons living with HIV/AIDS (PLHA) play a crucial role in the design and implementation of antiretroviral drug and HIV/AIDS prevention and care programs.

To arrest immune damage, combinations of at least three drugs from the various classes of antiretroviral drugs in a cocktail are used. This three-drug cocktail is called Highly Active Antiretroviral Therapy (HAART). Each class of anti-HIV drugs attacks the virus at different replication stage while it is growing in the human host lymphocyte cell (JAIDS, 2004). However, there are drug related issues that influence their use; firstly all ARVs are still costly even with recent dramatic price reduction. Secondly side effects of the drugs are common and need to be monitored which may lead to stopping, changing the drug, or death. Patients on ARV require counseling on the possible side effects and must be advised on any diet modifications that may be needed to enhance nutrient absorption and metabolism. Some medication, e.g. Protease Inhibitors can interact with certain nutrients in food affecting their absorption, metabolism, distribution and excretion, and hence reducing the antiviral drug efficiency. In addition, ARVs produce metabolic disorders including elevated levels of triglycerides, cholesterol, and fat maldistribution and insulin resistance. The latter may lead to diabetes. These changes require dietary modification such as avoiding foods high in cholesterol, exercising daily or taking medications to lower the lipids. Elevated lipids as well as diabetes are a risk factor for heart disease (JAIDS, 2004).
Many medications can cause side effects that can affect food intake and nutrient absorption. These side effects include nausea, vomiting, loss or change in taste, loss of appetite (anorexia), bloating and heartburn, constipation and diarrhea. These side effects can result in poor nutrient intake and absorption, weight loss and ultimately malnutrition. In turn, some ARVs can cause metabolic side effects that increase the risk of other nutrition-related conditions such as heart disease.

1.2 Problem statement and justification
ARVs use is on the increase and little is known about their side effects in children in Kenya. There are few studies which have been done on the adults which show varying biochemical changes and therefore the need for study in children. All the available ARV agents have significant potential toxicities. Interactions between ARV agents and other drugs that may be used in HIV-infected children can lead to additional and potentially more severe reactions. The adverse events can occur at any time, even after years of treatment. However, problems are usually noted the first few weeks or months after starting a new drug. So far, very few children have been put on ARV compared to adults and this is due to unavailability of pediatric formulations and lack of trained personnel in this field. There are fewer ARV drugs approved for use in children due to delay in obtaining safety, toxicological and pharmacokinetic data (GCH, 2004). This study will help fill a glaring gap in knowledge about biochemical changes in children as a result of ARV therapy.

1.3 Research questions
1. What are the effects of HAART treatment on viral load and CD4 count in HIV infected children?
2. What are the effects of HAART treatment on random blood sugar, lactate, calcium, phosphorous, alkaline phosphatase, alanine and aspartate aminotransferase in HIV infected children.

1.4 Null Hypothesis
HIV and HAART are not associated with changes in serum biochemical parameters in children.

1.5 Objectives
The general objective was to determine the effects of HAART treatment on some biochemical, immunological parameters and viral load in HIV infected children.

1.5.1 Specific objectives
1. To determine the effect of ARV treatment on viral load and CD4 count in HIV infected children.
2. To determine the effect of ARV treatment on random blood sugar, alkaline phosphatase, calcium, phosphorous, lactate, alanine and aspartate aminotransferase in HIV infected children.

1.6 Significance and anticipated outcome
This study has provided data on the presence and magnitude of the biochemical derangements in children who are HIV positive and those on HAART. The data obtained from the analytes was availed to the clinicians and would help design early intervention measures to be applied during management.
CHAPTER TWO
LITERATURE REVIEW

2.1 The disease

Human immunodeficiency virus (HIV/AIDS) is an immune related disease in which the infection causes depletion of the immune system rendering patients susceptible to opportunistic diseases. HIV is a retrovirus composed of RNA which can only replicate itself by invading host mammalian cells and by enlisting the cell. Lymphocytes derived from the thymus tissue, so called T-cells and a number of other cells in the body such as macrophages and microglial cells of CNS bear a genetic marker called CD4, which is an essential receptor for HIV to enter the cell. The part of the CD4 receptor which is necessary for this process, is called soluble CD4; the virus enters the body through breaks in mucous membranes after exchange of body fluids. Once the virus enters a host cell, it releases viral RNA, which is converted to DNA by a process catalyzed by the enzyme reverse transcriptase. Copies of viral RNA can be produced; and the viral DNA is subsequently integrated into the genome of the host cell where it remains dormant for long periods (JAIDS, 2004). Subsequently copies of viral RNA can be produced to form new viral particles.

2.1.1 Epidemiology of HIV

The number of people living with HIV/AIDS worldwide by the end of 2007 was 39.4 million, of which approximately 86% of them were in sub-Saharan Africa where about 3 million new infections occurred in 2004 alone. There were 3.1 million deaths from AIDS in the sub-Saharan Africa region in 2004 with young people between 15-24 years old, accounting for half of these
new infections. By the end of 2004, there were about 2.2 million persons under the age of fifteen years living with HIV/AIDS worldwide, 86% of whom were in the sub-Saharan Africa. During the same year, about 510,000 children in the same age group died from HIV. There were about 700,000 new infections; 88% in both categories were from sub-Saharan Africa. In Kenya, it is estimated that about 30,0000 infants acquire HIV annually and over 13 million orphans who have lost one or both parents to AIDS worldwide, 90% being in Africa (WHO, 2007).

2.1.2 Pediatric HIV infection and ARV therapy

Tremendous progress has been made over the past few years in diagnosing and treating infants and children with human immunodeficiency virus (HIV) infection. However, much remains to be done to effectively scale-up and sustain prevention efforts and treatment services for all in need. The most efficient and cost-effective way to tackle pediatric HIV globally is to reduce mother-to-child transmission (MTCT). In 2008, an estimated 45% of pregnant women living with HIV received antiretrovirals (ARVs) to prevent transmission of HIV to their children. However, every day, there are nearly 1,200 new infections in children less than 15 years of age, more than 90% of them occurring in the developing world and most being the result of transmission from mother to child. HIV-infected infants frequently present with clinical symptoms in the first year of life. Without effective treatment, an estimated one third of infected infants will have died by one year of age, and about half will have died by two years of age. While progress has been made in preventing new HIV infections in infants and children, greater efforts are needed to scale-up these effective preventive interventions as well as services for care and treatment. The 2009 progress report towards universal access: scaling up priority HIV/AIDS interventions in the
health sector, documents the progress made by countries in scaling up antiretroviral therapy (ART) for children. In 2008, over 275,000 children received ART, up from 127,000 in 2006. This is 38% of those in need using the previous 2006 recommendations for ART initiation in children. Given the new guidance contained in this document, estimates of the numbers of infants and children who qualify for ART will have to be revised. HIV-infected infants and children now survive to adolescence and adulthood, and the challenges of providing HIV care are evolving into the challenges of providing both acute and chronic, lifelong care (WHO, 2007).

2.1.3 Antiretrovirals and their mode of action
The Highly Active Antiretroviral Therapy (HAART) is a regimen that combines at least three ARV drugs. These drugs are usually combined to maximally reduce viral replication to undetectable level, to prevent viral break through and emergence of resistance and to elicit a durable therapeutic response and avoid treatment failure. For HAART to yield the desired effects there should be 95% adherence to the prescribed regimen, the drug combination must have proven effect when used together, administered at correct times with regard to food and drug interactions, and the choice of regimen should preserve future treatment options (GCH, 2005).

ARV drugs are classified according to the mode of action as follows: NRTIs (Nucleoside Reverse Transcriptase Inhibitors), inhibiting the reverse transcription by competitively blocking reverse transcriptase activity. NNRTIs (Non – Nucleoside Reverse Transcriptase Inhibitors), inhibiting the reverse transcription by binding directly to reverse transcriptase enzyme activity while protease Inhibitors (PIs), inhibit the cutting down of the core multi protein molecule to functional protein molecules. Fusion Inhibitors (FI), inhibit the fusion between the virus and
CD4 cell membranes hence preventing binding and entry (Dietrich et al, 2004). Over the past one year, ARV has become increasingly available to most HIV positive children in Kenya through various donor initiatives. It is therefore expected that an increasing number of children will be exposed to ARV and therefore exposure to side effects especially the biochemical anomalies.

2.1.4 The pathogenesis of HIV biochemical values
Biochemical change in body fluids including blood is always secondary to HIV infection and ARV treatment. In children metabolic and morphologic changes in persons receiving therapy are a key obstacle to the initiation and continuation of therapy because these body changes place individuals at risk of future vascular morbidity. Whilst probable contributions to the etiology of this condition have been described, the mechanisms by which these changes occur are not fully understood and no reliably effective therapy has been established (Desai et al., 2003)

Until the classic studies of Harrigan (1995), it was unclear whether most HIV patients died due to the biochemical changes or the virus. Differentiating between complicating consequences of HIV infections and toxicities of drug used in the management of HIV infection is challenging. However, the experience gained with combination anti-retroviral (ARV) drugs has led to the recognition of several distinct adverse drug events. These include mitochondrial dysfunction, hepatic toxicity, pancreatitis and peripheral neuropathy. While individual ARV drugs or classes of ARV drug are associated with specific toxicities, interaction of ARVs with other drugs used in the management of HIV/AIDS complications can result in altered pharmacokinetics and
additional drug toxicities. There are major drug events seen in children, considering that experience in children is more limited than in adults (Harrigan, 1995).

2.1.5 Pediatrics ARV drugs and drug formulations
A list of ARVs in the various classes that is available for children in NRTIs group include Abacavir, Didanosine, Lamivudine, and stavudine, Zidovudine, Fixed Dose Combinations, Emtricitabine and Tenofovir. NNRTIs include Nevirapine, Efavirenz and Delavirdine whereas PIs include Nelfinavir, Ritonavir, Lopinavir, Indinavir etc (WHO, 2005). Possible drug combination include, s 2 NRTIs and NNRTI (spares PIs), 2NRTI and 1 PI (spares NNRTI). 1 NRTI and 1 NNRTI+1 PI (does not spare any class). In children, first line treatment regimens mostly will combine NRTI and NNRTI. Protease Inhibitors are usually used when the first line drugs have failed and are therefore often spared for future use. The drug combination must balance efficacy, toxicity, palatability, and cost- effectiveness and maintain future options. (GCH, 2005).

Among the abnormalities noted with various ARVs in the class of NRTI, include lactic acidosis with steatosis, pancreatitis, increased liver enzymes and blood glucose levels as seen in Abacavir, Didanosine, Lamivudine and Stavudine. Zidovudine may also portray muscle wasting (Myopathy, myositis) in addition to the above drug adverse effects. Infants who have anaemia at birth or who are premature warrant more intensive monitoring. More intensive serum chemistry measurement during the first few weeks of life would be advised in these infants (CDC, 1994).
2.1.6 Common ART biochemical parameters and their risk factors

It is important that one establish if adverse side effects are due to ARV. One of the major adverse effects with NRTIs is lactic acidosis whose symptoms occur between 1 and 20 months later. The common observation in the laboratory include hyperlactatemia, increased anion gap, elevated aminotransferases, CPK, lipase, LDH and Amylase. Lipodystrophy is also common with these drugs which manifest in insulin resistance, hyperglycaemia with increased risk of diabetes mellitus and coronary Heart Disease (JAIDS, 2004).

2.1.7 Bone metabolism

Pablo Tebas (Tebas, 2000) from the Washington University School of Medicine looked at serum and urine markers in 73 HIV-positive patients on a PI-containing regimen to identify whether the pathogenesis of decreases in BMD previously reported by this group involved either the impaired bone formation or the resorption process. Bone is a living tissue, 10% of which is replaced each year in a continuous turnover. It is resorbed by osteoclasts that release calcium and collagen metabolites and formed by osteoblasts. An imbalance in these two processes leads to bone loss. Bone consists of mineralised organic matrix, 90% of which is type 1 collagen and calcification provides the degree of 'hardness'. Bone mineral loss may be more common in children with lipodystrophy and is recognised as one of the emerging metabolic complications of HIV infection in adults and children. Osteoporosis is characterized by severe loss of bone mass and disruption of skeletal micro architecture, which can lead to increased risk of spontaneous and traumatic fracture of the bone (Tebas, 2000). Decreased BMD by PI therapy is brought about by hepatic CYP 450 enzyme that mediates vitamin D metabolism to its most potent circulating metabolite, part of an essential process of vitamin D control of calcium homeostasis (Dusso, 2000).
There have been numerous studies over the last 2 years to evaluate different potential explanations, causative factors, or associations related to bone mineral density loss in HIV. These include prior corticosteroid use and elevated lactate (mitochondrial toxicity), lower weight prior to starting HAART, a greater rise in CD4 count on therapy, fat loss in leg, duration of HIV therapy, PI therapy, not associated with PI use, having lower body weight, age, total body fat, lean body mass, and immune changes (White, 2001). It remains unclear what causes bone loss in HIV since other viral diseases can be associated with manifestations such as this (Dresner-Pollak et al., 1996).

Childhood and adolescence are characterized by a rapid growth of the skeleton, through very high bone cell activity. Normal activity of maintenance of the mineralized bone matrix is coupled with the shaping and growth of the bones, a process called modelling (Frost, 1986). Bone modelling ceases at the end of the pubertal period, when the epiphyses close. The best method for evaluating bone metabolism is the hystomorphometric study of a bone biopsy; however, the procedure is very invasive and complex and, therefore, cannot be used routinely. Bone metabolic rate could instead be studied by serum and urine measurements of specific biochemical markers (Calvo et al., 1996). In children and adolescents, the concentrations of both bone formation and resorption indexes is markedly higher than in adults, and they change according to age, sex and pubertal stage.

Several studies have assessed the values of biochemical markers of bone formation and resorption in adult patients with HIV. Low levels of bone formation (Calvo et al., 1996) and increased concentrations of bone resorption indexes have been found in untreated patients.
Moreover, one study showed that HAART may induce normalization of bone remodeling processes in HIV-infected adults (Aukrust et al., 1999). However, the observed changes in biochemical parameters of bone metabolism during HAART may not be a direct proof of BMD increase; alternatively, such discrepancy may be attributed to the different response elicited by HAART treatment in a growing child. Most children are now receiving a combined triple antiretroviral treatment. It is important that BMD is evaluated in larger long-term studies in order to assess the dynamic of bone loss and the role of each individual medication included in antiretroviral regimen.

2.1.8 Liver enzymes
Elevations in liver enzymes with or without clinical hepatitis have been reported in 14 to 20% of HIV-infected adults receiving HAART. Differential diagnosis of liver dysfunction in an HIV-infected patient is complicated, as abnormalities in liver functions are common and may be caused by HIV itself, co-infections with hepatitis B or C viruses or opportunistic infections, malignancies, drug interactions, or drug induced hepatic toxicity. Hepatotoxicity has been reported with all of the available NRTI, NNRTI, and PI drugs (Montessori et al., 2003). However, hepatotoxicity has been commonly associated with non nucleoside reverse transcriptase inhibitors (NNRTIs) and PIs. When it results from nevirapine use it has an early onset, with the greatest risk occurring within the first two to six weeks of therapy. Hepatotoxicity is defined as a threefold to fivefold increase in serum transaminases (ALT) with or without clinical hepatitis, and is more common in women and patients with >250 cells/mm$^3$ CD4+ T cell counts. In fact, women with CD4+ T Cell counts of >250 cells/mm$^3$, including pregnant women receiving long
term treatment for HIV, have a considerable higher risk of developing hepatotoxicity than other women (Dietrich et al., 2004).

Most cases of hepatotoxicity are not associated with acute hepatitis, and symptoms may resolve within a few months. Patients should be therefore regularly monitored for the first 18 weeks of treatment with antiretroviral. Hepatotoxicity associated with ritonavir use is similar to that caused by nevirapine but can occur at any time during the course of treatment (Mallal, 1998).

Dietrich et al., (2004) established that symptomatic nevirapine liver toxicity consists of elevated liver enzymes plus at least one symptom, which is typically rash but may include flu-like symptoms of fever. The severity of symptomatic liver toxicity ranges from mild symptoms with liver enzyme abnormalities to rapidly occurring liver failure and death. Symptomatic nevirapine liver toxicity typically occurs after only a few weeks of dosing and may progress to liver failure despite monitoring of laboratory tests, which is not characteristic of other antiretroviral (Dietrich et al., 2004).

Nevirapine liver toxicity is less frequent (<2% for both males and females with CD4+ counts <250 cells/mm3) when started in patients with lower CD4 counts. The incidence of hepatic toxicities and their risk factors in pediatric population is hindered by the variability in reporting of hepatic events. Early studies with NRTI drug in pediatric patients with mild to moderate symptoms of HIV disease demonstrated that elevated liver function tests, including increase in serum transaminases (AST and ALT) were a relatively common event in children being treated with NRTI drug. In an early study of ZDV monotherapy 12.8% of patients developed ALT > 5
times. While in a combination NRTI therapies, 4% of the children developed ALT > 10 times (Dietrich et al., 2004).

Monitoring liver function tests, as part of routine periodic laboratory evaluation of HIV – infected children remains an important part of standard monitoring. Such monitoring is particularly important in the first few months after initiating antiretroviral therapy or changing therapies (Wit et al., 2002).

2.1.9 Blood sugar
Insulin resistance without fasting hyperglycaemia, asymptomatic fasting hyperglycemia, new onset diabetes mellitus, and exacerbation of pre-existing diabetes have all been reported in patients treated with ARV therapy, especially with PI containing regimens. Incidence estimates of 3 to 17% are suggested for hyperglycaemia in adults, with median onset approximately 60 days following initiation of ARV therapy (Bray et al., 2002). When starting PIs, guardians and patients should be educated about symptoms of overt diabetes mellitus (polyuria, polyphagia, polydipsia, weight loss). For adults the International Aids Society – USA recommends a fasting blood glucose measurement before starting treatment with PIs, at 3 to 6 months after institution of therapy, and yearly while on therapy. A random glucose concentration in children is most preferred and values above 7.7mmols/l should be followed (Bray et al., 2002).

Protease inhibitors are considered a crucial component of antiretroviral combination therapy for HIV-1 infection (Tebas, 2000). These drugs interfere with post-translational processing of viral precursor proteins, and thereby prevent the formation of new infectious virus particles (Nicoll,
A variety of side effects have been documented in patients treated with protease inhibitors. The new onset of diabetes mellitus has been observed in patients treated with all currently approved protease inhibitors (indinavir, nelfinavir, ritonavir, sequinavir). This suggests that class specific mechanism associated with the use of protease inhibitors are involved in the induction of these metabolic alterations. Peripheral insulin resistance has been suggested to contribute to the described changes in glucose metabolism because some of the findings resemble those seen in patients with non-insulin-dependent diabetes mellitus now called Type 11.

2.1.10 Lactate levels in metabolic acidosis
Chronic and symptomatic mild hyperlactatemia (2.2 to 5.0 Mmols/l) is relatively frequent among HIV infected individuals receiving nucleoside analogue reverse transcriptase inhibitors (NRTIs), occurring in approximately 15 to 35% of infected individuals receiving ARV treatment, usually for longer than 6 months. There are few data available in paediatric patients. In a cohort of 81 HIV infected Spanish children receiving ARV therapy, asymptomatic mild hyperlactatemia was observed in 17% of children. In USA asymptomatic hyperlactatemia was observed in 32% of 127 HIV- infected children receiving highly active antiretroviral therapy (Brinkman, 2000).

Increases in the level of lactic acid have also been recognised in both HIV-positive adults and children and is linked to all nucleoside analogues as well as in people not using ARV treatment (Ivers et al., 2006). Lactic acidosis (LA) can be fatal in over half the reported cases. Attention was focussed on this with the recent FDA requirement for a new emphasis on this in product information summaries and advertising for abacavir, d4T and ddI (Church et al., 2000). Acute,
severe lactic acidosis is a rare but potentially fatal complication of treatment with nucleoside analogue reverse transcriptase inhibitors (NRTI) in HIV-infected patients (Lenzo et al., 1997). Severe lactic acidosis is typically symptomatic with nausea, vomiting, severe malaise and prostration and may occur precipitously after months or even years of NRTI treatment recently, mild or moderate lactate elevation in NRTI-treated individuals has been reported (Bray et al., 2002).

The natural history of mild to moderate hyperlactatemia and its relationship to the risk of severe, life-threatening lactic acidosis is not known. As lactate is the product of anaerobic glycolysis, hyperlactatemia in normal aerobic conditions may indicate mitochondrial dysfunction (Brinkman et al., 1999). The mitochondrial basis of NRTI-induced hepatic steatosis, lactic acidosis and myopathy is well established, and several other adverse effects such as peripheral polyneuropathy and cardiomyopathy have also been linked to mitochondrial toxicity. It has been hypothesized that some features of the 'lipodystrophy syndrome' are also tissue-specific mitochondrial toxicities caused by NRTI treatment (Mallal, 2000).

In contrast to the common form of mild stable hyperlactatemia, severe lactic acidosis appears to indicate an extreme, decompensated metabolic state in which homeostasis is lost completely. Notably, NRTI-induced severe lactic acidosis is almost always accompanied by massive hepatic steatosis and frequently by hepatic failure (Mallal, 1998). The liver has limited lactate clearance capacity in these circumstances and hence becomes a net lactate producer. The extent of mitochondrial toxicity in hepatocytes may be the key determinant of risk of decompensation into severe lactic acidosis in NRTI-treated patients (Brinkman, 2000).
Lactic acidosis/hepatic steatosis is thought to be secondary to mitochondrial dysfunction induced by NRTI treatment. NRTI drugs have varying affinity for mitochondria DNA polymerase gamma in vitro highest for zalcitabine, ZDV, Abacavir have lower affinity for the mitochondrial polymerase. Inhibition of mitochondrial DNA polymerase gamma can result in inhibition of mitochondrial DNA replication, resulting in impaired synthesis of mitochondrial respiratory chain enzymes, deterioration of oxidative phosphorylation, and depletion of ATP levels. When a cell is unable to generate enough energy through oxidative phosphorylation, anaerobic respiration occurs via conversion of pyruvate to lactate in the cytoplasm (Brinkman, 1998). Lactic acidosis is the condition caused by over-accumulation of lactate in the bloodstream and tissues, which the body is unable to clear.

Lactic acid and lactate are produced when glucose is broken down by the body’s cells to produce energy. More lactate is produced when oxygen supply is limited, such as during exercise, or in certain types of cells, or when the mitochondria, organelles inside the cells that normally produce energy, are not functioning properly. Elevated lactate is rather common in patients treated with nucleoside analogues (up to 25%) but there are usually no symptoms. However, in some cases there can be an abnormal accumulation of lactic acid in the blood, ‘hyperlactataemia’ or lactic acidaemia, that may be associated with symptoms such as fatigue, breathlessness, abdominal pain and weight loss.

If the condition worsens, the patient may develop lactic acidosis, which may occur in conjunction with severe hepatomegaly (enlarged liver). The patient may suffer respiratory failure and fall into a coma. In patients on ART, lactic acidosis is attributed to nucleoside analogues,
particularly d4T, ddI (especially the combination of d4T/ddI) and AZT, which all can cause
damage the mitochondria. It primarily occurs in women, particularly pregnant women, who have
been on ART for several months.

A South African study reported at the Thirteenth Conference on Retroviruses and Opportunistic
Infections found that lactic acidosis is occurring at an unusually high frequency in patients
receiving either d4T or AZT-based antiretroviral therapy. The South African study found an
incidence of 15 cases per 1000 per year of patient follow-up (almost as high a frequency as that
reported for peripheral neuropathy in the same study). The risk of developing lactic acidosis
seemed to be greater in women with a higher body weight which, for cultural reasons, is much
more common in South Africa than in most other settings. Multivariate analysis found that
women weighing 75kg or more had an adjusted hazard ratio of 25 for lactic acidosis when
compared with males, while women weighing between 60 and 75kg had an AHR of 5.6 for lactic
acidosis. Weight gain of 5kg or more by month 3 of treatment carried an AHR of 2.5 for lactic
acidosis (p<0.01) (Boule, 2006).

Discussion following the presentation could not offer a biological explanation for this
phenomenon, but questions from the floor revealed that a similar trend has been seen in the
Botswana treatment programme. As already noted, asymptomatic lactate elevations are fairly
common and a number of studies have found that individual measurements of lactate in the
blood are a poor predictor of the subsequent risk of developing lactic acidosis. Routinely
screening lactate levels could therefore be misleading — and impractical in many settings. So
diagnosis starts with the symptoms. Initial signs of high lactate levels include loss of weight,
nausea and vomiting, lack of appetite and malaise, as well as fatigue and difficulty in breathing. Muscle pain and numbness or tingling sensations have also been reported. In lactic acidosis, the liver may become swollen and tender, and liver enzymes may be elevated. Symptoms of acute lactic acidosis include severe difficulty in breathing, hyperventilation and stupor.

A diagnostic algorithm has been developed by the South African HIV Clinicians Society, which just published “Guidelines for the prevention, diagnosis and management of nucleoside reverse transcriptase inhibitor-associated symptomatic hyperlactatemia and lactic acidosis”. Any patient presenting with signs of hyperlactatemia or lactic acidosis should have their lactate levels checked immediately as a delay in diagnosis can be life-threatening. Measuring lactate requires specialized equipment which can be a challenge in settings without convenient access to a reference laboratory (specimens must be centrifuged and transported on ice). Point-of-care devices have been developed, however, which can be used at primary care and rural facilities; these devices can reliably determine lactate levels within +/-1 mmol/l of the laboratory measurement. They are now being used to diagnose ART-related lactic acidosis in rural clinics across Haiti, according to a recent letter in AIDS (Desai et al., 2003).

If lactate levels are below 2.5mol/l, the diagnosis has been excluded and other causes should be investigated. However, when lactate levels are higher, action must be taken. If lactate is not available, an alternative is standard bicarbonate (< 15 suggests severe lactic acidosis). Venous or arterial pH also useful, but only becomes abnormal if severe lactic acidosis. Even if no laboratory is available, and these tests cannot be performed, – the clinician should err on the side of making the diagnosis of lactic acidosis and treating sepsis, while investigating other causes.
The South African guidelines are based on expert experience rather than prospective clinical trials and may change as more evidence becomes available, and practice may vary somewhat by setting. Management for moderate or severe lactic acidosis (lactate >5 mmol/l, with bicarbonate <15-20) typically consists of hospital admission, and maintaining adequate hydration and bicarbonate administration. Patients may also need respiratory support. All antiretroviral treatments should usually be discontinued until lactate levels have returned to normal. Severe NRTI-induced lactic acidosis/hepatic steatosis is a rare event in a centre where there is a high degree of awareness of this condition. A venous lactate concentration that is > 5 mmol/l indicates a state of widespread cellular energy deficit and metabolic acidosis. (Lonergan et al., 2000).

Uncommonly, patients may present with less severe symptoms, hepatic steatosis and only mild to moderate hyperlactatemia and tolerate a revision, rather than cessation, of NRTI. It is not certain if this represent an earlier phase in the natural history of severe lactic acidosis in some patients (Lonergan, 2000). However, the fulminant form may still occur very precipitously, so diagnosis ultimately relies on having a high index of suspicion about suggestive symptoms in any patient taking a NRTI. In contrast, the incidental finding of mild to moderate hyperlactatemia without symptoms in patients taking HAART does not necessarily portend a progression to severe decompensated lactic acidosis. Based on the 516 patient-years of observation in this study, asymptomatic hyperlactatemia is stable (maintained at an average of 1.5-3.5 mmol/l) in the vast majority of patients without revision of therapy. 1.9 Clinical manifestation of ARV side effects (Brinkman, 2000).
Among the side effects include abdominal pain, anorexia/vomiting, hyperventilation, myalgias or laboratory markers suggestive of lactic acidosis hepatic toxicity and lipodystrophy. CD4 percentages show less age variability and are typically used as a measure across different ages. During all periods of childhood, it is preferred to maintain a CD4 percentage $>$25% (JAIDS, 2004).

2.1.11 Clinical and laboratory monitoring

Recommendations

2.1.11.1 CD4 monitoring

- CD4 should be measured at the time of diagnosis of HIV infection, and every 6 months thereafter. Monitor with increasing frequency as CD4 count approaches threshold for starting ART.
- CD4 should be measured prior to initiating ART.
- CD4 should be measured every 6 months after initiating ART.
- Measure CD4 if new clinical staging events develop, including growth faltering and neurodevelopment delay.
- Where capacity for CD4 measurement is limited, target the use of CD4 monitoring to assess the significance of clinical events.

2.1.11.2 Viral load monitoring

- VL determination is desirable, but not essential, prior to initiating ART.
- VL should be assessed to confirm clinical or immunological failure where possible, prior to switching treatment regimen.
2.1.11.3 Baseline hemoglobin level (and white cell count, if available) should be assessed at initiation of ART.

- For infants and children, measure hemoglobin at week 8 after initiation of AZT-containing regimens or more frequently if symptoms indicate.
- Growth, development and nutrition should be monitored monthly.
- Laboratory monitoring for toxicity should be symptom directed.

Some of the reasons for monitoring include:

- Early detection of adverse drug events and carrying out appropriate interventions
- Reinforcing adherence and assessment of treatment efficacy.
- Identification and addressing of other needs/factors that augment or hinder efficacy of therapy.
2.11.4 Biochemical reference ranges

Table 1: Biochemical reference ranges

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Ref ranges</th>
<th>units</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT</td>
<td>0-40</td>
<td>U/L</td>
</tr>
<tr>
<td>ALP</td>
<td>54-369</td>
<td>U/L</td>
</tr>
<tr>
<td>AST</td>
<td>0-40</td>
<td>U/L</td>
</tr>
<tr>
<td>Calcium</td>
<td>2.02-2.60</td>
<td>Mmol/l</td>
</tr>
<tr>
<td>Glucose</td>
<td>3.5-7.00</td>
<td>Mmol/l</td>
</tr>
<tr>
<td>Lactate</td>
<td>0.5-2.30</td>
<td>Mmol/l</td>
</tr>
<tr>
<td>Phosphorous</td>
<td>0.81-2.26</td>
<td>Mmol/l</td>
</tr>
</tbody>
</table>

A reference range is a set of values used by a health professional to interpret a set of medical tests results. The range is usually defined as the set of values 95% of the normal population falls within. The reference range will vary, depending on the age, sex and race of a population, and even the instruments the laboratory uses to perform the tests. Furthermore, by definition, 5% of the normal population will fall outside the reference range.
CHAPTER THREE
MATERIALS AND METHODS

3.1 Experimental design
This was a prospective study undertaken at Gertrude’s Children’s Hospital (GCH), Nairobi environs.

3.2 Participants
The population consisted of Human immunodeficient (HIV) Positive confirmed Children on antiretroviral drugs or naïve. These children aged between 1 to 15 years old. All these children had known CD4/CD8 and viral load copies. These children came from different parts of Nairobi and its surroundings and those who gave written informed consent (Appendix 1) for this study were interviewed through a questionnaire (Appendix 2). Those who did not meet the inclusion criteria were excluded from the study.

3.2.1 Ethical consideration
Ethical approval was obtained from Gertrude’s Children’s Hospital Ethical and Scientific Review Committee (Appendix 3).

3.2.2 Inclusion and exclusion criteria
Highly immunodeficient virus (HIV) positive children males and female between 1 and 15 years were included in the study. HIV serum samples from children who suffered chronic illness e.g. those on anti- tuberculosis drugs  diabetes mellitus, chronic renal failure were excluded from the study. Also excluded were children whose parents did not consent to participate in the study.
Initial screening for anti HIV-1 antibody had been conducted before recruitment using Genetic Systems rLAV ELISA (BioRad Laboratories, Redmond, WA). Reactive samples were repeated in duplicates using Vironostika HIV-I Microelisa Systems (Organon Teknika, Durham, North Carolina). Samples repeatedly reactive by both ELISAs were tested using Genetic Systems Confirmatory Assay 3.0 (BioRad Laboratories, Redmond, WA).

3.3 Specimen collection

Blood from suitable HIV positive children was the specimen of choice and collection was done during the day between the months of Jan and May 2007. Four milliliters of blood was allowed to flow into the syringe using a scalp vein. Once the specimens were acquired, they were labeled with the patient demographics and study numbers and taken to the laboratory.

3.4 Specimen Transportation

Specimens were transported from the comprehensive care centre to the processing laboratory in cool boxes within one hour.

3.5 Specimen Processing and Storage

Once clotted, the blood specimens were centrifuged at 3000 revolutions per minute for five minutes and serum separated immediately into labeled cryovials in duplicate. Serum specimens were then stored at -20°C awaiting laboratory analysis at GCH clinical laboratory.
3.6 Laboratory analysis
Seven biochemical parameters were determined on the sera specimens: Lactate, Glucose, calcium, inorganic phosphorus, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase. All the assays were performed based on the standard operating procedures (SOPs) written and maintained in the GCH laboratory.

3.7 Equipment for analysis
Aleyon 300i automatic Chemistry Analyzer (Abbott Diagnostics, Mannheim, Germany) was used.

3.8 Reagents preparation
The reagents were commercially prepared and specifically tailored for Alcyon 300i plus analyzer. The reagents were supplied in liquid form in reagent bottles. All tests used the same reagent bottles. The reagent bottles labels were colour-coded for ease of identification, and bar-coded for fast, accurate and secure entry of reagent data, such as the number of tests, lot number, and expiration date. Each reagent was placed in reagent boats and placed on marked racks and automatically checked for correct filling once placed on the instrument.

3.9 Sample handling
Sera specimens were thawed at room temperature then placed in labeled Alcyon sample cups then placed in sample racks.

3.10 Operation of machine
To run tests on samples, orders were created each with unique order identification. The orders specified which tests were to be run, study number and demographics then saved. The machine was programmed on Auto Start and the system started processing as soon as a sample rack was inserted. The instrument carried out all test orders automatically and produced the results.
3.11 Calibration of tests
Calibrator for automated systems (C.f.a.s) was used. C.f.a.s is a lyophilized calibrator supplied in bottles. Before use, calibrator bottle was carefully opened and exactly 3 ml distilled water pipetted carefully into the bottle, closed, and carefully dissolved by gentle swirling within 30 minutes. This was then aliquoted into six cryovials and stored at -20°C. Calibrator used the same types of tubes and racks as samples. A refrigerated rack position in the machine improved the stability of on-board calibrators. The system performed calibrations automatically.

3.12 Quality control (QC)
Humatrol N (normal upper) and Humatrol P (pathological upper) for all the parameters all from Human Diagnostics were the quality control materials used during the study period. Before use, a QC bottle was carefully opened and exactly 5 ml distilled water pipetted carefully into the bottle, closed, and carefully dissolved by gentle swirling within 30 minutes. This was then aliquoted into six cryovials and stored at -20°C. Quality control used the same types of tubes and racks as samples. A refrigerated rack position in the machine improved the stability of on-board controls. The system performed controls automatically according to the specifications in the test definition.

3.13 Analytical Methods
3.13.1 Lactate
Lactate reagent was used to measure lactate concentration by timed end point reaction. In the reaction, the oxygen supply is insufficient, typically during intense muscular activity and energy is released through anaerobic respiration. Anaerobic respiration converts pyruvate to lactate by
lactate dehydrogenase. The fermentation regenerates NAD\(^+\) maintaining the NAD\(^+\) concentration so that additional glycolysis reactions can occur. The fermentation step oxidizes the NADH produced by glycolysis back to NAD\(^+\) transferring two electrons from NADH to reduce pyruvate into lactate. The concentration of NADH formed was proportional to the lactate concentration and was determined for 2 minutes by measuring increase in absorbance at 340 nm at 37\(^\circ\)C.

**Test principle**

\[ \text{L-Lactate + NAD} \xrightarrow{LD} \text{Pyruvate + NADH + H}^+ \]

### 3.13.2 GLUC

Glucose reagent was used to measure GLUC concentration by timed end point reaction. In the reaction, hexokinase (HK) catalyzed the phosphorylation of glucose by ATP to form glucose-6-phosphate and ADP. Glucose-6-phosphate dehydrogenase (G6PDH) catalyzed the oxidation of glucose-6-phosphate by NADP\(^+\) to form NADPH. The auto analyzer proportioned 2 u/l sample and 150 u/l GLUC reagent into the reaction cuvette. The concentration of NADPH formed was proportional to the glucose concentration at and was determined for 2 minutes by measuring increase in absorbance at 340 nm at 37\(^\circ\)C.

**Test principle**

\[ \text{D-Glucose + ATP} \xrightarrow{HK} \text{D-Glucose-6-phosphate + ADP} \]

\[ \text{D-Glucose-6-phosphate + NADP}^+ \xrightarrow{G6PDH} \text{D-6-Phosphogluconate + NADPH + H}^+ \]
3.13.3 CA

Calcium reagent was used to measure CA concentration by timed end point reaction. In the reaction, calcium ions (\(\text{Ca}^{2+}\)) reacted with O-cresolphthalein complexone (CPC) under alkaline conditions to form a violet colored complex. Addition of 8-hydroxyquinoline prevented interference by magnesium and iron. The autoanalyzer proportioned 3 u/l sample and 220 u/l CA reagent into the reaction cuvette. The colour intensity of the complex formed was directly proportional to \(\text{Ca}^{2+}\) concentration and was determined by measuring the increase in absorbance at 552nm for 2 minutes at 37°C. The system automatically calculated the CA activity and expressed it in mmol/L.

- Test principle

\[
\text{Ca}^{2+} + \text{CPC} \xrightarrow{\text{pH 10.7}} \text{Calcium-o-CPC complex}
\]

3.13.4 PHOS

Phosphorous reagent was used to measure PHOS concentration by timed end point reaction. In the reaction, inorganic phosphorous reacted with ammonium molybdate in an acid medium to form phosphomolybdate complex. The auto analyzer proportioned 2.5 ml sample and 128 ml PHOS reagent into the reaction cuvette. The concentration of phosphomolybdate was directly proportional to the inorganic phosphate concentration. It was determined for 2 minutes by measuring the increase in absorbance at 340nm at 37°C. The system automatically calculated the PHOS activity and expressed it in mmol/L.

- Test principle
Phosphate + Ammonium molybdate $\overset{H_2SO_4}{\longrightarrow}$ Ammonium-phosphomolybdate.

3.13.5 ALP

Alkaline phophatase reagent was used to measure ALP activity by kinetic method. Phosphatase cleaved p-nitrophenyl phosphate in the presence of magnesium and zinc ions, into phosphate and p-nitrophenol. The autoanalyzer automatically proportion 2.75 $\mu$l sample and 82 $\mu$l ALP reagent into cuvette. The p-nitrophenol released was proportional to the ALP activity measured at 450 nm for 3 minutes at 37°C. The analyzer automatically calculated the ALP activity and expressed it in U/L.

- Test principle

p-Nitrophenyl phosphate $+ H_2O \xrightarrow{ALP} \text{Phosphate} + \text{p-Nitrophenol}$

3.13.6 ALT

Alanine aminotransferase reagent was used to measure ALT activity by enzymatic rate method. In the reaction, ALT catalyzed the reaction between L-alanine and 2-oxoglutarate. The pyruvate formed was reduced by nicotinamide adenine dinucleotide (NADH) in a reaction catalyzed by Lactate dehydrogenase (LDH) to form L-lactate and nicotinamide adenine dinucleotide (NAD$^+$). The rate of NADH oxidation was directly proportional to the ALT activity. Cobas Integra® 400 plus analyzer automatically aliquoted 10 $\mu$l sample and 250 $\mu$l ALT reagent into the cuvette. The system determined ALT activity by measuring the decrease in absorbance at 340nm for 3 minutes at 37°C and automatically calculated the ALT activity and expressed it in U/L.

- Test principle
L-Alanine + α-Ketoglutarate + L-Alanine $\xrightarrow{ALT}$ Pyruvate + L-Glutamate

Pyruvate + NADH + H$^+$ $\xrightarrow{LDH}$ L-Lactate + NAD$^+$

3.13.7 AST

Aspartate aminotransferase reagent was used to measure AST activity by enzymatic rate method. In the reaction, AST catalyzed the reaction between L-aspartate and 2-oxoglutarate to form oxaloacetate and L-glutamate. The oxaloacetate formed then reacted with nicotinamide adenine dinucleotide (NADH) in the presence of malate dehydrogenase (MDH) to form L-malate and nicotinamide adenine dinucleotide (NAD$^+$). The rate of NADH oxidation was directly proportional to the activity of AST. Cobas Integra 400 plus analyzer automatically aliquotted 11 ml sample and 95 ml AST reagent into the cuvette. The system determined AST activity by measuring the decrease in absorbance at 340nm at 37°C for 3 minutes. The analyzer automatically calculated the AST activity and expressed it in U/L.

- Test principle

L-Aspartate + α-Ketoglutarate $\xrightarrow{AST}$ Oxaloacetate + Glutamate

Oxaloacetate + NADH + H$^+$ $\xrightarrow{MDH}$ L-Malate + NAD$^+$

3.13.8 ALP

Alkaline phosphatase reagent was used to measure ALP activity by kinetic method. Phosphatase cleaved p-nitrophenyl phosphate in the presence of magnesium and zinc ions, into phosphate and p-nitrophenol. The autoanalyzer automatically proportion 2.75 ml sample and 82 ml ALP reagent into cuvette. The p-nitrophenol released was proportional to the ALP activity measured at 450
nm for 3 minutes at 37°C. The analyzer automatically calculated the ALP activity and expressed it in U/L

- Test principle

\[
p\text{- Nitrophenyl phosphate } + \text{H}_2\text{O} \xrightarrow{\text{ALP}} \text{Phosphate } + \text{p-Nitrophenol}
\]

3.14 Data management and analysis

Data were double entered into a Microsoft Excel database, compared, and corrected for data entry errors then imported into Statistical Package for Social Sciences (SPSS).

3.14.1 Sample size

This being a prospective cross-sectional study with a one-sample situation, the appropriate formula for sample size calculation was based on Fisher’s, (1980) equation.

\[
n = z^2 \times (p \times (1-p))^{2}
\]

Where,

- \(n\) = the minimum sample size,
- \(z\) = 1.96 at 95% confidence level
- \(P\) = estimate rate from other studies (anticipated population proportion due to ART biochemical changes).

3.14.2 Treatment of outlying observations

The data was visually inspected for extreme values and one value for single parameters that appeared abnormally elevated removed.
3.14.3 Statistics

Statistical Package for Social Sciences (SPSS) was used. Non-parametrically, reference ranges, means and medians were directly obtained from the analyzed data separately for both males and females at 95% reference range (2.5th and 97.5th percentiles) and 90% confidence limit (5.0th and 95th percentiles) but 95% confidence limit was reported because it is the most recommended (NCCLS 2000). P-values for the difference between male and female participants were estimated using the Mann-Whitney test where \( p < 0.05 \) were considered significantly different.
CHAPTER FOUR

RESULTS

3.1 Demographic characteristics of the study population

Table 1 represents demographic characteristic of the 133 children involved in this study at Gertrude’s Children’s Hospital Comprehensive Care Centre. From January 2007 to May 2007, 150 children were enrolled at the Gertrude’s comprehensive care centre. All were approached to enroll in the study; parents of 7 children declined to give consent while parents of 10 patients did not have complete baseline tests at the time of recruitment. One hundred and thirty three patients who met the study criteria were therefore enrolled; seventy nine were males and fifty four were females giving a male to female ratio of 1.5:1. Eighty one patients were on antiretroviral therapy, majority receiving WHO/CDC recommended first line regimen (Table 1).

Table 2: Distribution of study population by Gender and ARV status (N=133)

<table>
<thead>
<tr>
<th></th>
<th>ARV NAIVE</th>
<th></th>
<th>ON ARV TREATMENT</th>
<th></th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NO (%)</td>
<td>NO (%)</td>
<td></td>
<td>NO (%)</td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>29 (36.6)</td>
<td>50 (63.3)</td>
<td></td>
<td>79 (100)</td>
<td></td>
</tr>
<tr>
<td>Females</td>
<td>23 (42.6)</td>
<td>37 (57.4)</td>
<td></td>
<td>54 (100)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>52 (39.1)</td>
<td>81 (60.9)</td>
<td></td>
<td>133 (100)</td>
<td></td>
</tr>
</tbody>
</table>

A total of 79 (59.3%) males and 54 (40.7%) females were included in this study. Majority age range of the ART clients and the naive in the study site was between 3-6 years. The mean age of the children was 5.9 years (range 6-12 years). A greater proportion of patients aged 3-6 years were ARV naive compared to those from the other age categories. Similarly, there were more patients who were in the category of 12 years of age on ARV treatment compared to those who were ARV naive (8.6% vs. 1.9%). There were more children on antiretroviral therapy in this study than the ARV naive patients (P<0.05).
Fig 3.1: Age category and ARV status

Bar chart showing the distribution of age categories (0-3 years, 3-6 years, 6-9 years, 9-12 years, >12 years) for NAİVE and ON ARV statuses.
3.2 The effects of Septrin on immunological, virological and biochemical parameters on ARV naïve subjects

To investigate the effect of Septrin on immunological, virological and biochemical parameters, two groups of study subjects were considered: one consisting of untreated HIV children and the other consisting of HIV children treated with Septrin (Table 2). Results show that HIV children treated with Septrin had significantly increased levels of glucose and the log viral load and a reduced activity of AST and ALT compared to the HIV untreated children.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>ARV Naïve (N=27)</th>
<th>ARV Naïve Plus Septrin (N=96)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>4.90±1.80</td>
<td>4.96±1.04*</td>
</tr>
<tr>
<td>ALT</td>
<td>41.54±45.80</td>
<td>24.28±16.04*</td>
</tr>
<tr>
<td>AST</td>
<td>51.08±33.72</td>
<td>37.23±17.85*</td>
</tr>
<tr>
<td>ALP</td>
<td>238.85±107.76</td>
<td>265.65±120.32</td>
</tr>
<tr>
<td>PHOS(^{2+})</td>
<td>1.71±0.61</td>
<td>1.67±0.42</td>
</tr>
<tr>
<td>CA(^{2+})</td>
<td>2.19±0.42</td>
<td>2.19±0.33</td>
</tr>
<tr>
<td>Lactate</td>
<td>2.18±0.91</td>
<td>1.70±0.73</td>
</tr>
<tr>
<td>CD4/CD8 %</td>
<td>23.71±10.37</td>
<td>17.09±8.27</td>
</tr>
<tr>
<td>Log Viral Load</td>
<td>8.19±4.56</td>
<td>9.46±3.31*</td>
</tr>
</tbody>
</table>

Results are expressed as Mean±SD of number of subjects shown in parenthesis. *p < 0.05 represents statistically significant values by T test.

3.3 Effects of HAART administration on the Immunological, virological and biochemical parameters of HIV infected Children

To investigate the effect of HAART on immunological, virological and biochemical parameters, two groups of study subjects were considered: one consisting of untreated HIV children and the other consisting of HIV children treated with HAART (Table 3). Results show that HIV children treated with HAART had significantly reduced activity of AST and ALT compared to the HIV untreated children.
Table 4: Immunological, virological, and biochemical profiles by ARV treatment Status

<table>
<thead>
<tr>
<th></th>
<th>ARV Naive (N=52)</th>
<th>On ARV Treatment (N=81)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>4.94±1.43</td>
<td>4.91±1.08</td>
</tr>
<tr>
<td>ALT</td>
<td>35.98±35.78</td>
<td>22.31±13.98*</td>
</tr>
<tr>
<td>AST</td>
<td>46.20±26.84</td>
<td>35.91±18.26*</td>
</tr>
<tr>
<td>ALP</td>
<td>233.37±103.81</td>
<td>278.32±121.25</td>
</tr>
<tr>
<td>PHOS²⁻</td>
<td>1.72±0.49</td>
<td>1.64±0.44</td>
</tr>
<tr>
<td>CA²⁺</td>
<td>2.22±0.33</td>
<td>2.17±0.36</td>
</tr>
<tr>
<td>Lactate</td>
<td>1.93±0.84</td>
<td>1.69±0.78</td>
</tr>
<tr>
<td>CD4/CD8 %</td>
<td>21.62±9.27</td>
<td>15.74±8.31</td>
</tr>
<tr>
<td>Log viral load</td>
<td>8.90±3.91</td>
<td>9.72±3.39</td>
</tr>
</tbody>
</table>

Results are expressed as Mean±SD of number of subjects shown in parenthesis. *p < 0.05 represents statistically significant values by T test.

3.4 The effect of age on immunological, virological and biochemical parameters of HIV naïve and HIV treated children

To investigate the effect of age on immunological, virological and biochemical parameters of HIV naïve and HIV treated children, both the HAART naïve and HAART treated study subjects were divided into five age categories: 0-3, 3-6, 6-9, 9-12, and over 12 years (Table 4). Results show that: in age category 0-3 years, log viral load was significantly increased in the HAART treated subjects; in age category 3-6 years, ALT was significantly decreased in the HAART treated subjects; in age category 6-9 years, AST and ALT were significantly decreased in the HAART treated subjects; and in age category 9-12 years, AST and ALT were significantly decreased while ALP was significantly increased in HAART treated subjects compared to the HAART naïve subjects. Results also show that for HAART treated subjects: log viral load in age category 3-6 years is significantly higher than age categories 6-9, 9-12 and over 12 years which are similar while age category 0-3 years is significantly higher than all the other age categories.
Table 5: Effect of age on immunological, virological and biochemical parameters of HIV naïve and HIV treated children

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Age</th>
<th>0-3 years</th>
<th>3-6 years</th>
<th>6-9 years</th>
<th>9-12 years</th>
<th>&gt;12 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV Naive</td>
<td>HIV ARV</td>
<td>HIV Naive</td>
<td>HIV ARV</td>
<td>HIV Naive</td>
<td>HIV ARV</td>
<td>HIV Naive</td>
</tr>
<tr>
<td>Viral load</td>
<td>9.40±4.89</td>
<td>11.42±3.09b*</td>
<td>8.47±3.25</td>
<td>10.52±2.80a</td>
<td>9.3±3.87</td>
<td>8.15±3.87</td>
</tr>
<tr>
<td>Biochemical parameters</td>
<td></td>
<td>Glucose (mM)</td>
<td>4.72±1.04</td>
<td>5.20±1.32</td>
<td>4.50±1.09</td>
<td>4.89±0.85</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lactate (mM)</td>
<td>2.40±0.79</td>
<td>1.96±0.82</td>
<td>1.51±0.58</td>
<td>1.70±0.85</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ALT (U/L)</td>
<td>34.23±20.34</td>
<td>30.60±19.06</td>
<td>24.28±19.05</td>
<td>18.72±9.79*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AST (U/L)</td>
<td>56.69±33.68</td>
<td>50.45±27.66</td>
<td>33.50±14.61</td>
<td>32.32±9.74a</td>
</tr>
<tr>
<td>Bone Metabolism</td>
<td></td>
<td>ALP (U/L)</td>
<td>224.38±72.36</td>
<td>240.8±80.90</td>
<td>221.67±103.08</td>
<td>278.44±82.30</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Phos (mM)</td>
<td>1.99±0.69</td>
<td>1.77±0.45</td>
<td>1.65±0.28</td>
<td>1.72±0.59</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ca (mM)</td>
<td>2.29±0.24</td>
<td>2.19±0.5</td>
<td>2.11±0.36</td>
<td>2.24±0.30</td>
</tr>
</tbody>
</table>

Results are expressed as Mean±SD (standard deviation) of the number of subjects shown in parenthesis. Viral load is expressed as log (n+1); n is the number of viral RNA copies. *p < 0.05 represents statistically significant values by T test; Means for subjects followed by similar case letters are not significantly different at p < 0.05 (SNK test).
CHAPTER FOUR
DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

4.1 Discussion

According to the results of this study, the glucose levels were not found to be elevated in both ARV naive and patients on ARVs. Early reports on metabolic effects of ARV treatment indicated that these drugs particularly protease inhibitors were associated with peripheral insulin resistance leading to a diabetic state i.e., hyperglycaemia. This study however, did not evaluate insulin sensitivity which may be a key factor related to class specific protease inhibitors (Carr et al., 1998). The insulin resistance reported in many studies would suggest that with time, the patients develop impaired glucose tolerance or diabetes mellitus. It is known in adults with type 11 diabetes that the affected individuals have insulin resistance for several years before manifesting overt hyperglycaemia (Moyle et al., 1996). A longitudinal study would have been useful to assess hyperglycaemia in the current study.

The possible reasons for the difference observed in this study and those reported from studies involving adults may be due to the fact that only a few of the patients in this study were on protease inhibitors; the duration on therapy with these drugs was variable and children also respond differently to these drugs. This study was also cross sectional; perhaps progressive increase in blood glucose could have been demonstrated had a longitudinal study design been carried out. There was no significant difference in the levels of venous lactate (RVL) between the subjects on ARV and those ARV naive. Like
hyperglycaemia, severe lactic acidosis, though a rare occurrence, is a complication of ARVs and is potentially fatal. It is a result of mitochondrial toxicity induced by ARVs particularly NRTIs. Approximately 13.5% of patients on antiretroviral therapy had lactate levels values above the normal range (> 2.1 mmol/L) while 4.9% had lactate levels values > 3.0 mmol/L. The rise in lactate may be observed in physiological circumstances and thus the contribution of ARV requires exclusion of these factors. These results are in agreement with the study by Didier (2005) whereby the overall frequency of lactate levels was estimated at 13% and did not vary according to the type of ARV exposure.

These results contradict those from a controlled study of 349 adult subjects of the western Australian HIV cohort (Lonergan et al., 2000) where 2 patients with severe lactic acidosis (plasma lactate > 5 mmol/l) and 5 with lactate values ranging from 2.8-4.1 mmol/l were observed. Similarly, Nogwera (2004) reported more than half of HIV exposed children on ARV to have elevated lactate. The predominant risk factor for developing chronic hyperlactatemia in this study was the use of stavudine compared to zidovudine as found in three separate analyses and after adjustment for the potential confounding effect of duration of past and total NRTI exposure. Indeed change from stavudine to zidovudine in 5 patients with moderate hyperlactatemia was reported to have prevented them from progressing to a state of fulminant lactic acidosis (Brinkman et al., 2000). This discrepancy could be explained by a longer exposure to NRTI drugs both for prenatal and post natal periods. However, the subjects in this study had been treated longer with NRTI. This may indicate that asymptomatic hyperlactetimia alone may not be enough to warrant revision of therapy in any patient since RVL values have been shown not to be
predictive for the development of lactic acidosis which has led to no recommendation for routine lactate monitoring. Although NRTI-induced lactate acidosis is a rare event a longitudinal study may have been useful to rule out occurrence of the same in this study.

All classes of ARV have been associated with asymptomatic elevations of alanine/aspartate aminotransferases levels and infrequently with serious life threatening clinical liver hepatotoxicity. In this study 1.2% and 3.8% of children on ART and the naive ones respectively had elevated liver enzymes. Among those on HAART 1.2% was in the nevirapine treatment and none was on efavirenz. All these patients had CD4 cell counts > 350 cells/μl. Hepatotoxicity resulting from nevirapine use has an early onset, with the greatest risk occurring within the first two to six weeks of therapy which therefore makes it difficult to predict serious drug related hepatic events in isolation of other parameters not measured in this study. This is in agreement with the findings of Dietrich (2004) where 10% and 17% of children on HAART and those naïve respectively had severe liver damage. Severe hepatic toxicity has rarely been reported and even more rarely resulted in treatment discontinuation. Liver enzyme elevations are common in HIV-infected adult patients, especially those treated with HAART from all classes of ARV drugs: protease inhibitors (PIs), nucleoside reverse transcriptase inhibitors (NRTIs) and non nucleoside reverse transcriptase inhibitors (NNRTIs) (Kontorinis et al., 2003).

In a study by Dietrich et al. (2004) on HIV infected patients, severe hepatotoxicity was not found on nevirapine and efavirenz after starting treatment. This is similar to findings
where no association of severe hepatotoxicity was found using the same drugs in children. Further study is necessary to determine long term effects of HAART in relation to the effects in the liver.

In this study the serum markers did not show any changes in the children under ART therapy for up to 60 months. This is in contrast to other studies on bone metabolism in HIV patients using radiological indices (DEXA) which reported osteopenia and osteoporosis in adult patients (Tebas et al., 2000). These differences could be explained by the fact that in growing children, bone formation exceeds bone resorption, resulting in increased bone mass.

There was no significant difference in the CD4 cell distribution among the patients on ARV as compared to non-treated infected group. In addition, this study found that children not on ARV had lower baseline CD4 counts whereas children on treatment had an increased CD4 counts after the initiation of HAART. It has been noted that younger children produce more CD4 cells in absolute numbers, they require relatively more CD4 cells to catch up and normalize their CD counts, these explains the margin between the ARV treated patients and the ARV naïve ones (Rompalo et al., 1999). It seems therefore that administration of HAART had a remarkable effect on immune reconstitution regardless of the high viral load observed. This is in agreement with the study by Rossum et al. (2000) where immune reconstitution in children was seen to be independent of age.
The observed viral load differences in the treated and non-treated groups were more pronounced in the antiretroviral treated patients than the naïve subjects. This difference was statistically significant (p<0.05). When Log viral load copies were stratified by age, levels were significantly higher in children less than 3 years on ARV with log 9.40±4.89 for the naïve subjects and log 11.42±3.09 for the subjects on treatment consecutively (p<0.05).

Septrin (Co-trimoxazole) prophylaxis for opportunistic infections (OIs) is essential elements for ART according to WHO guidelines. Several reports have confirmed the efficacy of OIs’ drugs in the prevention and clinical management of HIV patients (Chintu, 2004). The results of this study showed that a total of 96 (72%) patients were on Septrin. Glucose, ALT and AST was improved in the groups on Septrin and across, the viral load count range was found to be higher among the Septrin treated subjects than in the Septrin naïve category. The results are very similar in magnitude to those in HIV-infected adults with WHO stage 2 or 3 participating in trials in West Africa (Wiktor, 1999). As in those trials, co-trimoxazole seemed to be well tolerated in this study with no alteration in the biochemical, immunological and virological profiles (p>0.05). There was increase on viral load counts in patients treated with Septrin, as expected; ALT and AST counts were lower in the co-trimoxazole group than in the naïve group. There was no follow-up time in this study as in the West African group (Anglaret, 1999) which provides reassurance that giving the drug over an extended period for prophylaxis, as would be necessary if initiated early in the course of HIV infection, seems not to decrease its effectiveness, at least over an average of 18 months. One might expect to see benefit
confined to children with lower CD4 count, we did not evaluate or record this magnitude of benefit in children with CD4 counts above and below 15% of total lymphocyte count.
4.2 CONCLUSIONS
The conclusions of these study findings are:

- Based on the results of this study the CD4 count was found to be significantly increased whereas viral load was reduced.

- The evaluation of biochemical profile found ALT and AST to be significantly reduced in patients who were on HAART treatment. HIV-1 appears to be sensitive to first line drugs used at Gertrude’s Children’s Hospital facility.

- This study adopts the alternate hypothesis that indicated that HIV and HAART are associated with changes in serum biochemical parameters in children.
4.3 RECOMMENDATIONS FOR FURTHER WORK

- Although the study demonstrated positive trends among the ARV treated subjects, a longitudinal study is recommended to unmask possible metabolic derangements.

- Further studies require to be done including the non HIV infected subjects to assess whether the treatment returns the measured parameters to normal.

- Though CD4 cell count remains the current biomarker to ARV treatment prognosis, biochemical parameters associated with ART beneficial effects can serve as additional markers for optimal adherence to ARV therapy and should be exploited.
REFERENCES


Church, J. (2000). Near-Fatal Metabolic Acidosis, Liver Failure in Mitochondrial DNA Depletion in an HIV-infected Child Treated with Combination ARV Therapy. Abstract 58. 7th CROI.


APPENDIX I: CONSENT EXPLANATION FORM

My name is Peninah N. Gatuthu a student at Kenyatta University. I am carrying out a study on Biochemical evaluation of children with HIV and ready for the initiation of ARV therapy. A follow-up will also be done at 4-8 weeks, 3 and 6 months with blood collection for tests of the liver, pancreas and the kidney. I am kindly requesting you to participate in the study, which will also help your Doctor to further manage you. If you agree to enter into the study, the following will be expected of you:

(1) Sign a consent form
(2) Sign an assent form
(3) Answer a questionnaire
(4) The tests will be free of charge
(5) I will collect blood from you but there are no risks involved in the entire procedure only mild pain, which I will minimize.

All information about you will be treated in the strictest confidence and will be given to your Doctor to further manage you.

You are free to withdraw to join this research study at any time in which case please rest assured that the care you receive will not be interfered with the least.

Thank you.
APPENDIX 11: CONSENT FORM

I, ......... .......... ......... ......... ......... ......... ......... ......... ........., am a parent \ legal guardian of ......... .......... ......... ......... ......... ......... ......... ......... ........., I hereby freely consent for my child to participate in this research study which has been explained to me. It is understood that participation or otherwise in this study will not adversely affect my medical care. I also understand that all information about me shall be treated in the strictest confidence.

Witness

Signed: ............. ............. ............. ............. ............. ............. ............. ............. .............

Parent/Guardian

Witness ............. ............. ............. ............. ............. ............. ............. ............. .............

Dated: ............. ............. ............. ............. ............. ............. ............. ............. .............
APPENDIX 111: ASSENT FORM

I... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...

Agree to participate in this study which has been explained to my parents/guardian and me. It is Understood that participation or otherwise in this study will not adversely affect my medical care. I also understand that all information about myself will be treated in the strictest confidence.

Signed: ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...

Self

Witness ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...

Dated: ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...

Figure 3.4 Immunosuppressed Patients and the Threshold to CD4 Count

In the absence of a CD4 count, a total lymphocyte count can be substituted. A count of <3500/mcL for children 1-15 years old and <3500/mcL for children 1-15 months to 6 years; <1200/mcL for children 6 months to 15 years; and <700/mcL for children with immunosuppression, especially when HIV-related symptoms
## Appendix IV: Immunological Classification Based on Total and % CD4 Count

<table>
<thead>
<tr>
<th>Immunological Category</th>
<th>CD4/ul (%) &lt; 12 months</th>
<th>CD4/ul (%) 1-5 years</th>
<th>CD4/ul (%) 6-12 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>No evidence of suppression</td>
<td>≥1500 (≥25)</td>
<td>≥1000 (≥25)</td>
<td>≥500 (≥25)</td>
</tr>
<tr>
<td>Evidence moderate suppression</td>
<td>750-1499 (15-24)</td>
<td>500-999 (15-24)</td>
<td>200-499 (15-24)</td>
</tr>
<tr>
<td>Severe suppression</td>
<td>&lt;750 (&lt;15)</td>
<td>&lt;500 (&lt;15)</td>
<td>&lt;200 (15)</td>
</tr>
</tbody>
</table>

**Figure 3.4 Immunological Classification Based on Total and % CD4 Count**

In the absence of a CD4 count, a total lymphocyte count (TLC) can be substituted. A TLC of <3500/mm³ for children <18 months, <230/mm³ for children 18 months to 6 years, or <1200/mm³ for children over 6 years is indicative of immunosuppression, especially when HIV-related symptoms are present (CDC).
### Appendix V: Longevity of drug combination

<table>
<thead>
<tr>
<th>Final Combinations</th>
<th>Treatment duration (in months)</th>
<th>Treatment in Months</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Range</th>
<th>Mean</th>
<th>Median</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>1-20</td>
<td>21-40</td>
<td>41-60</td>
<td>61+</td>
<td>months</td>
<td>months</td>
</tr>
<tr>
<td>Not on ARV</td>
<td>52</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2NRTIs + PI-</td>
<td>0</td>
<td>6</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>3</td>
<td>52</td>
</tr>
<tr>
<td>2NRTIs + NNRT</td>
<td>1</td>
<td>55</td>
<td>6</td>
<td>5</td>
<td>3</td>
<td>0</td>
<td>121</td>
</tr>
<tr>
<td>3 NRTIs</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td>53</td>
<td>62</td>
<td>6</td>
<td>9</td>
<td>3</td>
<td>0</td>
<td>121</td>
</tr>
</tbody>
</table>

**Figure 3.5 The Longevity of drug classification**

From June 2006 to November 2006, 133 patients were enrolled: 79 males and 54 females. Their mean age was 6 years 9 months (range: 2 months - 15 years). It can be noted that children who are less than 1 year recorded the highest Viral load with a mean of 1.39 million copies and also showed the highest moderate evidence of suppression. The superior antiviral activity of NRTI + NNRTI HAART over PI-HAART was consistently demonstrated in all treatment duration. The modal group was 1-20 months.
Appendix VI: Laboratory Specimen Analysis

Photo 1

Photo 2
Watching is Dr J.N. Ngeranwa and Dr. A. Amayo.

Photo 1: The analyst, Peninah N. Gatuthu and the supervisor Dr J.N Ngeranwa looking on at the sample preparation on Alcyon biochemistry Analyzer.

Photo 2: The analyst, Peninah N. Chege and the Dr Amayo clinical Chemistry pathologist at Gertrude’s Children’s Hospital Laboratory preparing the equipment for analysis. Dr J.N Ngeranwa the supervisor is looking on.