PREVALENCE OF IIYPERLACTATEMIA IN CHILDREN W ITH HIV/A IDS OP NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITOR IiASEI) HK.HI.Y ACTIVE ANTIRETROVIRAL THERAPV AT THE KENVATTA NATIONAL HOSPITAL

DISSERTATION PRESENTED IN PART FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF MEDICINE IN PAEDIATRICS AND CHILD HEALTH, UNIVERSITY 0 NAIROBI.

BY

DR. PETER KIPKURUI MASHEP

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SUPERVISORS:

PROF. ELIZABETH MALECHE OBIMBO

M.B.Ch.B, MMED (Paediatrics and Child Health), MPH (Epidemiology),

Senior Lecturer in the Department of Paediatrics and Child Health, University of Nairobi

DR. AHMED M. R. LAVING

M.B.Ch.B, MMED (Paediatrics and Child Health), Gastroenterologist,

Lecturer in the Department of Paediatrics and Child Health, University of Nairobi.

DEPARTMENT OF PAEDIATRICS AND CHILD HE ALTH, UNIVERSITY OF NAIR(

DECLARATION

This dissertation is my original work and has not been presented for a degree in any university or published anywhere.

DR. PETER KIPKURUI MASHEP

M.B.Ch.B

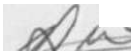
Signature: O fAltjoiU^ Date Z d s k o K

This dissertation is submitted to the University of Nairobi with our approval as supervisors.

PROF. ELIZABETH MALECHE OBIMBO

M.B.Ch.B, MMED, MPH (Epidemiology), Senior Lecturer

Department of Paediatrics and Child Health, University of Nairobi.

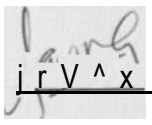


Signature:

DR. LAVING M. R. AHMED

M.B.Ch.B, MMED, Gastroenterologist, Lecturer

Department of Paediatrics and Child Health, University of Nairobi.

Signature:  jrV^x Date_ S

DEDICATION

I dedicate this work to my loving and supportive wife Alexandria and our children Hannah and Matthew for their inspiration, love, patience, and support.

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LIST OF ABBREVIATIONS (Alphabetically)

AIDS	Acquired Immune Deficiency Syndrome
ART	Anti Retroviral Therapy
ARV	Anti Retroviral Drugs
CCC	Comprehensive Care Center
HAART	Highly Active Anti-Retroviral Therapy
HIV	Human Immunodeficiency Virus
KNH	Kenyatta National Hospital
MtDNA	Mitochondrial DNA
nDNA	Nuclear DNA
NRTI	Nucleoside Reverse Transcriptase Inhibitor
NVP	Nevirapine
PMTCT	Prevention of Mother To child Transmission
SPSS	Statistical Package for Social Sciences
WHO	World Health Organization
GFR	Glomerular Filtration Rate

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ABSTRACT

Background: Children with HIV/AIDS live longer and healthier lives because of treatment advances such as highly active antiretroviral therapy (HAART) and treatment of opportunistic infections. In Kenya the national guidelines recommend the use of regimens based on a backbone of two nucleoside reverse transcriptase inhibitors (NRTI). Elevated lactate levels and life threatening hepatic dysfunction have been described in children and adults on HAART regimen containing NRTIs but few studies are available that evaluate lactate levels among African children. The final common pathway for most of these toxicities is thought to be through mitochondrial damage leading to impairment of the aerobic respiration leading to accumulation of lactic acid the dead end product of anaerobic respiration resulting in hyperlactatemia, life threatening lactic acidosis and hepatic steatosis in these individuals.

Objectives:

The primary objective was to determine the prevalence of hyperlactatemia among children on treatment with NRTI based HAART regimen attending the comprehensive care centre clinic at Kenyatta National Hospital. The secondary objective was to describe the factors associated with hyperlactatemia.

Study design:

This was a hospital based descriptive cross sectional study during the year 2010.

Methods:

Blood samples were drawn from these patients and analyzed for L-lactate. Treatment history and baseline data were corroborated from the patients' records. The primary objective was achieved by calculating the overall and age-specific prevalence. The secondary objective was achieved by analysis of the correlates for hyperlactatemia by description and comparison of the characteristics of patients with and without hyperlactatemia, with the aid of software programs (Epi-Info and SPSS). P-value of less than 0.05 was considered significant. Results were presented in tables, charts (pie), and graphs as appropriate.

Results:

A total of 271 children were recruited into the study. The overall prevalence of hyperlactatemia was found to be 11.8%. There were significant differences in the age specific prevalence. The highest prevalence was in the children of the 6-<10 years age group at 17.9% (OR 5.8; 95% CI 1.7 - 20.9), children aged 2-<6 years had a prevalence of 13.2% (OR 4.2; 95% CI 1.2 - 15.7) compared to children >10 years who had a prevalence of 3.6%. 89.9% of the children with hyperlactatemia had mildly elevated levels.

No significant correlation was found between hyperlactatemia with other factors explored.

Conclusion:

The prevalence of hyperlactatemia among children on NRTI-based HAART at Kenyatta National Hospital Comprehensive Care Centre is comparable to that found in other similar studies but children under ten years had the greatest prevalence and a 4.2 - 5.8 fold increased risk for hyperlactatemia. Most of the children with hyperlactatemia had a mild elevation of the lactic acid levels.

Recommendation: There is need to evaluate lactic acid levels at baseline for children on HAART and at three months follow-up visits for those children with increased levels to determine the trend especially those below ten years of age.

1. INTRODUCTION

The advent of highly active antiretroviral therapy (HAART) has had a significant impact in reducing morbidity and mortality among HIV infected children and has remarkably contributed to the observed decrease in infant and under-five mortality rates in Kenya [1] and worldwide. These children now live longer and healthier lives [2-5]. However as a result of the increasing experience, and the prolonged and intensified use of these therapies, related adverse effects are being recognized and are rapidly emerging as an important challenge to the management of these patients. Hyperlactatemia, which is one of the commoner toxicities, is thought to result from mitochondrial injury associated with the use of nucleoside reverse transcriptase inhibitors (NRTIs) and has been found to occur among children and adults on regimens containing NRTIs Few studies on lactic acid levels and its impact on the general health and growth of children have been done among the African paediatric population [6]. The importance of this study is underlined by the fact that NRTIs form a backbone for both first and second line highly active antiretroviral therapy (HAART) regimens used in sub-Saharan Africa. This study determined the prevalence, and explored the associated factors, and clinical features of hyperlactatemia among children receiving HAART at the comprehensive care clinic (CCC) in Kenyatta National Hospital (KNH).

2. BACKGROUND AND LITERATURE REVIEW

2.1 Normal Glucose Metabolism

The mitochondrion is the powerhouse of the cell and plays a vital role in the production of energy for normal cellular processes as well as in glucose and free fatty acid (FFA) metabolism. Physiologically during glycolysis, glucose is metabolized to pyruvate through a series of enzyme mediated reactions that occur in the cytoplasm resulting in the production of a small amount of ATP. Pyruvate is then taken up by mitochondria and metabolized to acetyl coenzyme A (Acetyl coA) a substrate for the Krebs cycle in which oxidative phosphorylation occurs. Free fatty acids are also converted to acetyl coA to enter the Krebs

cycle. Oxidative phosphorylation involves oxidation of NADP to NADPH (reduced and oxidized nicotine adenine dinucleotide, respectively) through an electron transfer process that is tightly coupled with phosphorylation of ADP resulting in the generation of most of the ATP molecules that are yielded from each quantum of glucose and free fatty acid. This process is catalyzed by mitochondrial cytochrome enzymes in the double membrane of the mitochondria and requires oxygen (Fig 1) <16). Under anaerobic conditions or in the presence of mitochondrial enzyme dysfunction or depletion, the metabolism of pyruvate to acetyl coA is inhibited. Pyruvate in these circumstances is then metabolized to lactic acid in cytoplasm by lactate dehydrogenase and is associated with significant reduction in energy production, accumulation of lactic acid, and ketogenesis from accumulating free fatty acids. Fig 1 <16)

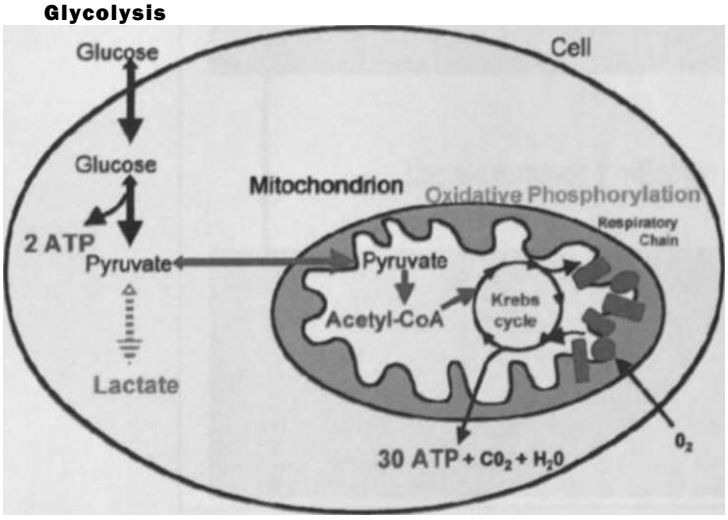

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Figure 1: Schematic representation of glycolysis and oxidative phosphorylation in the presence and absence of mitochondrial toxicity. ATP, adenosine triphosphate; CoA, coenzyme A. (16>

The normal basal turnover of lactate in the body is about 1mEq/minute (15- 20 mEq/kg/day) and most tissues are net producers of lactate. The main net consumers of lactate are the liver and the renal cortex which contribute 50% and 20% to the clearance of lactate in the body respectively through gluconeogenesis the remainder being cleared by other tissues such as myocardium. Fig 2 <[17];[18]>.

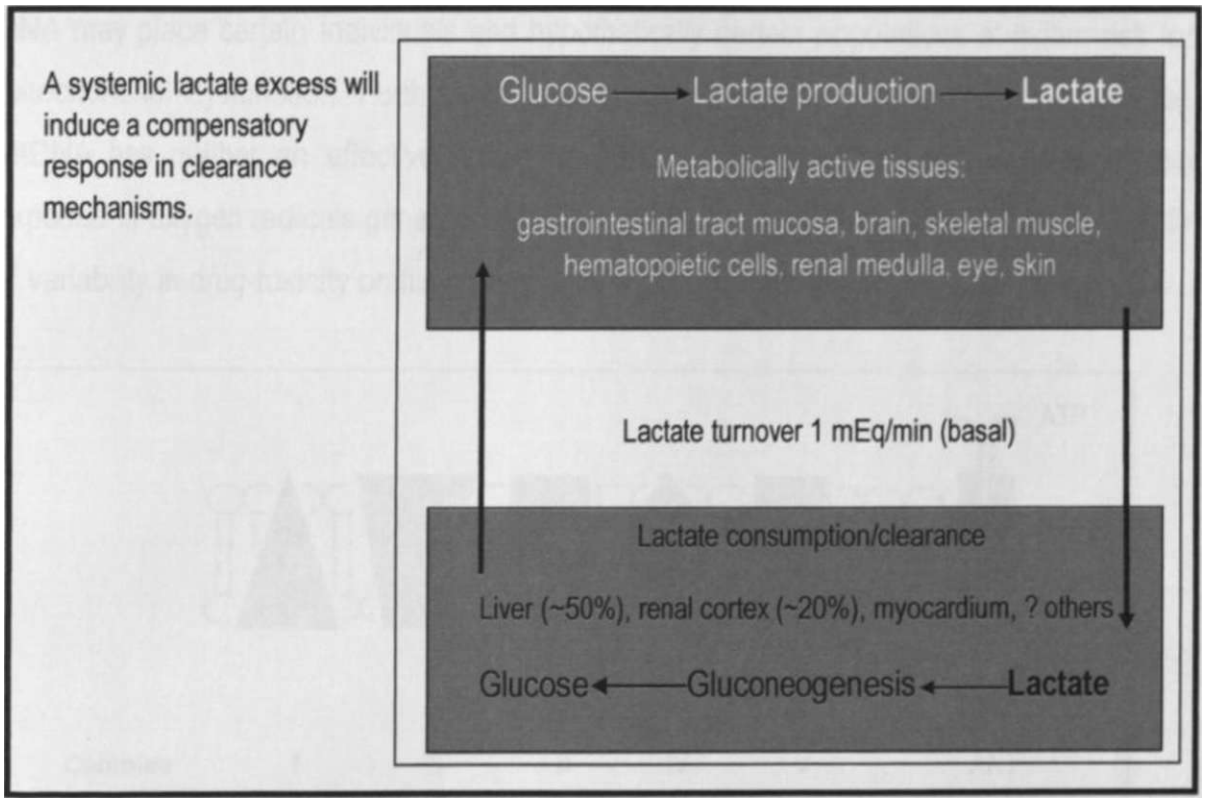
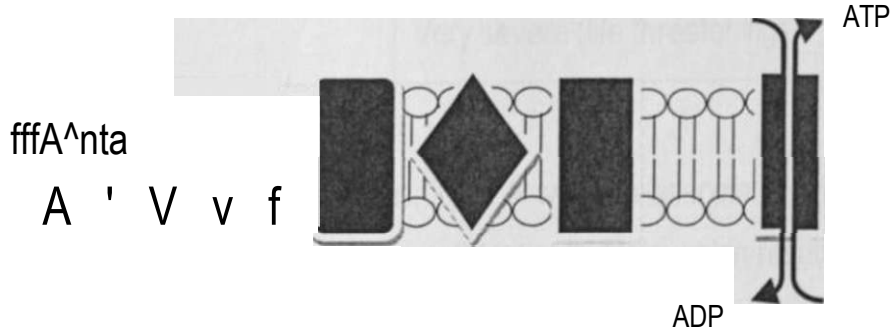


Figure 2: Homeostatic Regulation of Systemic Lactate. ([17];[18]>

2.2 Normal Mitochondrial Function

Five DNA polymerases annotated alpha to epsilon have been characterized in eukaryotic cells and serve different functions in DNA replication. DNA polymerase gamma enzyme is the only one involved in Mitochondrial DNA (MtDNA) replication. The mitochondrial enzymes are composed of five complexes annotated I - V and an adenine nucleotide translocator (ANT) each consisting of a variable number of subunits, thirteen of which are

encoded by MtDNA <[19]). Both nuclear DNA (nDNA) and M1DNA however contribute to formation of most subunits in each mitochondrial enzyme complex therefore inhibition of DNA polymerase-y affects most of the enzyme complexes (except complex II). fig 3 <[19]). MtDNA is maternally inherited and its replication rate is independent of that of nDNA occurring throughout the cell cycle even in the quiescent non-dividing cells. Basal MtDNA turnover varies in response to the energy demand of the cell or stress. There is also significant polymorphism in the MtDNA in the general population and some forms of the DNA may place certain individuals and hypothetical^ certain populations at higher risk for mitochondrial dysfunction. Furthermore, mutations and defects can easily occur because MtDNA has neither an effective repair mechanism nor protective histones, and is also exposed to oxygen radicals generated by the respiratory chain. This indicates the possibility of variability in drug-toxicity profile among different age groups (?:[8]).



Comolex	I			IV		ANT
Subunit	43		4	1	12	
MtDNA	7	1	0	3	2	
nDNA	36	9	4	10	12	

Figure 3: Diagram of the oxidative phosphorylation system, containing four respiratory chain complexes (I-IV), F1-F0 ATP synthetase (complex V) plus the adenine nucleotide translocator (ANT), located in the mitochondrial inner membrane. Each complex is composed of several subunits, each of which is encoded by either nuclear or mitochondrial DNA (nDNA or MtDNA). Adapted from Wallace <[19:20]>.

2.3 Definition, Classification, and Clinical Presentation of Hyperlactatemia

Lactic acidosis is defined as a plasma lactate level £ 5 mmol/L accompanied by pH < 7.35 in venous blood. It is almost always associated with hepatic steatosis and high mortality <21>. Hyperlactatemia is defined as plasma lactate £ 2.0 mmol/L and below 5mmol/L f22). Severity is classified as shown in the table 1 below:

Table 1: Grades of Severity of Hyperlactatemia <10,23)

Venous lactate (mmol/L)	**Interpretation**
0.9-2.0	Normal
2.1-3.5	Mild elevation
3.6- 5.0	Moderate elevation
5.1 - 9.9	Severe elevation
>10	Very severe (life threatening)

Lactic acidosis can be classified into two types; Type A which is associated with conditions resulting in impaired tissue oxygenation and type B lactic acidosis which results from those not associated with impaired tissue oxygenation <18>.

Type A lactic acidosis is the most common type and results from conditions such as hypo-perfusion in shock (secondary to hypo-volemia, septic shock, or cardiac insufficiency). Concurrent respiratory acidosis in these patients may contribute to the academia. Patients with type B lactic acidosis usually have no manifestations of systemic hypo-perfusion. Mechanisms involved in type B include toxin-induced impairment of cellular metabolism or regional areas of ischaemia. NRTI induced lactic acidosis falls in this group.

Patients with hyperlactatemia may present clinically in many varied ways depending on the tissues most affected by the impairment of oxidative - phosphorylation and are summarized in table 2 below:

Table 2: Clinical Manifestations of Oxidative Phosphorylation Disorders <[19'2427]).

Disorder	**Clinical Manifestations**
Neurological	Peripheral neuropathy, encephalopathy, dementia, seizures, stroke
Myopathy	Hypotonia, muscle weakness, exercise intolerance
Cardiac	Cardiomyopathy, conduction disorders
Endocrine	Diabetes Mellitus
Gastrointestinal	Colonic pseudo-obstruction, exocrine pancreas dysfunction, pancreatitis, hepatomegaly, steatosis, liver failure, lactic acidosis
Nephrological	Non-selective proximal tubular dysfunction with academia, phosphaturia and glucosuria, glomerulopathy
Hematological	Anaemia, thrombocytopenia, pancytopenia
Psychiatric	Depression
General	Multiple systemic lipomas, fatigue

2.4 Mechanisms of Anti-Retroviral Drug Associated Hyperlactatemia

Among the various classes of antiretroviral drugs currently available for treatment of HIV/AIDS infected children, only the NRTI class has been demonstrated to be associated significantly with hyperlactatemia. Other reverse transcriptase inhibitors (RTI) are also used as HIV inhibitors, including non-nucleoside analogues (NNRTI) such as nevirapine, delavirdine, efavirenz, and nucleotide analogues such as tenofovir and adefovir [9-(2-phosphomethoxyethyl) adenine (PMEA)]. Although data on the affinity for human DNA polymerases are less abundant for these compounds, it appears that non-nucleoside analogues do not interfere with any of these polymerases, whereas the currently available nucleotide analogues appear to have a strong affinity for DNA polymerases p and y in particular but no data of *in-vitro* mitochondrial toxicity have been reported to date for them. **(19; 28)**

NRTIs are phosphorylated successively by cellular kinases in various tissues resulting in triphosphate forms that are substrates of the viral DNA polymerase (reverse transcriptase enzyme) which is their primary target. The phosphorylated active forms of the NRTIs are 2',3'-dideoxy-nucleoside triphosphate analogues (ddNTP) of naturally occurring deoxynucleoside triphosphates (dNTP) but lack the 3'-hydroxyl group required for DNA chain elongation. When these analogues are incorporated into elongating viral DNA they either cause premature termination of the chain (dideoxy-sequencing) or formation of non-functional viral DNA thus inhibiting viral replication. These triphosphates are also substrates of the human DNA polymerase-y and cause inhibition of MtDNA replication through chain termination or formation of untranslatable MtDNA. This leads to depletion of MtDNA, reduction in production of respiratory chain enzymes, and consequent reduction in aerobic respiratory reserve of cells and consequently lactic acid accumulation. <19>

Mitochondrial toxicity has been suggested as the final common pathway for a variety of NRTI related adverse effects (19'.Toxicity related to nucleoside analogues was first observed

in muscle tissues of patients receiving zidovudine (AZT) OT. Different tissues are thought to have different threshold expressions for toxicity dependent on the ATP requirements of the tissue. Threshold expression is the minimum level of ATP production required to sustain normal cellular functions. However, any body system can be affected to varying degrees. Patients may present with variable patterns of hyperlactatemia including chronic compensated hyperlactatemia, intermittent hyperlactatemia, symptomatic decompensated hyperlactatemia, or rarely decompensated life threatening severe lactic acidosis <[19]).

The factors affecting the extent to which tissues are affected by NRTIs have been described by the so called 'phosphorylase y - hypothesis' which states that the toxicity of antiretroviral nucleoside analogues depends on (i) the sub-cellular availability and abundance of the antiretroviral nucleoside analogues in the target tissue; (ii) the ability of cellular nucleoside kinases to use the antiretroviral nucleoside analogue as a competitive alternative substrate resulting in monophosphorylation and later ^phosphorylation of the antiretroviral nucleoside analogues; (iii) the ability of the triphosphate of the antiretroviral nucleoside analogue to inhibit DNA polymerase-y either by serving as a competitive (ineffective) alternative substrate or by chain termination of the nascent MtDNA strand (non-competitive); and (iv) the metabolic reliance on oxidative phosphorylation in the target tissues. <[19; 2°]) In one In-vitro study the NRTIs were shown to have the following order of decreasing mitochondrial toxicity: zalcitabine (ddC), stavudine (d4T), zidovudine (ZDV) and then didanosine (ddl). Abacavir has minimal association with mitochondrial toxicity'[30]).

One factor thought to predispose patients to hyperlactatemia is prolonged use of NRTIs and this is well recognized especially in adult patients receiving HAART containing NRTIs. Mina J et al (2001) in a large prospective longitudinal study demonstrated that lactate levels rose and persisted at elevated levels in patients after initiation of therapy^[12]'. (Fig 4).

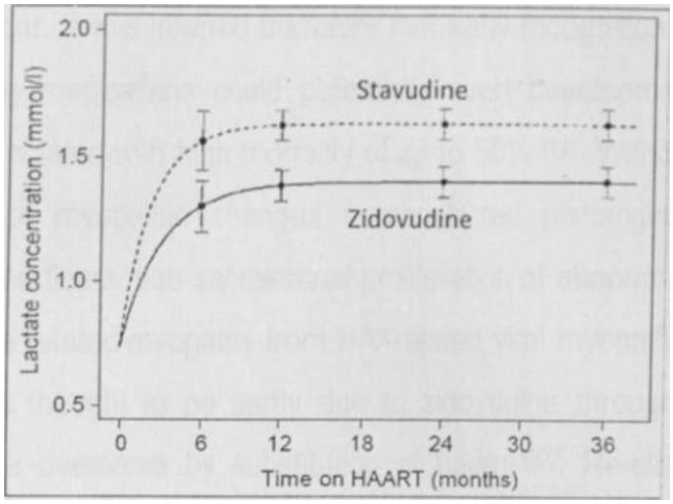


Figure 4: Rise in population average venous lactate concentrations after start of highly active antiretroviral therapy (HAART) ([12]).

The number of NRTIs that a patient is exposed to may also predispose to development of hyperlactatemia. Work done by Lonergan J et al (2001) revealed that the risk of hyperlactatemia increases with each additional NRTI used and that regimen containing stavudine bore highest incidence of lactic acidosis. The incidence rate of symptomatic hyperlactatemia among adults taking NRTIs increased about two fold for each additional NRTI in the regimen and the risk is lower in regimen without stavudine in both dual and triple therapy regimen. Abdominal pain, abdominal bloating, nausea, fatigue and increased alanine transaminase (ALT) in association with elevated lactic acid were the case defining symptoms [13].

Some toxicities attributable to NRTIs are reversible when the offending drug is withdrawn for example return of previously elevated lactate levels to normal ranges. In an earlier study. Lonergan J et al (2000) described a possible syndrome of early symptomatic hyperlactatemia in which patients presented with abdominal symptoms, increased ALT and lactate levels below 5 mmol/L. In these patients, there was no associated mortality and symptoms resolved on withdrawal of ART and on substitution with alternative regimen

symptoms did not recur. It was inferred therefore that early recognition and intervention by withdrawing offending medications could potentially avert development of severe lactic acidosis which is associated with high mortality of up to 50% <[14]). Withdrawal of zidovudine results in reversal of myopathic changes demonstrated histologically by substantial reduction in rugged-red fibers (sub-sacrolemnal proliferation of abnormal mitochondria) that distinguish zidovudine related myopathy from HIV-related viral myopathy <[31]). Bone marrow toxicity which is also thought to be partly due to zidovudine through inhibition of DNA polymerase y can be overcome by substitution of haem <[32]). Re-challenge with NRTIs different from the offending ones has not been shown to result in recurrence of hyperlactatemia in some cases <[14]>.

2.5 Epidemiology of Hyperlactatemia in HIV Infected Children

An extensive review of literature indicates that data on hyperlactatemia in African children with HIV on highly active antiretroviral therapy (HAART) is lacking. Available studies among children in USA and Europe indicate that hyperlactatemia occurs in high prevalence among patients on HAART. (Summarized in table 3 below.)

In a study in USA, Desai et al (2003) reported a prevalence of 32% hyperlactatemia among 127 children between ages 1 and 17 years receiving HAART in the Brooklyn pediatric AIDS network <[6]>. Elevated lactate levels were associated with use of nucleoside reverse transcriptase inhibitors (NRTI) and protease inhibitor (PI) therapy. However, stavudine and ritonavir alone or in combination and non-nucleoside reverse transcriptase inhibitors (NNRTI) were reportedly not associated with elevated lactate levels.

Table 3: Summary of Previous Studies on Prevalence of Hyperlactatemia.

STUDY	STUDY DESIGN	COUNTRY	SAMPLE	INCLUSION CRITERIA	RESULTS
Desai et al (2002) (6)	Cross sectional	USA (Brooklyn)	127 children (1-17 years)	HIV positive on HAART	Hyperlactatemia Prevalence 32%
Noguera et al (2003)ro	Prospective cohort study	Spain	80 children (9mo-17years)	HIV positive on various PMTCT regimen	Incidence of hyperlactatemia was 29% on at least one occasion
Rhoads et al (2006) (8)	Retrospective longitudinal study	G. Britain	146 children (<16 years)	HIV positive on HAART	Age <3 years associated with hyperlactatemia
Ekouevi et al (2003) 0>	Prospective cohort study	Ivory coast	338 infants	Babies on PMTCT	Hyperlactatemia Prevalence 14.3%
Baubaker Ketal (1999) <0>	Cross sectional	Switzerland	880 adult patients	Swiss Cohort study Patients >16 yrs	Prevalence 8.3%

In a longitudinal study by Noguera et al (2003) conducted in Spain of 80 HIV-infected children of between 9 months to 17 years of age receiving antiretroviral therapy (ART), 29% of the children were reported to have hyperlactatemia on at least one occasion during the period of follow up ro.The incidence was 8.7 per 100 patient-years. However, the study did not investigate the relationship between the risks of hyperlactatemia with each drug Younger age at start of ART and a higher CD4 count were significantly associated with hyperlactatemia. It was however underpowered to conclusively establish the relationship between hyperlactatemia and each antiretroviral drug.

Similarly, in a study investigating the changes in metabolites including lactic acid in children on antiretroviral therapy by Rhoads et al (2006) conducted in Great Britain of 146 children, found higher median levels in children <3 years of age on combination ART than those £3 years of age on combination ART and higher than children not on combination ART in any age group [8]. The study however was not designed to study the relationship between each regimen and elevated lactate levels. Adult studies indicate the range of prevalence of hyperlactatemia to be 8-35% [11].

One study was done in Africa by Ekouevi et al (2006) on lactate levels of neonates exposed to antiretroviral drugs through prevention of mother to child transmission programs (PMTCT). Babies exposed to single dose nevirapine (NVPsd) as well as those whose mothers received various regimens for PMTCT (ZDV only or ZDV+3TC from 32 wks gestation, together with intrapartum NVP & baby got NVPsd) had comparable prevalence of hyperlactatemia 13.1% and 14.3% respectively. Although none of the children had symptomatic hyperlactatemia or other signs of mitochondrial toxicity some children were noted to have persistently elevated lactate levels up to six months of follow up [9].

Although studies among adults reveal that stavudine is associated with elevated lactic acid levels [10-13], Desai et al observed that stavudine and ritonavir alone or in combination were not associated with elevated lactate levels in children [6]. This observation, although not reported in the other paediatric studies done in Spain (*) and Great Britain, W could be a reflection that first, there is an age related MtDNA polymorphism accounting for the observed variation in effects of these drugs on MtDNA in paediatric and adult patients. Secondly, it could reflect the regional polymorphism in MtDNA of the different populations of children studied in USA and Europe. Thirdly the study may have been underpowered to conclusively examine this question due to the small sample size relative to those among adult patients.

2.6 Other Causes of Hyperlactatemia

Drugs other than NRTIs that have been reported to cause hyperlactatemia include biguanides (currently metformin and phenformin in the past) used in type 2 diabetics. The incidence of hyperlactatemia in patients on biguanides is however is very low J[33]).

Malignancy in rare occasions is also associated with lactic acidosis especially leukemia, lymphoma, and solid malignancies but the pathogenesis is unclear. Anaerobic metabolism due to dense clusters of tumor cells and/or metastatic replacement of the hepatic parenchyma has been proposed, although lactic acidosis has occurred in patients with relatively small tumor burdens. Increased lactate production by the neoplastic cells has also been suggested, but this would not explain the rarity of tumor-induced lactic acidosis Thiamine or riboflavin deficiency and altered Insulin growth factor (IGF and IGF-Binding Protein) have also been suggested in some of these cases. Regardless of the mechanism, removal of the tumor (by chemotherapy, irradiation, or surgery) leads to correction of the acidosis i[34]).

A mild degree of lactic acidosis may be observed with alcoholism in which lactate production is usually normal but lactate utilization is diminished because of impaired hepatic gluconeogenesis. Although lactate levels generally do not exceed 3 mEq/L in these patients, alcohol ingestion can potentiate the severity of other disorders that are associated with the overproduction of lactate f[35]'.

D-lactic acidosis, a unique form of lactic acidosis can occur in patients with jejuno-ileal bypass or, less commonly, small bowel resection or other cause of the short bowel syndrome. In these settings, glucose and starch are metabolized in the colon into D-lactic acid, which is then absorbed into the systemic circulation. The ensuing acidaemia tends to persist, since D-lactate is not recognized by L-lactate dehydrogenase, the enzyme that catalyzes the conversion of the physiologically occurring L-lactate into pyruvate I[36]'.

Inherited mitochondrial disorders cause either deficiency or impaired function of mitochondrial enzymes leading to lactic acidosis. Other risk factors associated with hyperlactatemia or severe lactic acidosis are unknown although HIV infection has independently been associated with mitochondrial necrosis. Obesity and female gender are also thought to predispose to hyperlactatemia <[19'28])

2.7 Determination of Mitochondrial Toxicity and Hyperlactatemia

Currently there is no widely available and cost effective single test that has been shown to give accurate prediction of, or to measure the mitochondrial toxicity due to NRTIs. Cote et al (2002) demonstrated that mitochondrial DNA to nuclear DNA ratio (MtDNA: nDNA) is reduced in muscle and liver biopsy specimen of patients who present with symptoms of hyperlactatemia and is currently the gold standard ([37]). However, routine biopsy may not be practical and DNA tests not widely available or cost effective for this to be useful for monitoring these patients especially in resource limited settings like the sub-Saharan Africa.

Montaner J et al (2004) demonstrated that symptomatic hyperlactatemia correlated well with reduced MtDNA and therefore, serum lactate could be a useful marker for mitochondrial toxicities <[16]>. Some studies also demonstrate that clinically important hyperlactatemia can be picked through routine measurement of plasma lactic acid levels which impact on patient's quality of life, adherence to therapy, and ability to maintain effective long-term antiretroviral therapy [38]).

The measurement of lactate levels is a delicate and technical process. Accurate measurements may theoretically be affected by the patient's level of physical activity, however, rest for at least thirty minutes, avoiding arm exercise prior to sample collection, and application of tourniquet for less than 2 minutes have been shown to have negligible effects on plasma lactate levels <[39; 40]>. Sodium fluoride is a useful and effective inhibitor of glycolysis for preserving blood specimen for lactic acid determination, and has been shown

to have a sustained efficacy for up to 72 hours. Separation of red ceils from plasma by centrifuging the blood samples within 15 minutes of collection further helps to prevent a potential initial increase of lactic acid in the sample after collection caused by anaerobic respiration of red blood cells since the maximal inhibitory effect of sodium fluoride is achieved after one hour of incubation of blood samples <39>.

3. STUDY RATIONALE

Almost all HIV infected children in Sub-Saharan Africa are on treatment with NRTI based regimens which are associated with hyperlactatemia, a known debilitating and potentially fatal adverse effect of this class of drugs. There is paucity of information regarding the prevalence of hyperlactatemia, the associated factors, and how it presents among African children. Lactate levels are not routinely monitored in most setups and late recognition of this condition exposes children to severe morbidity and risk of death.

It is therefore important to determine the magnitude of hyperlactatemia among children infected with HIV on treatment with nucleoside analogue based highly active antiretroviral drugs. This information would inform guidelines on the monitoring of HIV infected children on HAART containing nucleoside analogues.

4. RESEARCH QUESTION

What is the prevalence of hyperlactatemia among HIV infected children on nucleoside reverse transcriptase inhibitor-based highly active antiretroviral therapy at the Kenyatta National Hospital?

5. STUDY OBJECTIVES

5.1 Primary Objective

To determine the prevalence of hyperlactatemia among HIV infected children receiving nucleoside reverse transcriptase inhibitor based highly active antiretroviral therapy enrolled for care at the Kenyatta National Hospital.

5.2 Secondary Objective:

To describe and compare the socio-demographic and clinical characteristics of HIV infected children on treatment with nucleoside reverse transcriptase inhibitor based highly active antiretroviral therapy with and without hyperlactatemia. These include: socio-demographic; baseline clinical, immunologic and virologic status; nutritional status; treatment regime, duration, and dose; other drugs/supplements taken and presenting symptoms and signs.

6. METHODOLOGY

6.1 Study Design

This was a descriptive cross-sectional study during the year 2010 including the school holidays so that children from boarding schools could be included.

6.2 Source Population

The sample population was derived from paediatric patients attending the paediatric HIV clinic at Kenyatta National Hospital Comprehensive Care Center. Over 1100 children of age birth to fourteen years attend the clinic regularly and of these 650 are on antiretroviral therapy. A few adolescent patients between the age of 14 and 17 years who prefer to continue visiting the paediatric clinic are allowed to continue care. Out of the 650 patients on therapy, about 550 are on first line therapy (AZT+3TC+NVP) while 91 are on alternative therapy (AZT+3TC+Kaletra) due to perinatal nevirapine exposure or nevirapine intolerance. The remaining children are on various other combination therapies due to other factors including treatment failure and toxicity.

6.3 Study Population

The study population included children infected with HIV on treatment with HAART containing NRTIs who attend the KNH CCC paediatric HIV clinic. Patients who were considered for recruitment into the study had to fulfill the following criteria:

- ***Inclusion criteria***
    - HIV infected
    - Must have been on HAART including an NRTI for at least 6 months
    - Age 6 mo to 17 years
    - Must have been on follow-up at the KNH-CCC for at least 6 months
    - Signed informed consent

- ***Exclusion criteria***

  - Children with obvious or known co-morbid conditions that can cause hyperlactatemia such as malignancy.

Only in - patients admitted through this paediatric HIV clinic were eligible for inclusion. It was noted that other HIV positive paediatric patients were admitted through the paediatric filter clinic at this same hospital as emergency cases, during hours when the clinic was closed or as referrals from other HIV care centers for in-patient care but these were excluded to avoid double inclusion and because they were not from the study population.

6.4 Study Site

The study was conducted at the Kenyatta National Hospital (KNH) comprehensive care clinic (CCC) for HIV infected children. KNH is situated in Nairobi the capital city of Kenya and is the largest referral hospital in East and Central Africa. Both the adult and paediatric HIV clinics are co-located in the same building but run separately. Most of the patients who attend the clinic are from the city and its environs although some are referred from other parts of the country. The paediatric clinic runs on weekdays from 8am to 5pm and 20 to 40 patients are seen per day. When patients arrive at the clinic they report to the records office where their record files are retrieved from storage and the patient registered in a clinic attendance register by the records clerks. Generally the clinicians see the patients in the order of registration with exception of very sick children who are triaged and seen without waiting, or those delayed due to other unusual reasons such as a lost file or pending laboratory results. Once seen by a clinician those requiring laboratory investigations are sent to the laboratory for sample collection. Stable patients are reviewed with the results during the next appointment.

During the first year of initiation of HAART the children are reviewed monthly and thereafter stable patients are seen typically on 3 monthly appointments and routine monitoring

laboratory tests done during each of these visits include a full blood count, creatinine level, and alanine transaminase level (AST). CD4 count and percentage are done every 6 months. Viral load is not done routinely due to cost, but a few patients may have it done especially in assessing treatment failure.

6.5 Sample Size Determination

The sample size estimation was based on the primary objective to give adequate power to determine the prevalence of hyperlactatemia in the study population.

Using Fischer's formula <[41]):

$$n = \frac{(Z(i-g/2))^2 x p x (1-p)}{d^2}$$

- n = Minimum sample size
- a = Degree of precision set at ± 5%
- p = Estimated prevalence of hyperlactatemia in HIV infected children on HAART of 32%. Desai et al 2003[16])
- d = significance level set at 5%
- Z (i,/2) = 1.96, critical value at a significance level of 5%.

$n = 1.96^2 \times 0.32 \times 0.68/0.05^2 = 334$

The sample was stratified by age as follows:

Table 4: Age groups

Age	Number
< 2 Years	83
2 - <6 Years	83
6 - 1 0 Years	83
> 10 Years (Adolescents)	83

6.6 Sampling Procedure

Sequential sampling was used to select patients to be considered for recruitment into the study using the clinic attendance register as the sampling frame. The investigator visited the study site from 8am to 5pm and selected the study subjects in the order indicated in the clinic attendance register and assessed their eligibility, obtained their consent for recruitment into the study, interviewed them, examined them and then sent the patients to the laboratory for blood sample collection. This process of selection was repeated every day until the sample size was achieved for each age group. Concerns raised by the patient or issues regarding the visit were dealt with by the investigator in consultation with the clinic physicians to avoid further delaying the patient in the clinic.

7. STUDY PROCEDURE

7.1 General Flow

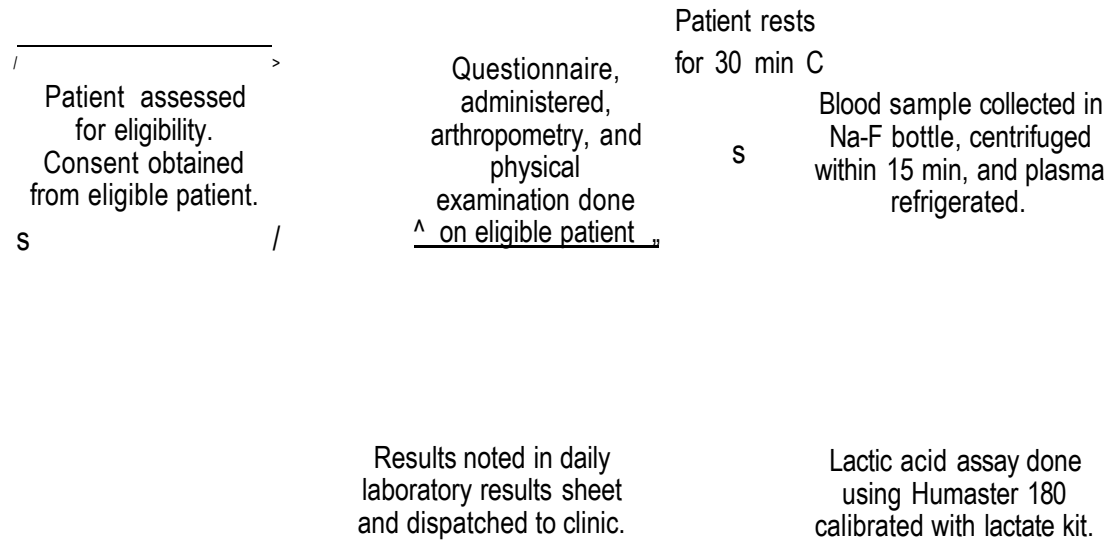


Figure 5: Flow chart of study procedure

7.2 Clinical Procedures

7.2.1 Enrolment

The investigator visited the KNH-CCC paediatric clinic on weekdays from 8 am to 5 pm until the minimum sample size is attained for each age group. The investigator identified HIV infected children who are aged six months to seventeen years. The age was ascertained to **the** nearest month using parent/guardian report, birth certificate or growth chart (the road to Health card). The investigator confirmed whether or not the child was on HAART by checking the child's follow-up notes in the file and collaborating with information from the parent/guardian. He also checked for pre-existing medical conditions that could cause hyperlactatemia that the child is on treatment for. If the child was found to meet the inclusion criteria, assent was sought from children above 7 years where possible in addition to an

informed consent from the parent/guardian after explaining to him/her about the study. Assent was indicated by the child writing his name in annotated area of the assent form (Appendix I) while consent was indicated by the parent/guardian signing the annotated area of the consent form. (Appendix II).

7.2.2 *Interview*

A questionnaire (Appendix III) was then administered and filled by interviewing the caregiver and/or child and filling in all the required information as fully as possible. Data was corroborated from the patient's record file regarding past clinical and laboratory data. These had been structured into the following areas: socio-demographic data; baseline characteristics including WHO staging, CD4 count and percentage, and viral load; treatment history which included initial HAART regimen, dosages, duration, compliance, treatment changes and reason, adverse effects, use of other medications, and presence of any current symptoms or known concurrent illnesses.

7.2.3 *Weight and Height Measurement*

The investigator measured the weight of the child using 25 Kg Salter scale or a calibrated step-on weighing scale for older children and adolescents >25 Kg recording the measurement on the questionnaire to the nearest 0.1 Kg. The Length (for children less than 2 years of age) or Height for children above 2 years of age was measured using a measuring board; the measurement was recorded to the nearest 0.1 cm. All measurements were taken using the WHO measurement standards [42]. (Appendix VI).

7.2.4 Physical Examination

The investigator or his assistant then examined the patient carefully noting all relevant physical examination findings and recorded these in the questionnaire appropriately and accurately.

7.3 Laboratory Procedures

7.3.1 Blood Collection Procedure

Key observations during blood collection included:

1. Sample collection was done at the laboratory for stable patients. Samples from very sick patients were collected at the clinic.
2. 4ml of venous blood was collected into vacutainers containing sodium fluoride (gray corked) once.
3. The patient was allowed to rest for at least thirty minutes before the blood sample is collected. The area for venepuncture was cleaned thoroughly with a spirit swab.
4. Unnecessary tourniquet application was avoided if superficial veins were easily located. Where tourniquet was applied it was done for a maximum of two minutes from application time to sample collection. Forearm or hand exercise during location of blood vessel for venepuncture was avoided. Once free flow of blood was established the tourniquet was released and the blood sample collected.
5. Other samples required for routine tests were then collected after this.
6. Collected sample was mixed with the fluoride by inverting vacutainer and centrifuged within 15 minutes of collection. Plasma collected in the clinic was transported to the lab in ice.
7. Plasma kept at room temperature (15-25 °C) was assayed for lactate levels within 72 hours from collection.
8. Where delay was anticipated plasma was refrigerated at 2 - 8 °C and remained viable for 14 days (kit specification - appendix IX).

7.3.2 Lactic Acid Determination Procedure (Appendix IX)

Collected samples were analyzed at the University of Nairobi Paediatric department laboratory. Plasma lactic acid level was determined using Humaster 180 analyzer which is a fully automated processor. The machine was calibrated using the lactate kit (C.f.a.s. -

Calibrator for automated systems) solution provided by Roche diagnostics. Each blood sample was loaded through a specimen port and the assay runs and results printed automatically.

Expected values for plasma lactate when using this particular kit was 0.5 to 2.2 mmol/L. Lactate levels above 2.2 mmol/L were therefore communicated to the primary clinician as elevated for necessary action.

Standardization: One machine was used to run all the tests. Sample collection was done by only one phlebotomist trained on the required precautions.

Quality assurance: Each day, an internal quality control specimen of known value was analyzed for L-lactate. The value obtained was compared to the known value and a standard Deviation Index (SDI) calculated. According to the manufacturer of the kit, only assays whose values were within 2 SDI were acceptable. The analyzer had a measuring range of 0.22 - 15.5 mmol/L when using the lactate kit. Samples with extremely high lactate levels could give falsely low results in the measuring range, however these were flagged 'LIMT2' to indicate the necessity for a rerun with diluted sample. The laboratory also had external quality assurance scheme with the Human Quality Assessment Services (HUQAS) affiliated to Digital PT of Canada.

Results of the other routine investigations taken at the same time were obtained for correlation later. The laboratory tests conducted routinely included full blood count, CD4 count, liver function and renal function tests. In addition adherence level as evaluated during routine evaluation and viral loads were recorded where available.

8. STUDY DEFINITIONS

8.1 World Health Organization HIV Infection Definition (2007) («>

For children < 18 months HIV infection was defined as a positive virologic test (HIV RNA/DNA PCR) done more than four weeks after birth.

For children > 18 months HIV infection it was defined as a positive antibody test (rapid/ELISA) confirmed by a second HIV antibody test using different antigens or operating characteristics; and/or positive virological test for HIV or its components (HIV-RNA or DNA or ultra sensitive HIV p24 antigen) confirmed by a second virological test obtained from a separate determination. In the study clinic, Determine and Uni-gold for rapid antibody HIV tests and Amplicor RNA/DNA PCR (Roche) were used.

8.2 Hyperlactatemia

Hyperlactatemia was defined as serum lactate level above 2.2 mmol/L as determined by the manufacturer's kit specification. Grading of severity was as shown in table 2.

Table 5: Study Definitions for Interpretation of Lactate Levels.

Interpretation	**Venous lactate (mmol/L)**
Normal	0.5 -2.2*
Mild elevation	2.3-3.5
Moderate elevation	3.6- 5.0
Severe elevation	5.1 - 9.9
Very severe (life threatening)	>10

'Using reference range described by the manufacturer of the lactate kit (Appendix IX).

8.3 WHO Immunologic Staging (2007) («)

The World Health Organization (2007) immunologic staging was used as indicated the table below:

Table 6: WHO Immunological Classification for Established HIV Infection <43).

HIV related immunodeficiency	Age related CD4 values			
	<11 months (%CD_4+)	***12-35 months (%CD 4^+)***	***36-59 months (%CD<+)***	***>5 years*** (absolute number per mm3 or %CD4+)
None/ not significant	>35	>30	>25	>500
Mild	30-35	25-30	20-25	350-499
Advanced	25-29	20-24	15-19	200-349
Severe	<25	<20	<15	< 200 or <15%

8.4 WHO Clinical Staging (2007) <45>

The WHO (2007) clinical staging characteristics for patients with confirmed HIV infection was used as indicated in appendix IV (children) and appendix V (adults and adolescents). For the purposes of HIV case definitions for reporting and surveillance by WHO, children are defined as younger than 15 years of age, and adults and adolescents as 15 years or older.

Table 7: WHO Clinical Staging for Established HIV Infection (2007) (43>

HIV Associated Symptoms	Clinical Stage
Asymptomatic	1
Mild symptoms	2
Advanced symptoms	3
Severe Symptoms	4

9. DATA MANAGEMENT

9.1 Storage

Data collected during the interviews was filled in the questionnaires and laboratory results recorded in the request forms and noted on the laboratory results section of questionnaire by the investigator subsequently. The data was entered and stored in a computer database (Epi-Info). Copies of request forms with recorded results were forwarded to the clinic for filing in the patients' notes and were available for review during the next visit.

9.2 Analysis

Data was collected, continuously cleaned and stored until the sample size was achieved for each age group. Data was then entered into Microsoft Access database and analyzed using Epi-Info nutrition software and SPSS statistical software.

The primary objective of the study was achieved by calculation of the overall prevalence of hyperlactatemia by dividing the number of patients with hyperlactatemia by the total number of patients assessed and expressed as a percentage. Age-group specific prevalence was also determined similarly by dividing the number of children with hyperlactatemia in each age group with the total number of children in the age group.

The secondary objective of the study was achieved by analyzing the correlates of hyperlactatemia. This was done by describing and comparing the socio-demographic (age, gender, ethnic group, and economic status), nutritional status, clinical and immunologic disease stage, treatment variables (each drug and combination regimen of HAART, dose, dose appropriateness, duration of use, compliance, and other concurrent drugs/supplements used), and clinical characteristics (symptoms and signs) of those children with and those without hyperlactatemia. This was done for the overall study population and also for each age-group.

The nutritional status of the children with and without hyperlactatemia was analyzed using Epi-Info 3.2 Nutrition software. The height for age Z-scores (HAZ), weight for age Z-scores (WAZ), and weight for height Z-scores (WHZ) will be computed. The Z-scores represented the number of standard deviations of the measured parameters (HAZ, WAZ, and WHZ) for each child from the mean of a normal population. BMI will be calculated for children two years and above as weight (kg)/height^2 (m^2) and the BMI percentiles for age computed based on the CDC 2000 charts.

Overall and age-group specific proportions of children with hyperlactatemia were determined within the 95% confidence interval, compared, and analyzed for correlation in various categories based on the patient characteristics (age, gender, disease stage, nutritional status, treatment variables, and clinical characteristics). The means with standard deviations and standard errors, and the medians with inter-quartile ranges, were derived for descriptive data and presented in frequency tables, pie charts, graphs, and bar graphs.

Associations between hyperlactatemia and the patients' characteristics and clinical features were tested independently using Chi-square test and the Fisher Exact test where numbers are less than five. A p-value of less than 0.05 was considered significant. Occurrence of hyperlactatemia was modeled using each of the drug/ drug combination (regimen), and clinical features to determine any confounders. Correlates were determined for the whole study population and for various age-groups.

10. ETHICAL CONSIDERATIONS

The study was submitted for approval by the Department of Paediatrics and Child Health of the University of Nairobi and the KNH - UON ethical committee to which it was presented and approval obtained.

Confidentiality of data collected was ensured. Both electronic and hard data were stored and accessed sorely by the principal investigator. It was available to the following other persons when required; the supervisors, the ethics board, and the attending physician accessing the patient notes or by direct notification of results that need urgent intervention.

11. RESULTS

Between August 2010 and December 2010, a total of 303 patients who met the inclusion criteria were recruited into the study. Data from 31 of these patients was excluded from the final analysis due to various reasons. 29 patients were omitted due to failure to turn up for blood sample collection, while 3 patients had some data missing from their files. Therefore, a total of 271 were included in the analysis.

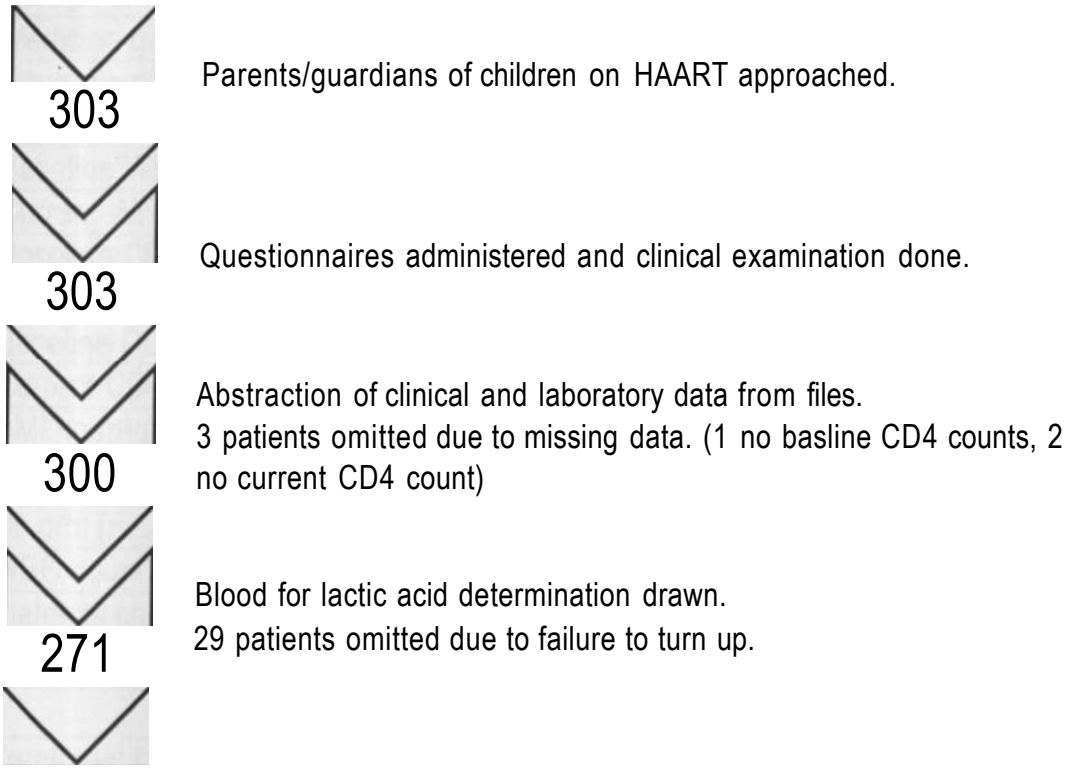


Figure 6: Flow chart showing the selection process of study subjects.

11.1 Characteristics Of Study Subjects

The demographic and clinical characteristics of the study population were as shown in table 8 below.

Table 8: Demographic and Clinical Characteristics of the Study Population (N = 271).

Characteristic	Number (%)
Socio-demographic features	
Age (years) Median (IQR)	7(5-7)
Male	133(49.1)
Mother's community(Kikuyu)	115(42.4)
Caretaker (Parent)	222(81.9)
Medicine giver (Parent/Self)	231(85.2)
Have regular family income	158(58.3)
Disease Stage and Nutritional Status	
Baseline WHO disease stage (1 & 2)	12(4.4)
Current WHO disease stage (1 & 2)	193(71.2)
Baseline CD4 Count cells/ml (median, IQR)	522 (186- 922)
Current CD4 Count cells/ml (median, IQR)	933 (528-1331)
Baseline CD4 %(Median, IQR)	12(6-17)
Current CD4% (Median, IQR)	26(18-32)
BMI kg/m^2 (median, IQR)	16.1(16.08-17.63)
Monitored Laboratory Indices	
Hb g/dl (mean, IQR)	12(11-13)
WBC count x10^6/L (mean, IQR)	6.78 (4.65-8.23)
Platelets count x10^6/L(mean, IQR)	272 (219-350)
LFT ALT U/L (mean, IQR)	38 (21 -40)
Treatment characteristics	
Treatment (1st Line Regimen)	237(87.5)
Treatment duration, months (Mean, IQR)	35 (19-48)
Multivitamin supplements	253(93.3)

11.1.1. Socio-demographic characteristics of the study group.

The mean and median ages for the patients in the study were 8 and 7 years respectively. There were 133 males (49.1%) in the study population therefore the male: female ratio was 1:1. There were very few children below 2 years of age in the study population and only 11 were eligible for recruitment of whom only 4 turned up for blood sampling. The gender composition by age groups is as shown in figure 7 below.

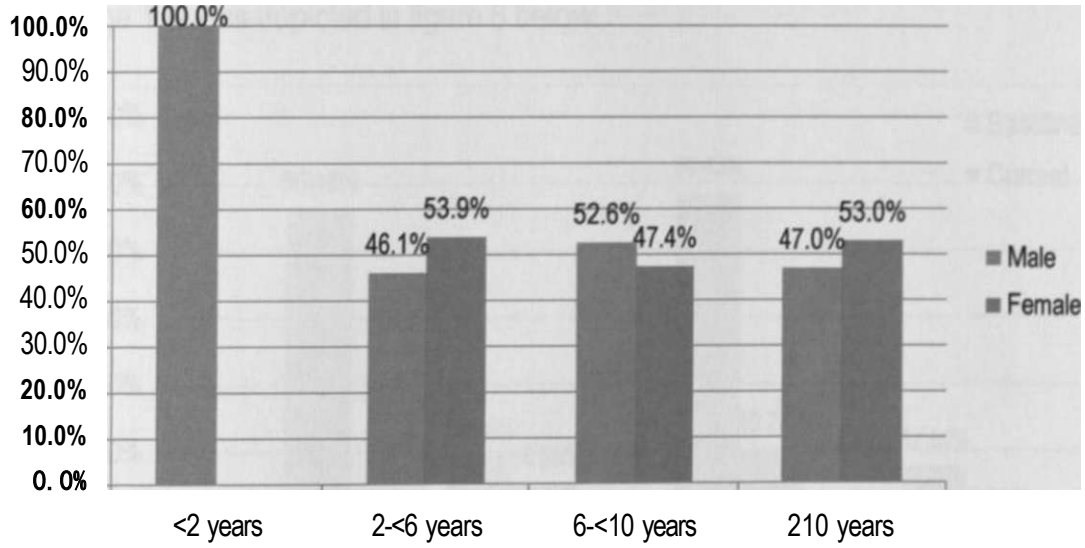


Figure 7: Gender Composition of Study Population by Age Group.

97% of the patients were from families residing in Nairobi and its environs. About 42.4% of the patients had maternal heritage from the Kikuyu community, Luo 22.2%, Kamba 12.2%, and Luhya 8.4% while rest were spread among 38 various other communities in Kenya in smaller proportions of less than 2% each. 81.9% of the patients had at least one parent as the primary caretaker and 85.2% had a parent or themselves (especially children > 10 years) administering the medications to them.

67.9% of primary care-takers had completed primary level of education and 58.3% of the children recruited in the study came from households that had at least one member with a stable monthly income. 51.5% of the families lived in single room houses.

11.1.2. WHO Disease stage and nutritional status

Majority of the children had WHO stage 3 & 4 disease at initiation of therapy (76.0%) and at the point at which the study was conducted 71.2% of the subjects were now in WHO disease stage 1 & 2 as depicted in figure 8 below.

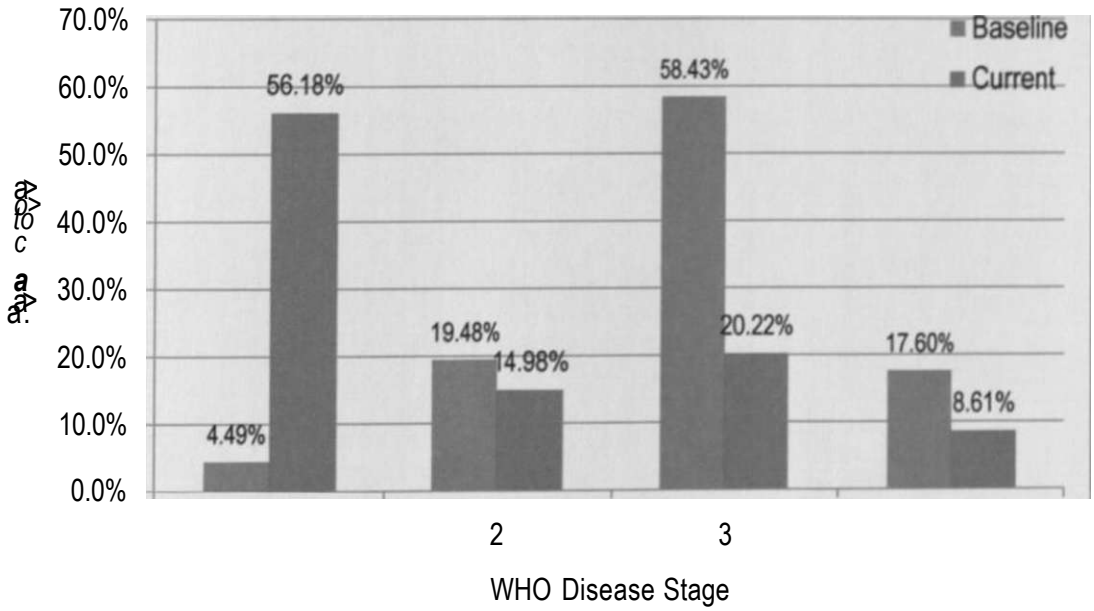


Figure 8: WHO Disease Stages at Baseline and Current.

The mean Body Mass Index for the group was 16.39. However, 94.46% of the patients had Z-score above -3 SD for height-for-age (HAZ) and 96.31% for weight-for-height (WHZ).

11.1.3. Monitored Laboratory Indices

The mean hemoglobin level in the study group was 12 g/dl (IQR 11 - 1 3 g/dl), mean white blood cell count was 12 x 10^6/L with an IQR of 4.65 to 8.23 x 10«/L, and the mean platelet count was 272 x 10^6/L (IQR 219 -350 x 10^5/L). The majority of the patients had indices within normal values. A minority of the patients had viral load determination done at various points in the course of their treatment most being for evaluation of treatment failure.

11.1.4. Treatment characteristics

The HAART regimen taken by the children in the study group are represented in figure 9 below. Most of the children (87.1%) were also still on first line treatment according to the Kenyan National Guidelines for HIV/AIDS treatment and had been on treatment for a mean duration of 35 months. All patients selected for the study had appropriate dosage for each drug.

The NRTI used by most children in the study was Lamivudine (84.5%), followed by zidovudine (56.8%), abacavir (29.8%), stavudine (12.5%), and didanosine (7.7%). Only one patient was on zalcitabine. Figure 9 below.

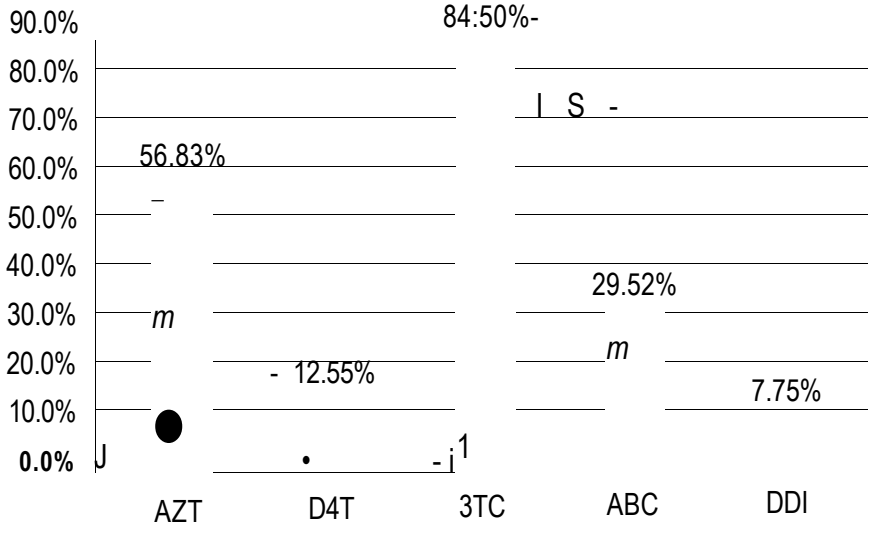


Figure 9: Proportion of Patients on Various NRTIs.

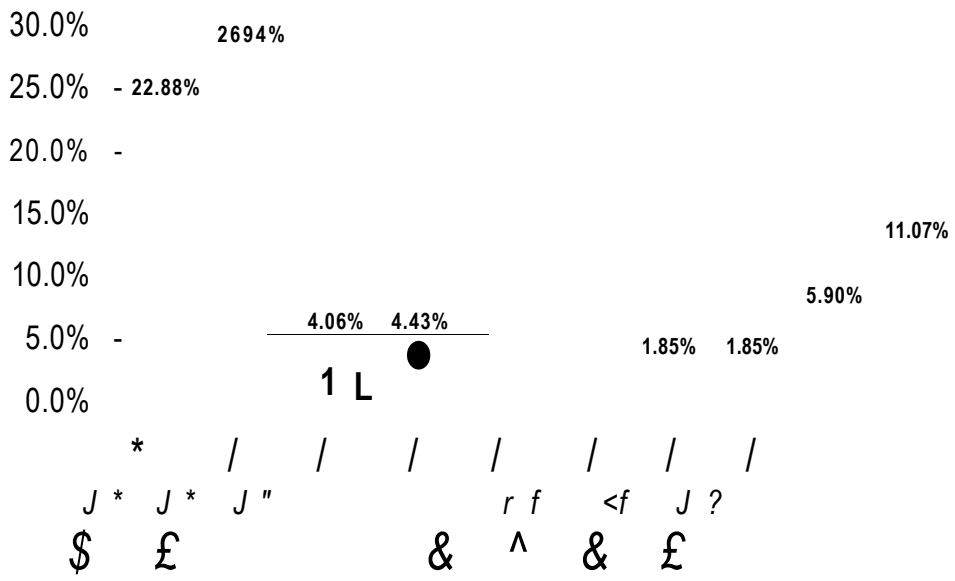


Figure 10: Proportion of Study Subjects on Various NRTI combinations.

Majority of the patients (49.82%) were on regimen containing zidovudine and lamivudine while 21.03% were on abacavir and lamivudine based regimen and 8.49% were on stavudine and lamivudine as shown in Fig 10 above.

11.2 Prevalence Of Hyperlactatemia

The prevalence of hyperlactatemia among children receiving nucleoside reverse transcriptase inhibitors (NRTI) as part of their highly active anti-retro-viral therapy (HAART) regimen was found to be 11.8% in the study population. 10.7% of the patients with hyperlactatemia had moderate elevation of lactic acid while the remaining 1.1% had mild elevation. Similar studies among children reported prevalence rates of between 13.4% and 32.3% <6,7).Table 9 below.

Table 9: Prevalence of Hyperlactatemia.

Lactate level	**Proportion of patients**
Mild elevation	1.1%
Moderate elevation	10.8 %
Normal	88.2 %

11.3 Correlates Of Hyperlactatemia

The secondary objective of the study was to determine factors associated with hyperlactatemia among children receiving NRTI containing treatment regimen. The factors that were studied were socio-demographic, WHO disease stage, nutritional status, treatment factors such as drug regimen taken (individual drugs and combinations), length of therapy, and adherence level, multivitamin and other nutritional supplements, and organ functional status especially liver and renal function.

11.3.1. Socio-demographic characteristics

a) Age

There was significant difference in the characteristics of the patients with and without hyperlactatemia in terms of age table 10 below.

The highest prevalence of 17.9% was among children between ages 6 to 10 years and the lowest prevalence of 3.6% found was among children over ten years old. Children in the age group 2 - < 6 years and those 6- <10 years were 4.3 times (p = 0.029) and 5.2 times (p = 0.006) more likely to have hyperlactatemia than those £10 years and this was statistically significant. The reason for this difference in prevalence rates in the different age groups is not apparent from this study.

Table 10: Correlation of Hyperlactatemia with Age

Age groups	**N (%)**	**P value**	**OR**	**95% C.I.for OR**	
				Lower	Upper
<2 years	0	0.999	.000	.000	
2 - <6 years	12(13.6)	0.029	4.263	1.158	15.694
6 - <10 years	17(17.9)	0.006	5.885	1.659	20.874
>10 years	3(3.6)	Reference group			

This provides a snap shot of the patients' lactic acid homeostatic status but the trend may need to be elucidated through prospective studies i.e. whether these patients had a onetime elevation of lactic acid, a rising trend, or intermittent hyperlactatemia.

b) Other Characteristics

There were no significant differences in other socio-demographic features among patients with and those without hyperlactatemia as summarized in table 11 below.

Table 11: Correlation of Hyperlactatemia with Socio-demographic Characteristics.

Characteristic	Lactic acid level N (%)		p - Value
	Normal	Abnormal	
Male	120(50.2)	13(40.6)	0.308
Mother's tribe (Kikuyu)	102(42.7)	13(40.6)	0.489
Caretaker (Parent)	192(80.3)	30(93.3)	0.064
Medicine giver (Parent/Self)	201(84.1)	30(98.8)	0.319
Regular family income	138(57.7)	20(62.5)	0.715
Baseline WHO disease stage (1 &2)	11(4.7)	1(3.1)	0.179
Current WHO disease stage (1 & 2)	168(70.6)	25(78.2)	0.289
Treatment (1st Line Regimen)	211(88.3)	26(81.3)	0.259
Body Mass Index (Mean ± SD)	16.59 ±3.02	16.20 ±2.11	0.477

11.3.2. Symptoms and Signs Exhibited by Patients

The symptoms and signs of patients with and without hyperlactatemia were also analyzed for any correlation and the results depicted in table 12 below. There were no differences in the symptoms and signs observed in patients with and without hyperlactatemia at the time of the study therefore none of these may be useful in determining which patients are likely to have hyperlactatemia.

Table 12: Correlation of Hyperlactatemia with Clinical Symptoms & Signs.

Symptoms and signs	**Lactic acid level N (%)**		**p- Value**
	Normal	**Abnormal**	
Abdominal pain	10(4.2)	1(3.1)	0.769
Nausea	1(0.4)	1(3.1)	0.095
Vomiting	6(2.5)	0(0)	0.363
Reduced Appetite (anorexia, refusal to feed)	9(3.9)	1(3.1)	0.837
General Examination findings*	16(6.7)	2(6.3)	0.924
Respiratory system findings	29(12.1)	2(6.3)	0.326
Cardiovascular system findings	3(1.3)	0(0)	0.524
Central nervous system findings	2(0.8)	0(0)	0.603
Abdominal findings	11(4.6)	0(0)	0.215

* Only abnormal physical findings are shown

11.3.3. Characteristics of Laboratory Parameters

An analysis of the hematologic parameters, indices for liver (Alanine transaminase, ALT level) and renal function (creatinine level, and estimated glomerular filtration rate, GFR) as well as markers for immunologic status (CD4 count, CD4 percentage) was done for the patients with and without hyperlactatemia and the results are depicted in table 5 below. There was no statistically significant difference in these characteristics between the patients with and without hyperlactatemia. Table 13 below.

Table 13: Correlation of Hyperlactatemia with Laboratory Indices.

Laboratory parameter	**Lactic acid level mean(SDJQR)**		
	Normal	**Abnormal**	**p Valu<**
Hb (g/dL)	12.33(11.1-13.2)	12.31(11.7-14.2)	0.94
WBC (count x 109/L)	21.4(6.9-26.7)	14.7(8.4-20.6)	0.60
Platelets (count x 10^9/L)	285.4(219.6-350.1)	286.5(213.9-362.5)	0.98
ALT (U/L)	38.3(21.4-43.5)	36.1(24.7-42.8)	0.78
Current CD4 count (per mL)	959(736 - 1327)	1020(618- 1289)	0.66
Current CD4 %	25.2(10.9)	24.2(11.3)	0.62
Abnormal GFR*, N (%)	113(87.6)	16(12.4)	0.87

'Abnormal GFR = <90 ml/min/1.73m^2

It was noted that both groups of patients exhibited high prevalence of renal dysfunction. Although the kidneys account for up to 30% of lactic acid clearance from the body the prevalence of chronic kidney disease as depicted by the estimated creatinine clearance rate of <90ml/min/1.73m^2 was similar in the patients with and without hyperlactatemia at 46.7% and 45.1% (O.R 1.2; 95% CI: 0.5 - 3.1, p = 0.866) respectively and was not statistically

significant. The degree of renal dysfunction as estimated by GFR is illustrated in the table 14 below.

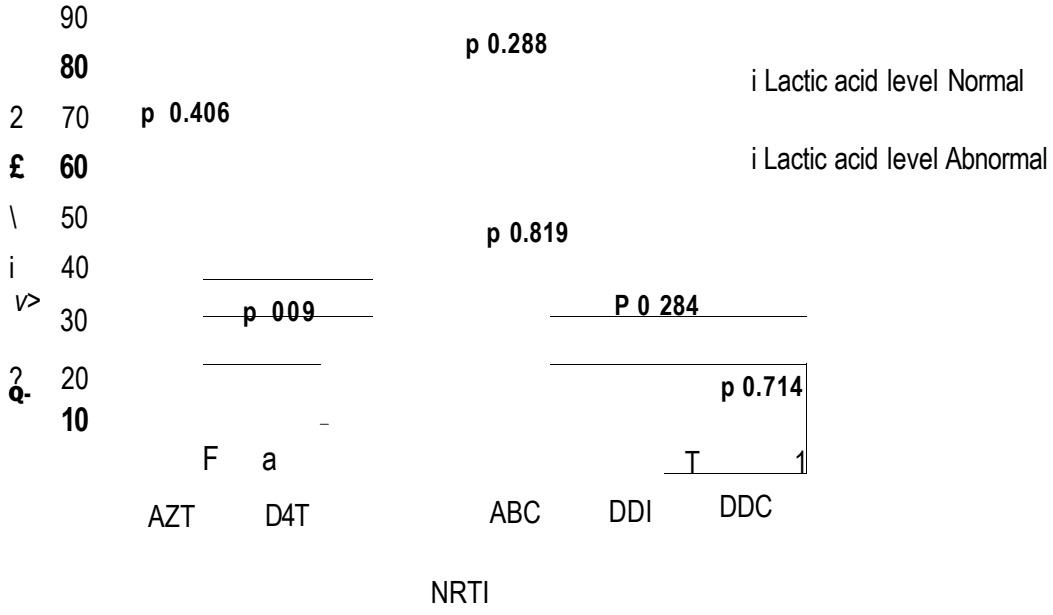
Table 14: Degree of Renal Dysfunction in Children in the Study.

CKD Stage*	**Lactate level N (%)**	
	Normal (n=240)	**Abnormal (n=31)**
1 (normal)	119(49.6)	14(45.2)
2	96(40.0)	13(41.9)
3	16(6.7)	1(3.2)
Unknown	9(3.8)	1(3.2)

*CKD -Chronic Kidney Disease, Stage 1 (GFR >90ml/min/1.73m2), Stage 2 (60 - 89), Stage 3 (30 - 59), Stage 4 (15- 29), Stage 5 (<15). GFR was estimated using the Schwartz formula.

11.3.4. Characteristics of Treatment Variables

Treatment variables were then analyzed for patients presenting with hyperlactatemia and those without. Both groups were analyzed by each drug taken and then according to the combinations of NRTIs taken by the patients and the results are depicted in fig 12 and table 15 below respectively. No statistically significant difference was found between the two groups of patients. In studies involving adults, patients on stavudine were reported to have higher prevalence of hyperlactatemia. Zalcitabine was only used in one patient who had treatment failure after drug resistance tests.



***AZT = Zidovudine, D4T= Stavudine, 3TC = Lamivudine, ABC = Abacavir, DDI = Didanosine, & DDC = Zalcitabine**

Figure 11: Correlation of Hyperlactatemia with each NRTI

Table 15: Correlation of Hyperlactatemia with **Various** NRTI corr **t** • at / s

NRTI combination*	**Lactate -evel N (%)**		**95% C.I**			
	Normal	**Abnormal**	**P value**	**OR**	**lower**	**upper**
AZT-3TC-NVP/EFV	122 (90.4%)	**13(9.6%)**	**Reference group**			
fe3TC-NVP/EFV	18(78.3%)	**5(21.7%)**	**.101**	**2.607**	**.830**	**8183**
ABC-3TC-AZT/NVP/EFV/Kal	52(91.2%)	**5(8.8%)**	**.852**	**902**	**306**	**2661**
Other	4(80.0%)	**1(20.0%)**	**.460**	**2.346**	**244**	**22689**
'AZT = Zidovudine, **3TC = Lamivudine, NVP = Nevirapme EFV E**lavrence. **ABC** B Abacavir, Kal = Kaletra **(Ritonavir boosted Lopinavir)**						

Patients receiving regimen **containing stavudine were noted to have increassd rtok (OR 2 6)** of developing **hyperlactatemia relative to those on zidovudine but this was slat stocai y** insignificant. The **patients receiving other NRTIs included those on didanosme. lenofovtr** and zalcitabine and **although as a grouped had an increased risk, this was st.itist.cany** insignificant.

12. DISCUSSION

The prevalence of hyperlactatemia among children receiving HAART consisting of NRTIs at Kenyatta National Hospital was found to be 11.8% majority of whom had mild elevation (89.9%).

The prevalence was found to be significantly highest in the children who were aged 6-<10 years (17.9%), followed by those aged 2 - <6 years (13.6%) compared to patients who were >10 years. These prevalence rates were similar to those reported in studies done in USA, Europe, and West Africa (W). Children who were 6 -<10 years were also 5.8 times (O.R 5.8; 95% CI 1.7 - 20.9, p = 0.006) and those 2-<6 years were 4.3 times (O.R 4.3; 95% CI 1.2 - 15.7, p= 0.029) more at risk for hyperlactatemia than those who were >10 years. One prospective study reported a higher incidence of hyperlactatemia among children less than 3 years (8). The reason for this difference is not apparent from this study but may indicate the need for surveillance in children below 10 years.

Hyperlactatemia did not have any correlation with the baseline or current WHO disease stage and immunologic status (CD4 count or percent). There was also no correlation with nutritional state of the patients.

Analysis of the monitored laboratory data for these patients did not reveal any correlation with hyperlactatemia. Despite the fact that a high prevalence of renal dysfunction was observed among the children in this study and that renal function accounts for 30% clearance of lactic acid, there was no correlation between the occurrence of hyperlactatemia and renal dysfunction. Urinalysis for proteinuria was not evaluated for these patients during the study and most patients had no available previous results for further analysis. Correlation between hyperlactatemia and other laboratory indices was also explored including liver function (monitored using ALT),

hematologic parameters (Hemoglobin level, White cell count, Platelet count). No correlation was found between hyperlactatemia and any these other variables.

Analysis of the various symptoms and signs exhibited by patients found to have hyperlactatemia in comparison to those without hyperlactatemia did not show any correlation. Therefore no symptoms or signs can specifically be used to clinically pick out patients likely to have hyperlactatemia in children taking NRTI-based HAART from the results of this study.

Generally the children recruited into the study had received treatment for a mean of 35 months and showed marked improvement in clinical staging of disease. This was indicated by an increase in the median CD4 count and CD4 percent from 522 cells/ml and 12% respectively to the current values of 933 cells/ml and 26% respectively. In addition the proportion of patients who had a baseline WHO disease stage of 3 or 4 had reduced from 88% to 29% with most now in stage 1 and 2 (71%).

No prospective studies in children have been published yet to indicate the trend of lactic acid levels in children, although those in adults show a mild increase in baseline levels in adult patients on similar medications.

Limitations of this study include the cross-sectional design; therefore it provides only a snap-shot of the lactic acid levels in these children without an indication of the trend. There was also a lack of reference values for lactic acid level in African children on treatment with HAART. Some data had to be abstracted from patient's files which made it difficult to verify some of the information hence the assumption that all the data had been recorded with due accuracy.

13. CONCLUSIONS

1. There is a high prevalence of hyperlactatemia among children receiving NRTI-based HAART at Kenyatta National Hospital with the overall prevalence being 11.8% for the study population. Most of these patients (89.9%) had mild hyperlactatemia.
2. Prevalence of hyperlactatemia was relative highest among children less than ten years (2 to <6 years = 13.6%, 6 to <10 years = 17.9%) who also had a relatively higher risk for developing hyperlactatemia of 4.2 and 5.8 times respectively compared to children above ten years.

14. RECOMMENDATIONS

We recommend the determination of baseline lactic acid level for patients on NRTI-based antiretroviral therapy and follow-up levels three months later for those found to have increased levels to determine the trend especially for children below ten years. While it is reassuring that the rate of life threatening lactic acidosis is low, there is need for further prospective studies to evaluate the trend of lactic acid levels in children receiving NRTI-based therapy.

REFERENCES

1. *Kenya Demographic and Health Survey 2008-2009, Preliminary Report* .Central Bureau of Statistics (CBS) (Kenya), Ministry of Health (MOH) (Kenya), and ORC Macro. 2009. Calverton, Maryland: CBS, MOH, and ORC Macro, 2009.

2. Melvin A, Mohan K, Arcuino L, et al. *Clinical, virologic and immunologic responses of children with advanced HIV-1 disease treated with protease inhibitors.* 1997, Pediatr Infect Dis J, Vol. 16, pp. 968-974.

3. Resino S, Sanchez-Ramon S, Bellon J,et al. *Immunological recovery after* 3 years' *antiretroviral therapy in HIV-1-infected children.* 2002, AIDS, Vol. 16, pp. 483-486.

4. Van Rossum A, Niesters H, Geelen S, et al. *Clinical and virologic response to combination treatment with indinavir, zidovudine and lamivudine in children with HIV-1 infection: a multi center study in the Netherlands.* 2000, J Pediatr, Vol. 136, pp. 780-788.

5. Verweel G, Hartwig N, Wolfs T, et al. *Treatment with highly active anti-retroviral therapy in HIV-1 infected children is associated with a sustained effect on growth.* 2002, Pediatrics, Vol. 109, pp. 1-7.

6. Desai N, Mathura M. and Weedon J. *Lactate levels in children with HIV/AIDS on highly active antiretroviral therapy.* 2003, AIDS, Vol. 17(10), pp. 1565-1567.

7. Noguera A, Fortuny C, Sanchez E, et al. *Hyperlactatemia in human immunodeficiency virus-infected children receiving antiretroviral treatment.* 2003, Pediatr Infect Dis J, Vol. 22(9), pp. 778-82.

8. Rhoads MP, Smith CJ, Tudor-Williams G, et al. *Effects of highly active antiretroviral therapy on paediatric metabolite levels.* 2006, HIV Med, Vol. 7(2), pp. 16-24.

9. Ekouevi DK, Toure R, Becquet R, et al. *Serum lactate levels in infants exposed peripartum to antiretroviral agents to prevent mother-to-child transmission of HIV: Agence Nationale de Recherches Sur le SIDA et les Hepatites Virales 1209 study, Abidjan, Ivory Coast.* 2006, Paediatrics, Vol. 118(4), pp. e1071-7.

10. Boubaker K, Flepp M, Sudre P, et al. ***Hyperlactatemia and antiretroviral therapy: the*** *Swiss HIV Cohort Study.* 2001, Clin Infect Dis, Vol. 33, pp. 1931-1937.

11. Calza L, Manfredi R and F C. *Hyperlactataemia and lactic acidosis in HIV infected patients receiving antiretroviral therapy.* 2005, Clin Nutr, Vol. 24(1), pp. 5-15.

12. John M, Moore CB, James IR, et al. *Chronic hyperlactatemia in HIV infected patients taking anti-retroviral therapy.* 2001, AIDS, Vol. 15, pp. 717-723.

13. Lonergan J, Havlir D, Babber E, et al. *Incidence and outcome of hyperlactatemia associated with clinical manifestations in HIV infected adults receiving NRTI containing regimens.* Chicago. 2001.8th Conference on Retroviruses and Opportunistic Infections.

14. Lonergan J, Behling C, Pfander H, et al. *Hyperlactatemia and hepatic abnormalities in 10 human immunodeficiency virus-infected patients receiving nucleoside analogue combination regimens.* 2000, Clinical Infectious Diseases, Vol. 31, pp. 162-6.

15. Delgado J, Harris M, Tesiorowski A, et al. *Symptomatic elevations of lactic acid and their response to treatment manipulation in HIV infected persons: a case series.* 2001, Clin Infect Dis, Vol. 33, pp. 2072-2074.

16. Montaner JSG, Co* te' HCF, Harris M, et al. *Nucleoside-related mitochondrial toxicity among HIV-infected patients receiving antiretroviral therapy: insights from the evaluation of venous lactic acid and peripheral blood mitochondrial DNA.* 2004, Clinical Infectious Diseases, Vol. 38(Suppl 2), pp. S73-9.

17. Mina John and Simon Mallal. *Hyperlactatemia syndromes in people with HIV infection* 2002, Current Opion in Infectious Diseases, Vol. 15, pp. 23-29.

18. Madias NE. *Lactic acidosis.* 1986, Kidney Int, Vol. 29, p. 752.

19. Brinkman K, ter Hofstede H, Burger D, et al. *Adverse effects of reverse transcriptase inhibitors: mitochondrial toxicity as common pathway.* AIDS, 1998, AIDS, Vol. 12, pp. 1735-1744.

20. Wallace DC. *Diseases of the mitochondrial DNA.* 1992, Annu Rev Biochem, Vol. 61, pp. 1175-1212.

21. Luft D, Deichsel G, Schmulling RM, et al. *Definition of clinically relevant lactic acidosis in patients with internal diseases.* 1983, Am J Clin Pathol, Vol. 80, pp. 484 - 9.

22. G Moyle. *Hyperlactatemia and lactic acidosis during antiretroviral therapy: causes, management and possible aetiologies.* 2001, AIDS Rev, Vol. 3, pp. 150 -6.

23. Working Group on Antiretroviral Therapy and Medical Management of HIV-infected Children. *Guidelines for the use of antiretroviral agents in pediatric HIV infection Supplement III: Pediatric adverse drug events.* February 28,2008, pp 6, Table 1.

24. Johns DR. *The other human genome: mitochondrial DNA and disease.* 1996, Nat Med, Vol. 2, pp. 1065-1068.

25. Johns DR. *Seminars in medicine of the Beth Israel Hospital, Boston.. Mitochondrial DNA and disease.* 1995, N Engl J Med, Vol. 333, pp. 638-644.

26. Wallace DC. *Mitochondrial genetics: a paradigm for aging and degenerative diseases?* 1992, Science, Vol. 256, pp. 628-632.

27. Munnich A, Rustin P, Rotig A, et al. *Clinical aspects of mitochondrial disorders.* 1992, J Inherit Metab Dis, Vol. 15, pp. 448^55.

28. Powderly WG. *Long-term exposure to lifelong therapies.* 2002, J Acquir Immune Defic Syndr, Vol. 29 (Suppl.), pp. S28-S40.

29. Arnaudo E, Dalakas M, Shanske S, et al. *Depletion of muscle mitochondrial DNA in AIDS patients with zidovudine-induced myopathy.* 1991, Lancet, Vol. 337, pp. 508-10.

30. Chen CH, Vazquez PM, Cheng YC. *Effect of anti-human immunodeficiency virus nucleoside analogs on mitochondrial DNA and its implication for delayed toxicity.* Mol Pharmacol, 1991, Vol. 39, pp. 625-628.

31. Peters BS, Winer J, Landon DN, et al. *Mitochondrial myopathy associated with chronic zidovudine therapy in AIDS.* 1993, QJM, Vol. 86, pp. 5-15.

32. Lutton JD, Mathew A, Levere RD, et al. *Role of heme metabolism in AZT-induced bone marrow toxicity.* 1990, Am J Hematol, Vol. 35, pp. 1-5.

33. Stang M, Wysowski DK, and Butler-Jones D. *Incidence of lactic acidosis in metformin users.* June 1999, Diabetes Care, Vol. 22(6), pp. 925-7.

34. Sillos EM, Shenep JL, Burghen GA, et al. *Lactic acidosis: a metabolic complication of hematologic malignancies: case report and review of the literature.* Nov 1,2001, Cancer. Vol. 92(9), pp. 2237-46.

35. Halperin ML, Hammeke M, Josse RG, et al. *Metabolic acidosis in the alcoholic: a pathophysiologic approach.* Mar 1983, Metabolism, Vol. 32(3), pp. 308-15.

36. Uribarri J, Oh MS, and Carroll HJ. *D-lactic acidosis. A review of clinical presentation, biochemical features, and pathophysiologic mechanisms.* Mar 1998, Medicine (Baltimore), Vol. 77(2), pp. 73-82.

37. Co* te' HCF, Brumme ZL, Crabbe KJP, et al. *Changes in mitochondrial DNA as a marker of nucleoside toxicity in **HIV** infected patients.* 2002, N Engl J Med, Vol. 346, pp. 811-820.

38. Blanco F, Garcia-Benayas T, Gomez J, et al. *Factors associated with hyperlactatemia in HIV+ patients under antiretroviral therapy (abstract 520).* Buenos Aires. 2001. 1st International AIDS conference on HIV pathogenesis and treatment.

39. Chan A.Y.W, SwamInathan A, and Cockram C. S. *Effectiveness of sodium fluoride as a preservative of glucose in blood.* 1989, Clin. Chem, Vol. 35(2), pp. 315-317.

40. Schambelan M, Benson CA, Carr A, et al. *Management of metabolic complications associated with antiretroviral therapy for HIV- 1 infection: recommendations of an International AIDS society-USA panel.* 2002, J Acquir Immune Defic Syndr, Vol. 31(3), pp. 257-75.

41. Lemeshow S, Hosmer DW, Klar J, et al. *Adequacy of sample size in health studies.* Chichester. John Wiley, 1990.

42. World Health Organization -Regional office for the Eastern Mediterranean. *Field guide on rapid nutritional assessment in emergencies.* WHO, 1998. pp. 20-21.

43. World Health Organization. *WHO case definitions of HIV for surveillance and revised clinical staging and immunological classification of HIV - related disease m adults and children.* WHO, 2007.

Appendix I

ASSENT FORM

(For Children 7 to 13 years)

Study Title: Prevalence of Hyperlactatemia among Children with HIV/AIDS on Nucleoside Reverse Transcriptase Inhibitor based Highly Active Antiretroviral Therapy.

Principal Investigator: Dr. Peter K. Mashep (Tel: 0720 241707. or address below)

Supervisors: Dr. Elizabeth M. Obimbo and Dr. Ahmed M.R. Laving

What is a research study?

We wan! to tell you about a research study we are doing. A research study is a wa, to leam more about something.

Why are you being asked to be a part of this study?

We would like to find out more about a prcblem called hyperlactatemia which " sick and take medicine like the ones you are taking My day. You are ^ ^ J ^ J * „ because you take these medicines every day. About three hundred other oh,Wren are expected ,o study.

If you join the study what will happen to you?

You Wn be asked some questions by one o. the dxtors to answer with the M p of your about how you are feeling and how you feel when you take ^ / ^ J ^ t S and.all you are and then exartne you by looking a, your head, face ^ be 9 done only Afterwards we will use a needle to take some blood from your amn be wjth you once and we will no. require you to visit again for this research. Your parent/guard,an throughout the process.

Will any part of the study hurt?

You will feel a pinch when we use the needle to 9 ^ ® ^'^o^way a ^a ^days^^ ^ ^ ^ re the needle touched your skin. Like other black marks, it will go away after a aays

Will the study help you and other children?

The study wil, help you by showing any bad effects of^ ^ X S ^ T a ^ doctor can do something about it. We may also leam someinmy

s

and have to take medicine like yours every day. This study will also help us learn more about your sickness and the medicine we use to treat you and other children so that we can treat all of you better.

Do you have to be in the study?

You do not have to join this study. It is up to you. You can say okay now and change your mind later. All you have to do is tell us you want to stop. No one will be mad at you if you don't want to be in the study or if you join the study and change your mind later and stop.

What choices do you have if you say no to this study?

This study is optional and additional. If you choose not to join nothing will change about your treatment/care.

What about your privacy?

This study has explained to your parents and they said that we could ask you if you want to be in it. You can talk this over with them before you decide. The doctor will not about what you what you tell us to anyone else except people working with him/her. If the doctor needs to talk to anyone else about you he/she will ask you and your parents if it is ok.

What if you have any Questions?

Before you say yes or no to being in this study, we will answer any questions you have. If you join the study, you can ask questions at any time. Just tell the doctor attending to you that you have a question.

If you have any questions about this study later please feel free to contact principal investigator or any of the supervisors through the contacts provided above.

Other information about the study.

- If you decide to be in the study, please write your name in the space below.
- You can change your mind and stop being part of it at any time. All you have to do is tell the person in charge. It's okay. The researchers and your parents won't be upset.
- You will be given a copy of this paper to keep.

(Sign your name here) Date

Principal investigator's name Signature of Investigator & Date

APPENDIX VIII

CONSENT FORM

[For Parents/Guardians and Children > 14 years)

Study **Title:** Prevalence hyperlactatemia in children on highly active antiretroviral therapy at the Kenyatta National Hospital comprehensive care centre.

Investigator: Dr. Peter K. Mashep (Tel: 0720-241707)

(MMED Student, Department of Paediatrics and Child health, University of Nairobi, P.O. Box 30197 - 00100, Nairobi.)

Supervisors: Dr. Elizabeth M. Obimbo and Dr. Ahmed M.R. Laving

(Lecturers, Department of Paediatrics and Child Health, University of Nairobi, P.O. Box 30197 - 00100, Nairobi.)

Investigators Note: The aim of this form is to give you some information about the study to help you decide whether or not your child will be part of the study.

Introduction: Treatment of HIV with antiretroviral drugs has improved the lives of many infected children and many of them are living healthier and longer lives. However just like other medications for other medical conditions, these drugs do have adverse effects a number of which your child is being monitored for regularly. Increased lactic acid in blood is one of the known side-effects of these medications which in some patients may be very high and cause illness. The purpose of this study is to find out how common this problem is and the symptoms such children with high lactate levels have to enable us identify them earlier and accurately for us to treat them.

Procedure: I will ask you some questions about you and your child. Then I will conduct a physical examination on your child and take his weight and height/length. Finally, I will draw a 4ml blood sample once while blood for other regular tests if any is being taken. The sample will be taken to the laboratory for testing of lactic acid level.

Risks: Your child might experience some pain when the blood sample is taken because of being pricked No other risk is foreseen.

Benefits: You will not be charged for the blood test and the result will be communicated to the doctor so that your child may be treated if necessary.

Confidentiality: The information you give will be held in the strictest confidence and will only be shared with the doctor treating your child for the benefit of the child in terms of treatment and care.

Reassurance: Should you opt not to participate the treatment of your child will not be interfered with in any way. You may withdraw from the study at any time without explanation or consequence.

Ethical consideration: I have approval from the Ethics and Standards Committee to carry out this study. If you have any enquiries concerning this study you may contact the ethical committee through the address below:

The Chairperson, KNH/UON-Ethics Review Committee, Kenyatta National Hospital, Hospital Rd, along Ngong Rd. P.O. Box 20723, Nairobi, Tel: 726300-9, Fax: 725272

ASSENT/ CONSENT FORM FOR PARTICIPATION IN THE RESEARCH STUDY

I/my parent or legal guardian have read the previous page(s) of the consent form and the investigator has explained the details of the study, I/my parent or legal guardian understand that I am free to ask additional questions.

If I/my parent or legal guardian wish to get additional information regarding this research and my rights as a research subject, or if I/my parent or legal guardian believe I have been harmed by this study, I/my parent or guardian may contact the: The Chairperson, KNH/UON-Ethics Review Committee, Kenyatta National Hospital, Hospital Rd, along Ngong Rd. P.O. Box 20723, Nairobi, Tel: 726300-9, Fax: 725272

I/my parent or legal guardian is aware that this is a research project and that unforeseen side effects may occur.

I/my parent or legal guardian understand that there is no formal program for compensating patients for medical injuries arising from this research. Medical treatment will be provided for injuries at the usual charge to me or to my insurer unless payment is otherwise provided for in this consent form.

I/my parent or guardian understand that participation in this study is voluntary and I/my parent or legal guardian may refuse to participate or may discontinue participation at any time without penalty, loss of benefits, or prejudice to the quality of care which I will receive.

I/my parent or legal guardian, acknowledge that no guarantees have been made to me regarding the results of the investigations involved in this study, and I agree to participate in the study and have been given a copy of this form.

To indicate that you understand the conditions of this study and that you consent (accept) for your child to participate in it, please sign or put your thumbprint in the space provided below.

STUDY SUBJECT DATE

PARENT OR LEGAL GUARDIAN DATE *(subject is a minor)*

The subject has been given the opportunity to read a description of the protocol, to ask questions before signing, and has been given a copy.

PRINCIPAL INVESTIGATOR'S NAME SIGNATURE OF INVESTIGATOR & DATE

APPENDIX III

QUESTIONNAIRE

a) Socio-Demographic Data

Study Code:_____ IP No_____ PEPFAR NO.

Age:_____ D.O.B:_____ Sex:

Father's tribe_____ Mother's tribe

Residence:_____ (District, Location, Sub location, Estate/Village)

Mobile:

1. Who is (are) the primary caretaker(s)?_____ (specify relationship)
2. Educational level of the caretaker (Indicate number of years completed)
3. Does anyone in the household have a regular income?
    a. Yes • No •
    b. If yes. how much?_____ KSH
4. How many rooms do you live in? •

b) Disease Stage

	Baseline		Most Recent	
	Value	Date	Value	Date
WHO stage				
CD4 count				
CD4 percent				
Viral load				

c) Nutritional status (Appendix V)

Measurement		Actual value
Height/Length (nearest 0.1 cm)		
Weight (nearest 0.1 kg)		
Surface Area {m^2=	$\frac{Height\ (m) \times Weight\ (Kg)}{3600}$	

d) Treatment History:

5. When did the child start taking HAART?. (Date)

6. What regimen of ART is the child currently on?
   1st Line_____ *2nd* Line_____ Salvage

Drug	Dose received (mg)	Dose mg/kg or mg/m²	Appropriateness'. (H=High; L= Low; A=Appropriate)	Duration received (nearest Months)
NRTI				
Zidovudine (A2T)				
Stavudine (D4T)				
Lamivudine (3TC)				
Abacavir (ABC)				
Didanosine (DDI)				
Emtricitabine (FTC)				
NtRTI				
Tenofovir (TDF)				
NNRTI				
Nevirapine (NVP)				
Efavirenz (EFV)				
PI				
LPV/r LPV				
(Kaletra) R				
Nelfinavir NFV)				
Ritonavir (RTV)				
Indinavir (IDV)				
Atazanavir (ATV)				
Saquinavir soft gel(SQV)	.			
Saquinavir hard gel(SQV)				
Amprenavir (APV)				
Other (specify)				

'Check Ministry of Health paediatric and adolescent dosage charts Appendix VI & VII

7. Was the child on a different regimen before?

   Yes • No •

   If yes

   a) What regimen and for how long did the child take it?

   Duration_____Months

   b) Why was the regimen changed?

8. Did the child miss to take ARV in the last 7 days?

   Yes • No •

   If yes, how many times and why?

   times, due to

9. Who usually gives the child the ARVs?
10. Is the child on multivitamin supplements?

    Yes • Non

    If yes specify_

11. Is the child on any nutritional supplements? (e.g Astymine, Vitamins, Chinese herbal preparations etc)

    Yes • No •

    If yes, specify, duration__

12. Is the child on medication for any other condition? (e.g. TB, Diabetes, Cancer)

    Yes • Nou

    If yes, specify; the condition, medication and duration

13. Has your child ever had jaundice (yellowness of eyes)?

    Yes • **Nod**

14. Have you noticed any of the following symptoms in your child in the last one month?

Symptom/sign	*Yes*	*No*
Weight loss in last one month(quantify kg)		
Difficulty in breathing		
Easy fatigabilityAinng with activities (s)he used to do well before		
Abdominal pain		
Abdominal bloating		
Nausea		
Vomiting		
Reduced appetite (anorexia, refusal to feed)		
Others:		

e) Physical examination (Abnormal findings)

15. General examination

16. Respiratory findings.

17. Cardiovascular system findings.

18. CNS system findings.

19. Abdominal findings.

f) Laboratory data sheet

Test	**Result**	**Date**
Plasma Lactic acid level		
Hemogram (Hb, WBC count, Platelet count)		
LFT (at least ALT)		
U/E/Cr (at least creat)		
CD4 count		
CD4 Percent		
Viral load		

APPENDIX VIII

WORLD HEALTH ORGANIZATION CLINICAL STAGING FOR CHILDREN WITH CONFIRMED HIV INFECTION (2007) W.

Clinical stagel

Asymptomatic

Persistent generalized lymphadenopathy

Clinical stage 2

Unexplained persistent hepatosplenomegaly
Papular pruritic eruptions
Fungal nail infection
Angular cheilitis
Lineal gingival erythema
Extensive wart virus infection
Extensive molluscum contagiosum
Recurrent oral ulcerations
Unexplained persistent parotid enlargement
Herpes zoster
Recurrent or chronic upper respiratory tract infections
otitis media, otoritioea, sinusitis or tonsillitis)

Clinical stage 3

Unexplained' moderate malnutrition or wasting not adequately responding to standard therapy
Unexplained persistent diarrhoea (14 days or more)
Unexplained persistent fever (above 37.5°C intermittent or constant,
for longer than one month)
Persistent oral candidiasis (after first 6-8 weeks of life)
Oral hairy leukoplakia
Acute necrotizing ulcerative gingivitis or periodontitis
Lymph node tuberculosis
Pulmonary tuberculosis
Severe recurrent bacterial pneumonia
Symptomatic lymphoid interstitial pneumonitis
Chronic HIV-associated lung disease including brochiectasis
Unexplained anaemia (<8 g/dl), neutropaenia (<0.5 * 109 per litre)
and or chronic thrombocytopaenia (<50 * 109 per litre)

i Unexplained refers to where the condition is not explained by other causes.

Children

Clinical stage **4 j**[1]

Unexplained severe wasting, stunting or severe malnutrition not responding to standard therapy
Pneumocystis pneumonia
Recurrent severe bacterial infections (such as empyema, pyomyositis, bone or joint infection or meningitis but excluding pneumonia)
Chronic herpes simplex infection (orolabial or cutaneous of more than one month's duration or visceral at any site)
Oesophageal candidiasis (or candidiasis of trachea, bronchi or lungs)
Extrapulmonary tuberculosis
Kaposi sarcoma
Cytomegalovirus infection: retinitis or cytomegalovirus infection affecting another organ, with onset at age older than one month
Central nervous system toxoplasmosis (after one month of life)
Extrapulmonary cryptococcosis (including meningitis)
HIV encephalopathy
Disseminated endemic mycosis (coccidiomycosis or histoplasmosis)
Disseminated non-tuberculous mycobacterial infection
Chronic cryptosporidiosis (with diarrhoed)
Chronic isosporiasis
Cerebral or B-cell non-Hodgkin lymphoma
Progressive multifocal leukoencephalopathy
Symptomatic HIV-associated nephropathy or HIV-associated cardiomyopathy

"Some additional specific conditions can also be included in regional classifications (such as reactivation of American trypanosomiasis [meningoencephalitis and/or myocarditis] in the WHO Region of the Americas, disseminated penicilliosis in Asia and HIV-associated rectovaginal fistula in Africa).

APPENDIX V

WORLD HEALTH ORGANIZATION CLINICAL STAGING FOR ADULTS AND ADOLESCENTS WITH CONFIRMED HIV INFECTION' (2007) W.

Clinical stage 1
Asymptomatic
Persistent generalized lymphadenopathy
Clinical stage 2
Moderate unexplained weight loss
(<10% of presumed or measured body weight)l
Recurrent respiratory tract infections sinusitis, tonsillitis, otitis media and pharyngitis)
Herpes zoster
Angular cheilitis
Recurrent oral ulceration
Papular pruritic eruptions
Seborrhoeic dermatitis
Fungal nail infections
Clinical stage 3
Unexplained" severe weight loss (>10% of presumed or measured body weight)
Unexplained chronic diarrhoea for longer than one month
Unexplained persistent fever (above 37.6°C intermittent or constant,
for longer than one month)
Persistent oral candidiasis
Oral hairy leukoplakia
Pulmonary tuberculosis (current)
Severe bacterial infections (such as pneumonia, empyema, pyomyositis,
Done or joint infection, meningitis or bacteraemia)
Acute necrotizing ulcerative stomatitis, gingivitis or periodontitis
Unexplained anaemia (<8 g/dl), neutropaenia (<0.5 x 109 per litre)
or chronic thrombocytopaenia (<50 * 109 per litre)

'Assessment of body weight in pregnant woman needs to consider the expected weight gain of pregnancy.

" Unexplained refers to where the condition is not explained by other causes.

Adults and Adolescents

Clinical stage 4"" '

HIV wasting syndrome
Pneumocystis pneumonia
Recurrent severe bacterial pneumonia
Chronic herpes simplex infection (orolabial, genital or anorectal of more than one month's duration or visceral at any site)
Oesophageal candidiasis (or candidiasis of trachea, bronchi or lungs)
Extrapulmonary tuberculosis
Kaposi's sarcoma
Cytomegalovirus infection (retinitis or infection of other organs)
Central nervous system toxoplasmosis
HIV encephalopathy
Extrapulmonary cryptococcosis including meningitis
Disseminated non-tuberculous mycobacterial infection
Progressive multifocal leukoencephalopathy
Chronic cryptosporidiosis (with diarrhoed)
Chronic isosporiasis
Disseminated mycosis (coccidiomycosis or histoplasmosis)
Recurrent non-typhoidal Salmonella bacteraemia
Lymphoma (cerebral or B-cell non-Hodgkin) or other solid HIV-associated tumours
nvasive cervical carcinoma
Atypical disseminated leishmaniasis
Symptomatic HIV-associated nephropathy or symptomatic HIV-associated cardiomyopathy

"Some additional specific conditions can also be included in regional classifications (such as reactivation of American trypanosomiasis [meningoencephalitis and/or myocarditis]) in the WHO Region of the Americas and disseminated penicilliosis in Asia).

APPENDIX VIII

THE WORLD HEALTH ORGANIZATION MEASURING TECHNIQUES m

Weight

- A 25 Kg Salter scale is used. A bathroom scale will be used for children over 25 Kg.
- Every morning before the scale is used; it is checked against a known weight of 10 Kg or less.
- The weighing pants are attached to the lower hook of the scale, and the instrument is adjusted to zero.
- The clothes of the child are removed and the pant is put on.
- The weight is read to the nearest 0.1 Kg with the scale at eye level.
- The measurer reads the value out loud; the assistant repeats it and records it on the recording form.

Height:

A measuring board is used for measuring height in children who are less than two years of age (less than 85 cm in length).

- The child is gently placed on the board with the soles of the feet flat against the fixed vertical part.
- The head is put near the moving part (cursor)
- The child is made to lie straight in the middle of the board, looking directly up.
- The assistant holds the feet firmly against the feet board and places one hand and the knees of the child.
- The measurer gently holds the child' head places the curser against the crown of the head and reads out the length to the nearest 0.1 cm.
- The assistant repeats the reading and records it the recording form.
- Length is slightly more (0.5 cm) than height due to the effect of gravity. In view of that 0.5 cm is subtracted from all lengths taken.

For children 2 years of age and above (over 85 cm in length):

- The child is made to stand on a horizontal surface against a vertical measuring device (measuring board or calibrated tape measure stuck to a wall)
- The assistant makes sure that the child stands straight with the heels, knees against the wall.
- The cursor is then lowered onto the child' crown of the head.
- The length is read to the nearest 0.1 cm.

The measurer reads out loud; the assistant repeats it and records it on the recording form.

APPENDIX VII

Table 12 Antiretroviral Drugs and TMP/SMZ Paediatric Dose Chart for use in Resource-constrained Settings.

Weight	Abacavir (Ziagen®)		Stavudine (Zerit®, d4T)	Lamivudine (Epivir®, 3TC)		Zidovudine (Retrovir®, ZDV, AZT)		Didanosine (Videx® EC, ddI)	Nevirapine (Viramune®, NVP)						Efavirenz (Stocrin®, Sustiva®, EFV)		Lopinavir/ritonavir (Kaletra®)		Nelfinavir (Viracept)
	8 mg/KG twice daily		1 mg/KG twice daily	4 mg/KG twice daily		240 mg/m² twice daily		120 mg/m² twice daily	Induction dose: 4 mg/KG once daily for first 14 days, then give maintenance dose →		Maintenance dose				Dose as shown once daily		< 15 KG = 12 mg lop/KG ≥ 15 KG = 10 mg lop/KG twice daily (lop = lopinavir, r = ritonavir)		60 mg/KG twice daily
											< 8 yrs 7 mg/KG - twice daily		≥ 8 yrs 4 mg/KG twice daily						
KG	Liquid 20 mg/ml	Tablet 300 mg	Capsules 15, 20, 30 mg	Liquid 10 mg/ml	Tablet 150 mg	Liquid 10 mg/ml	Capsule 100 mg	Chewable tablets 25, 50, 100 mg	Liquid 10 mg/ml	Tablet 200 mg	Liquid 10 mg/ml	Tablet 200 mg	Liquid 10 mg/ml	Tablet 200 mg	Liquid 30 mg/ml	Capsules 50, 100, 200 mg	Liquid 80 mg lopinavir/ml[5]	Capsule 133.3/33.3 mg lopinavir/r	Tablet 250 mg
5 - 6.9	2 ml			2 ml		7 ml			2 ml		4 ml								2 tabs[7]
7 - 9.9	3 ml		15 mg	3 ml		9 ml	1 cap	25mg + 25mg	3 ml		6 ml						1.5 ml		2 tabs[7]
10-11.9	4 ml		15 mg or (20 mg[1])	4 ml		12 ml	1 cap	25mg + 25mg	4 ml		8 ml	½ tab			9 ml	200 mg	2 ml		2 tabs
12-14.9	5 ml		15 mg or (20 mg[1])	5 ml		14 ml	1 cap	50mg + 25mg	5 ml		9 ml	½ tab			9 ml	200 mg	2 ml		3 tabs
15-16.9	6 ml		15 mg or (20 mg[1])	6 ml	½ tab	15 ml	2 caps	50mg + 25mg	6 ml		10 ml	½ tab			10 ml	200 mg + 50 mg	2.5 ml	1 cap	3 tabs
17-19.9	7 ml	½ tab	20 mg	7 ml	½ tab	17 ml	2 caps	50mg + 50mg	7 ml		13 ml	1 tab AM + ½ tab PM[4]			10 ml	200 mg + 50 mg	2.5 ml	2 caps[4]	4 tabs
20-24.9	9 ml	½ tab	20 mg	9 ml	½ tab	20 ml	2 caps	50mg + 50mg	9 ml	½ tab	16 ml	1 tab AM + ½ tab PM[4]	9 ml	½ tab	12 ml	200 mg + 100 mg	3 ml	2 caps	5 tabs
25-29.9	25 - 27.9 KG	11 ml ½ tab	30 mg	11 ml	1 tab[7]	24 ml	3 caps or 300 mg tab	100mg + 25mg	11 ml	½ tab	20 ml	1 tab	11 ml	½ tab	15 ml	200 mg + 100 mg + 50 mg	3.5 ml	2 caps	5 tabs
	28 - 29.9 KG	12 ml 1 tab																	
30-34.9	13 ml	1 tab	30 mg	13 ml	1 tab	27 ml	3 caps or 300 mg tab	100mg + 25mg	13 ml	1 tab[7]			13 ml	1 tab AM + ½ tab PM[4]	30 - 32.9 KG	15 ml 200 mg + 100 mg + 50 mg	4 ml	3 caps	5 tabs
															33 - 34.9 KG	17 ml 200 mg + 200 mg			
35-40	16 ml	1 tab	30 mg	15 ml	1 tab	30 ml	3 caps or 300 mg tab	100mg + 25mg	15 ml	1 tab[7]			15 ml	1 tab AM + ½ tab PM[4]	17 ml	200 mg + 200 mg	5 ml	3 caps	5 tabs

Abacavir - Tablets may be swallowed whole or crushed and dispersed in water or onto a small amount of food and immediately ingesiec'

Ministry of Health, Republic of Kenya. *Guidelines for Antiretroviral Drug Therapy in Kenya.* Third edition. NASCOP. 2005. Table 12; pp 125.

APPENDIX VIII

ADULT ANTIRETROVIRAL DRUG DOSAGES *

I Drug	Dosage
m m m m m m m a m m	
Stavudine (d4T)	40mg BD for patients > 60kg; 30mg BD for patients <30kg
Zidovudine (AZT orZDV)	300mg/dose BD
Lamivudine (3TC)	150mg/dose BD
Stavudine + Didanosine (d4T + ddI)	>60kg: 200mg/dose BD or400mg/dose OD <60kg; 125mg/dose BD Or 250mg OD
Abacavir (ABC)	300mg/dose BD
Tenofovir disoproxil fumarate (TDF)	300mg/dose OD
	! *m m r n ^ t ^ i -*
Nevirapine (NVP)	200mg/dose OD for first 2 wks then 200mg/dose BD
Efavirenz (EFV)	600mg OD
Lopinavir/ritonavir (LPV/r)	(LPV 400 mg + RTV 100 mg) 3 capsules BD with EFV or NVP (LPV 533 mg + RTV 133 mg) 4 capsules BD
Indinavir (IDV)	800mg/dose TDS with RTV: IDV 800mg BD + RTV 100mg BD **preferred**
Nelfinavir (NFV)	750mg TDS or1250mg BD
Saquinavir (hard gel formulation)(SQV))	Only for combination with ritonavir in dose of SQV 1000mg and RTVIOOmg
Saquinavir (soft gel formulation)(SQV))	Not recommended; 1200 mg TDS or 1600mg BD. With RTV: (rtv 100MG + SQV-sgc 1000mg) BD
Ritonavir (RTV)	WHO recommends that RTV be used as a booster for other Pis at low dosage.
Amprenavir (APV)	1200mg BD
Atazanavir (ATV)	400mg OD
Preferred boosted with ritonavir ATV/RTV	ATV 300mg/RTV 100mg

'Extracted for drugs available in Kenya from **Ministry of Health, Republic of Kenya.** ***Guidelines for Antiretroviral Drug Therapy in Kenya.*** **Third edition. NASCOP. 2005. Table 12; pp 91-99.**

Lactate

f Lactate

cobas

o Indicates Roche/Hitachi analyzer(s) on which kit(s) can be used

Cat No.	Bottle	Contents	902	904	911 912	917	MODULAR P	MODULAR D
. , —837, 90 1,822837 190	1 2	|REAGENT| 2 X 20 mL fREAGENTI 2 x 5 mL	e	9	•	0	•	

Some analyzers and kits shown may not be available in all countries. For addiliional system applications, contact your local Roche Diagnostics representative.

English

System information

For Roche/Hitachi 904/911/912/917/MODULAR P analyzers: ACN 040.

Intended use

For the quantitative determination of L-ladate in olasma, cerebrospinal fluid or whole blood on Roche/Hitachi automated clinical chemistry analyzers.
L-lactate levels that evaluate the acid-base status are used in the diagnosis and treatment of lactic acidosis.

Summary

Anaerobic glycolysis markedly increases blood lactate and causes some increase in pyruvate levels, especially with prolonged exercise. The common cause for increased blood lactate and pyruvate is anoxia resulting from such conditions as shock, pneumonia and congestive heart failure. Lactic acidosis may also occur in renal failure and leukemia. Thiamine deficiency and diabetic ketoacidosis are associated with increased levels of lactate and pyruvate. Lactate levels in cerebrospinal fluid (CSF) are increased in bacterial meningitis. Increased CSF levels also occur in hypocapnia, hydrocephalus, brain abscesses, cerebral ischemia and any clinical condition associated with reduced oxygenation ol the brain and/or increased intracranial pressure. Lactate measurements that evaluate the acid-base status are used in the diagnosis and treatment of lactic acidosis (abnormally high acidity in the blood). In recent years, enzymatic methods lor the determination of lactate have gained favor over colorimetric and titrimetnc methods. Enzymatic methods are generally simple and provide greater specificity, accuracy and reproducibility. The first enzymatic method described for the determination of lactate was based on the transfer of hydrogen from lactate to potassium ferncyanide by lactate dehydrogenase. However, the procedure was cumbersome and did not receive wide acceptance.
Subsequent methods involved the UV measurement of the formation of NADH. In 1974, Gutmann and WahleteW descnbed a lactate procedure that measures the NADH formed by the oxidation of lactate catalyzed by LD. using hydrazine as a trapping agent for pyruvate. A method descnbed by Noll[2] is also based on the catalytic action of LD but includes ALT in the reaction mixture to more rapidly remove the pyruvate formed from the conversion of lactate.
The method presenled here uses an enzymatic reaction to convert lactate to pyruvate. The hydrogen peroxide produced by this reaction is then used in an enzymatic reaction to generate a colored dye.[14] This method offers longer reagent stability than the previous UV enzymatic methods.

Test principle

L-lactate is oxidized to pyruvate by the specific enzyme lactate oxidase (LOO).

$$\text{L-lactate} + O_2 \xrightarrow{\text{LOO}} \text{pyruvate} + H_2O_2$$

Peroxidase (POD) is used to generate a colored dye using the hydrogen peroxide generated in the first reaction.[3-4]

$$H_2O_2 + \text{H donor} + \text{4-aminoantipynne}^* \xrightarrow{\text{POO}} \text{chromogen-} + 2\ HjO$$

The intensity of the color formed is proportional to the L-lactate concentration.

Reagents • working solutions

R1 Hydrogen donor; ascorbate oxidase (cucumber): 2 30 U/mL (500 pkat/L); buffers; preservatives

"2 4-aminoantipyrine: 1 mg/mL (4.9 mmol/L|; LOD (microorganism): 2 15 U/mL (250 pkat/L); peroxidase (horseradish): 2 24 U/mL (400 pkat/L); buffers; preservatives

Precautions and warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagenls.
Safety data sheet available for professional user on request.
Disposal of all waste material should be in accordance with local guidelines.

Reagent handling

R1: Ready for use
R2: Ready for use

Storage and stability

Unopened kit components: Up to the expiration date at 2-8 'C
R1: 90 days opened and refrigerated on the analyzer
R2:90 days opened and refrigeraled on the analyzer

Specimen collection and preparation

For specimen collection and preparation only use suitable tubes or collection containers.
Only the specimens listed below were tested and lound acceptable.
Serum: Do not use serum.
Plasma: Sodium fluoride/potassium oxalate and sodium fluoride/sodium heparin plasma.
Centrifuge within 15 minutes of collecting the specimen.
Cerebrospinal fluid (CSF): May be used as obtained.
Whole blood: Use whole blood collected with sodium fluoride/potassium oxalate anticoagulant.
The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer. Centrifuge samples containing precipitates before performing the assay.

NOTES

1. The lactate level increases rapidly with physical exercise. The time required for return to normal lactate values depends on the physical fitness of the sublet. Thirty minutes at rest is usually sufficient lor this purpose.
2. Blood samples should be drawn from a stasis-free vein. However, minimal hemostasis (less than 30 seconds) win not affect lactate levels. Avoid the use of a tourniquet, if possible.[5]
3. Glycolysis in blood samples can rapidly increase lactate levels. Cells contnbute to the glycolysis and their quick removal is essential for accurate lactate analysis.[6] Hepannized plasma is acceptable, but precautions must be taken to retard glycolysis by keeping the whole blood on ice and then separating the plasma from the cells within 15 minutes of collection.

Whole blood must be deproteinized by the following procedure prior to assay:

Pipette into polypropylene micro luge lube:

Trichloracetic acid (TCA) (10 % w/v)	200 pL
Whole blood sample	200 pL

Stopper tightly, vortex, and centnfuge at 1500 RCF for 10 minutes.
Remove stopper.
Pipette into centrifuged microfuge tube: 46

0.9 % saline	IOO pL

Stopper tightly and invert tube gently several times. Transfer supernatant into a clean sample cup for analysis. Supernatant should be dear to slightly cloudy and colorless. Some particulate matter may be observed; this should settle to the bottom of the sample cup within sewal minutes. Multiply the whole blood supernatant result by 2.5 in order to correct for dilution.

Liactate

Lactate

Subility: Plasma (separated): 8 hours a! 15-25 °C or
14 days at 2-8 °C.[7]
CSF: 3 hours at 15-25 °C,
24 hours at 2-8 °C or
1 month al (-15)—(-25) °C.
Deprot. whole blood: 8 days at 2-8 °C[8]

Materials provided

See "Reagents • working solutions" section lor reagents.

Materials required (but not provided)

- C.f.a.s. (Calibrator for automated systems),
  Cat. No. 10759350 190. 10759350 360 (foi USA)
- Precmorm U, e.g. Cat. No. 10171743 122. or
  Precinorm U plus, Cat. No. 12149435122.12149435160 (for USA):
  Predpath U. e.g. Cat. No. 10171778 122, or
  Precipath U plus, Cat. No. 12149443122,12149443 160 (for USA)
- 0.9 % NaCl
- General laboratory equipment

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.
The performance of applications not validated by Roche is not warranted and must be defined by the user.

Calibration

Traceability: This method has been standardized against a primary standard
S1: 0.9 % NaCl
S2: C.f.a.s. (Calibrator for automated systems).

Calibration frequency

Two-point calibration is recommended

- after bottle change
  after reagent lot change
- as required following quality control procedures

Quality control

For quality control use the control material as listed in the 'Materials required" section. Other suitable control material can be used in addition. The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the limits.
Follow the applicable government regulations and local guidelines for quality control.

Calculation

The analyzer automatically calculates the analyte concentrauon of each sample.
Conversion factor:[5] mg/dL x 0.111 = mmol/L

Limitations - interference

Criterion: Recovery within ± 10 % of initial value.

Plasma

icterus: No significant interference up to an I index of 60 lor unconjugated bilirubin (approximate unconjugated bilirubin concentration: 60 mg/dL. 600 mg/L, or 1026 pmol/L) and up to an I index of 28 for conjugated bilirubin (approximate conjugated bilirubin concentration: 28 mg/dL. 280 mg/L. or 479 pmol/L).

Hemolysis: No significant interference from hemoglobin up to an H index of 1000 (approximate hemoglobin concentration: 1000 mg/dL, 10000 mg/L. or 621 pmol/L).

Ipemia (Intralipid): No significant interference from lipemia up to an L *index* of 1000. There is poor correlation between the L index (corresponds to turbidity) and tnglycendes concentration.

35 commonly used pharmaceuticals were tested in vitro. Dopamine (10 mg/L: 65 nmol/l) Levadopa (20 mg/L; 101 nmol/L) and Methyldopa (20 mg/L- 95 nmol/L) significantly reduced the lactate results. However, Dopamine at 1 mg/L (6.5 nmol/L), Levadopa at 4 mg/L (20 nmol/L) and Methyldopa at 2 mg/L (9.5 nmol/L) do not significantly affect lactate results. No interference with the assay was found with any of the other drugs tested.

Glycolate, a metabolite of ethylene glycol, causes a positive interference which is variable from lot to lot of reagent.

In very rare cases gammopathy, in particular type IgM (Waldenstrom's macroglobulinemia), may cause unreliable results.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

ACTION REQUIRED

Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on Roche/Hitachi analyzers. Refer to the latest version of the Carry-over evasion lists and the operator manual lor further instructions. US users refer to the Special Wash Programming document (located on MyLabOnline website) and the operator manual for special wash instructions.
Where required, special wash/carry-over evasion programming must be implemented prior to reporting results with this test.

Measuring range

2.0-140 mg/dL (0.22-15.5 mmol/L)

Specimen dilution

Samples with extremely high lactate concentrations may give falsely low results in the measuring range. However, these results are flagged 'LIMT2' to indicate the necessity of a rerun with diluted sample.
Determine samples with lactate concentrations > 140 mg/dL via the rerun function. Dilution of samples via the rerun function is a 1:1.5 dilution. Results from samples diluted by the rerun function are automatically multiplied by a factor of 1.5.
On instruments without renin function, manually dilute samples with 0.9 % NaCl (e.g. 1 + 1). Multiply the result by the appropriate dilution factor (e.g. 2).

Expected values

Plasma:[5]	4.5-19.8 mg/dL	(0.5-2.2 mmol/L)	venous
CSF:[5]	10-60 mg/dL	(1.1-6.7 mmol/L)	neonate
	10-40 mg/dL	(1.1-4.4 mmol/L)	3-10 days old
	10-25 mg/dL	(1.1-2.8 mmol/L)	> 10 days old
	10-22 mg/dL	(1.1-2.4 mmol/L)	adult
Whole blood:[5]	8.1-15.3 mg/dL	(0.9-1.7 mmol/L)	venous
	< 11.3 mg/dL	(< 1.3 mmol/L)	arterial

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Specific performance data

Representative performance data on the analyzers are given below.
Results obtained in individual laboratones may differ.

Precision

Precision was determined for plasma using human samples and controls in an internal protocol (within-run n = 21; between-run n = 63).
The following results were obtained:

	Within-run					Between-run				
Sample	Mean		SD		CV	Mean		SD		CV
	mg/dL	mmol	mg/dL	mmol	%	mg/dL	mmol	mg/dL	mmol	%
Human plasma	17.6	1.9	0.07	0.01	0.4	17.8	2.0	0.18	0.02	1.0
Control 1	14.2	1.6	0.09	0.01	0 6	14.3	1.6	0.191	0.02	1.3
Control 2	38.7	4.3	0.10	0.01	0.3	39 0	4.37	0.44	10 0351	1.1

Lactate

Precision was determined (or CSF using CSF controls in an internal protocol (within-run n = 21; between-run n = 20).
The following results were obtained:

	Within-run					Between-run				
Sample	Mean		SD		CV	Mean		SD		CV
	mg/dL	mmol /L	mg/dL	mmol /L	%	mg/dL	mmol /L	mg/dL	mmol H	%
Control 1	17.2	1.9	0.07	0.01	0.4	17.3	1.9	0.12	0.01	0.7
Control 2	62.4	6.9	0.36	0.04	0.6	62.8	7.0	0.46	0.05	0.7

Analytical sensitivity (lower detection limit)
Detection limit: 2 mg/dL (0.22 mmol/L)
The detection limit represents the lowest measurable analyte level that can be distinguished from zero. Il is calculated as the value lying three standard deviations above thai of the lowest standard (standard 1 + 3 SD. within-run precision, n = 21).

Method comparison
A comparison of the lactate determination using the Roche Diagnostics lactate assay on a Roche/Hitachi 917 analyzer (y) with the same assay on a Roche/Hitachi 717 analyzer (x) gave the following correlation (mg/dL):

Plasma

Passing/Bablok'	Linear regression
y = 1.015*-0.11	y = 1.01S* + 0.01
T = 1.00	r = 1.00

Number of samples measured: 37
The sample concentrations were between 7.0 and 133.9 mg/dL (0.78-14.9 mmol/L).

CSF

Passing/Bablok'	Linear regression
y = 1.012X + 0.30	y = 1.011*+ 0.32
T = 1.00	r = 1.00

Number of samples measured: 42
The sample concentrations were between 11.8 and 129.1 mg/dL (1.31-14.3 mmol/L).

Whole blood

Passing/Bablok[9]	Linear regression
y = 1.036* + 0.04	y = 1.040x + 0.13
T = 0.997	r = 0.997

Number of samples measured: 29
The sample concentrations were between 5.5 and 90.5 mg/dL (0.61-10.0 mmol/L).

References

1. Gutmann I, Wahlefeld A. Methods of Enzymatic Analysis. 2nd ed. Bergmeyer HU, ed. New York. NY: Acad Press Inc 1974:1464.
2. Noll F. Methods of Enzymatic Analysis. 2nd ed. Bergmeyer HU, ed. New York, NY: Acad Press Inc 1974:1475.
3. Tnnder P. Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. Ann Clin Biochem 1969:6:24.
4. Bartian D. Trinder P. An improved colour reagent for the determination of blood glucose by the oxidase system. Analyst 97;1972:142.
Tietz NW. Clinical Guide to Laboratory Tests. 3rd ed. Philadelphia, PA: WB Saunders Co 1995:382-383.
6. Burtts CA, Ashwood ER, eds. Tietz Textbook of Clinical Chemistry. 2nd ed. Philadelphia, PA: WB Saunders Co 1994:976.
7. Westgard JO, Lahmeyer BL, Bimbaum ML. Clin Chem 1972; 18:1334-1338.
8. Burns CA, Ashwood ER, eds. Tietz Textbook of Clinical Chemistry. 3rd ed Philadelphia, PA: WB Saunders Co 1999:788.
- Passing H, Bablok W el al. A general regression procedure for method transformation. J Clin Chem Biochem 1988;26:783-790.

Instrument settings
US users:
Refer to Die application sheet and Special Wash Programming document ocaied on MyLabOnline website) for additional operating information.

Roche/Hitachi 904, 911,912, 917 and MODULAR users:
Enter the application parameters via lhe application diskette, settings sheet or barcode sheet, as appropriate.