

CHAPTER 1: BACKGROUND AND LITERATURE REVIEW

1.1 HIV INFECTION

Acquired Immunodeficiency Syndrome (AIDS) is currently a growing, worldwide pandemic. It is caused by the Human Immunodeficiency Virus (HIV) which is a retrovirus of the lentivirus family that was unknown until the early 1980's [1]. Sub-Saharan Africa remains hardest hit by the pandemic, as illustrated in Figure. It has just over 10% of the world's population but it is home to more than 60% of all people living with HIV [1].

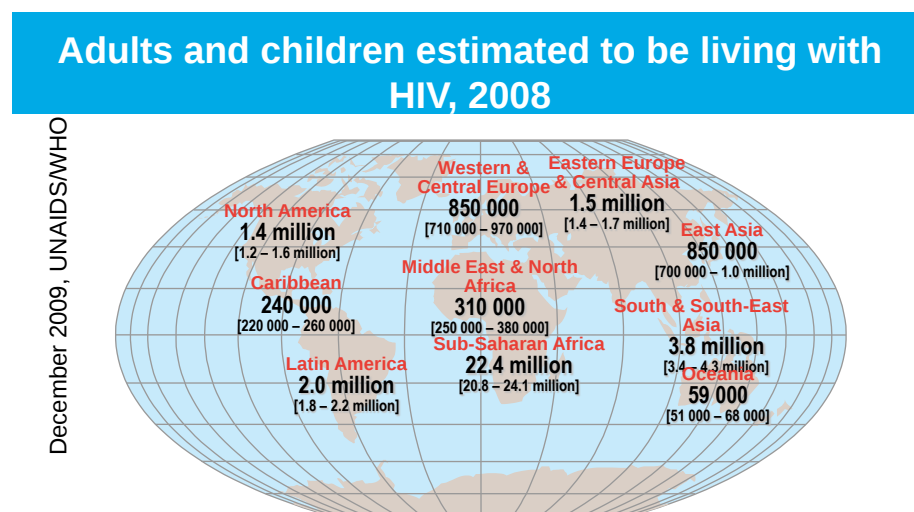


Figure 1: Estimates of people living with HIV worldwide

In Zimbabwe, according to the 2009 Antenatal Clinic HIV Estimates, the HIV prevalence in the age groups 15 to 49 years olds was 13.71% [2]. By the end of 2009, about 1.2 million

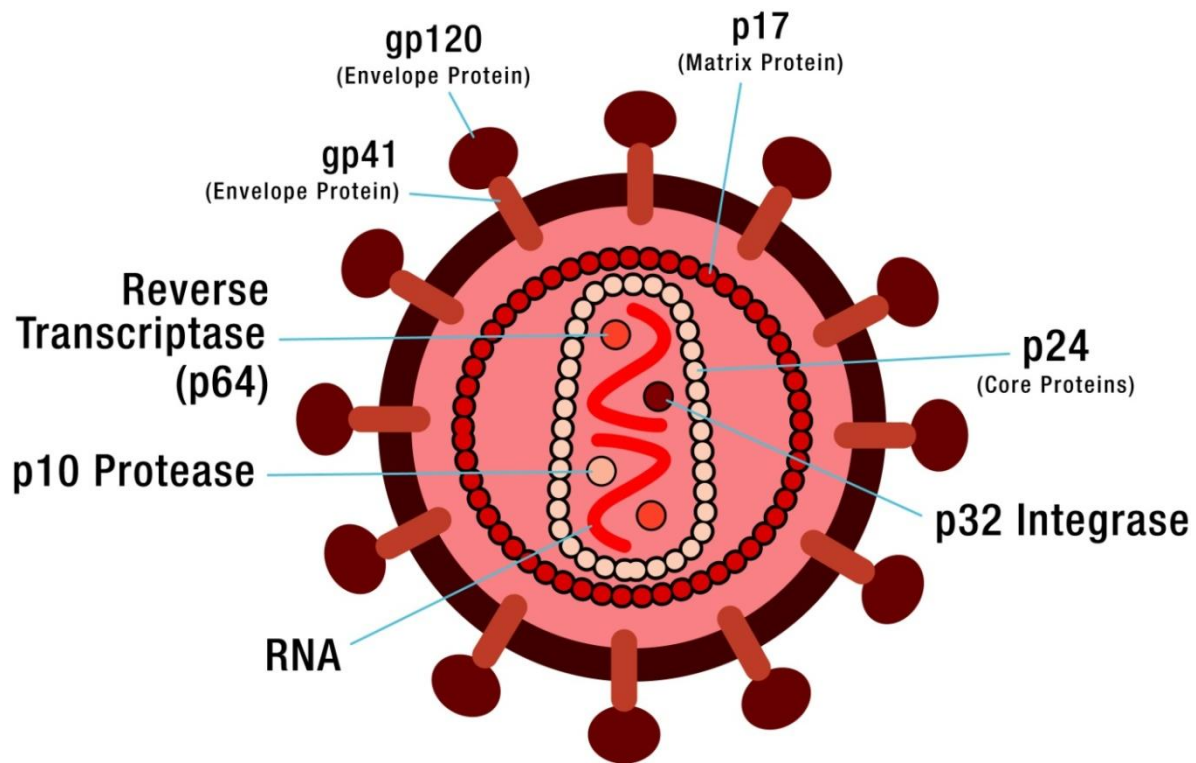
Zimbabweans were estimated to be living with HIV and AIDS [4]. HIV is the top most indirect cause of maternal mortality in Zimbabwe.

HIV is most commonly spread by body fluids during sexual contact with an infected partner [3]. It can also be transmitted through infected blood and shared needles or syringes contaminated with the virus.

1.2 STRUCTURE OF HIV AND MECHANISM OF INFECTION

The mature virus consists of a bar shaped electron dense core containing the viral genome, two short strands of RNA(Ribose Nucleic Acid) along with the enzymes, reverse transcriptase, protease, ribonuclease and integrase. All these are encased in a lipid envelope with 72 surface projections containing an antigen, gp120, that helps in the binding of the virus to target cells with CD4 (Clusters of Differentiation 4) receptors. The genome of HIV contains 3 major genes namely gag, pol and env [1]. The structure of the HIV virus is illustrated is shown in Figure 2 on page 3.

The binding of HIV to target cells involve the interaction of gp120 of the virus envelope with the CD4 molecule on the cell surface of T-helper lymphocytes, monocytes and macrophages [3]. After binding, the viral envelope fuses with the membrane of the target cell and the contents of the virus enter the cytoplasm.



[Adapted from: www.hiv.lanl.gov/./Gelderblom.pdf]

Figure 2: Structure of HIV

The viral RNA and the RNA-dependent DNA polymerase (reverse transcriptase) are released into the target cell. Reverse transcriptase then directs the synthesis of a complimentary strand of DNA on the RNA template from the virus. The same enzyme also directs the synthesis of a second strand of DNA complementary to the initial DNA strand. The double-stranded viral DNA copy (proviral form) is then randomly integrated into the host's DNA and it replicates coordinately with it. When the HIV-infected cell undergoes activation or stimulation, the provirus is also activated resulting in the production and release of infectious virions.

1.3 ANTIRETROVIRAL THERAPY

When AIDS was first recognized, patients with the disease were unlikely to live longer than a year or two [3]. Since then, scientists have developed drugs that can help people infected with HIV live longer and healthier lives. Since the mid 1990's, more than 20 antiretroviral drugs belonging to 4 classes have been approved by the Food and Drug Administration (FDA) [4].

Eradication of HIV infection has proved elusive, thus lifelong treatment must be administered to combat viral replication. Highly Active Antiretroviral Therapy (HAART) is often effective at maintaining suppression of viral replication. The goal of HAART is to reduce morbidity and mortality due to HIV and AIDS as well as to improve the quality of life of people living with HIV and AIDS.

Despite significant advances in HAART during the past few years, challenges such as virologic and immunologic failures, adverse effects of antiretroviral medications, drug-drug interactions and viral resistance, still pose significant obstacles to successful treatment [5].

HAART drugs fall into 6 categories which are as follows [2]:

1. Nucleoside reverse transcriptase inhibitors (NRTIs)

These drugs provide faulty nucleotides thereby halting the viral DNA chain synthesis. Examples of NRTIs are: Stavudine (d4T), Lamivudine (3TC), Zidovudine (AZT), Tenofovir (TDF), Abacavir (ABC), Didanosine (ddI) and Efavirenz (FTC).

2. Non-nucleoside reverse transcriptase inhibitors (NNRTIs)

These drugs bind reverse transcriptase in a hydrophobic pocket located next to the active site so that the enzyme cannot carry out its copying function. Examples of NNRTIs are: Nevirapine (NVP), Efavirenz (EFV) and Etravirine.

3. Protease inhibitors (PIs)

PIs block the enzyme, protease thereby preventing the assembly and release of HIV particles from infected cells. Examples of PIs are: Lopinavir (LPV), Atazanavir (ATV), Indinavir (IDV), Saquinavir (SQV), Ritonavir (RTV) and Darunavir.

4. Fusion inhibitors (FIs)

FIs interfere with the virus' ability to fuse with the cellular membrane proteins thereby blocking entry into the host cell. The only FI approved by FDA is Enfuvirtide [2]

5. Integrase inhibitors (IIs)

These drugs target HIV's integrase protein thereby blocking its ability to integrate its genetic code into human cells. The only drug in this class approved by FDA is Raltegravir [2].

6. CCR5 Inhibitors

These drugs block the CCR5 co-receptor that HIV uses to enter and infect the cells. The only drug in this category approved by FDA is Maraviroc.

These six classes of ARV drugs are used as first-line regimen and second-line regimen. The first-line regimen consists of drugs which are used to start HAART. WHO has recommended that the first-line regimen for adults and adolescence must contain two NRTIs plus one NNRTI. This regimen is efficacious, have generic formulations, is often available as FDCs (Fixed Dose

Combinations) and do not require a cold chain. When a treatment plan is being designed it is important to maximize the durability and efficacy of any first-line regimen [4].

The second-line regimen is only used when the first-line regimen has failed. There are quite a number of options of second-line regimens available since most of them are determined by the composition of the failed first-line regimen. Patients who fail to respond to first-line regimen should be treated with a different regimen that contains drugs that were not included in the first-line [4]. The recommended second-line regimen should contain two NRTIs plus one PI.

When selecting appropriate ARV regimens for a country, the following factors should be taken into consideration [4].

- Suitability of the drug formulation, especially the availability of FDCs
- Licensing approval by national drug regulatory authorities for the product and recommended dose.
- Toxicity profile.
- Laboratory monitoring requirements.
- Potential for maintenance of future treatment options(sequencing of ARVs).
- Promotion of adherence.
- Prevalent coexistent conditions (TB and hepatitis B).
- Availability from local and international manufacturers, including procurement and supply chain logistics.

- Price and cost-effectiveness.
- Special considerations for women of childbearing potential or who are pregnant.
- Specific ARV requirements for HIV-2 infections which are naturally resistant to NNRTIs.

1.4 ANTIRETROVIRAL THERAPY IN ZIMBABWE

In 2002, the Government of Zimbabwe declared AIDS an emergency in order to mobilise and increase efforts to make the treatment of AIDS a reality [2]. This, together with the development of generic formulations of ARVs globally made it possible for Ministry of Health and Child Welfare to establish systems to introduce ART in Zimbabwe. OI clinics were established in 2003 in preparation for the introduction of ART. Zimbabwe initiated its national ART programme in April 2004 in a phased approach from 5 initial sites to all central and provincial hospitals and has now been expanded to a number of mission and district hospitals [2].

Currently the only ARV drug classes available in Zimbabwe are NRTIs, NNRTIs and PIs. IIs, FIs and CCR5 inhibitors are not yet in use in Zimbabwe. Therefore, the ARV regimens currently in use in Zimbabwe are composed of NRTIs, NNRTIs and PIs.

The first-line regimens which are currently in use in Zimbabwe for adults and adolescents are as follows.

1. Tenofovir (300mg) + Lamivudine(300mg) + Nevirapine (200mg)

*This is the preferred first-line regimen in Zimbabwe.

2. Zidovudine (300mg) + Lamivudine(150mg) + Nevirapine (200mg)
3. Stavudine (30mg) + Lamivudine(150mg) + Nevirapine (200mg)
4. Tenofovir (300mg) + Lamivudine (300mg) + Efavirenz (600mg)

*Used on patients who are co-infected with TB or who react to Nevirapine.

Although WHO has recommended for the removal of stavudine in all regimens, the majority of Zimbabweans are still being commenced on the first-line regimen containing stavudine since Tenofovir is very expensive.

The second-line regimens which are currently in use in Zimbabwe for adults and adolescents are as follows.

1. If the first-line regimen contained Tenofovir, the second-line regimen used contain Zidovudine (300mg), Lamivudine (150mg) and Lopinavir (2 tablets twice a day)
2. If the first-line regimen contained Zidovudine or Stavudine, the second-line regimen used contains Tenofovir (300mg), Lamivudine (150mg) and Lopinavir (2 tablets twice a day).
3. Abacavir (300mg) + Didanosine (400mg) + Lopinavir (2 tablets twice a day) or Atazanavir (300mg).

1.5 COMPLICATIONS OF ANTIRETROVIRAL THERAPY

Although HAART has brought in a relief to the scourge of HIV, the complications associated with it have presented a great challenge to the implementation of antiretroviral therapy. The

complications associated with the use of HAART can be divided into class-specific side effects and individual drug side effects. NNRTIs and some PIs are associated with some dermatological complications. Gastrointestinal problems are a major complication of PIs and some NRTIs.

Nearly all ARV drugs have some individual side effects as shown in Table 1 below.

Table 1: Side effects of ARV drugs

DRUG	SIDE EFFECTS
Zidovudine	Anaemia, neutropenia, headache, myopathy, lactic acidosis
Stavudine	Lactic acidosis, peripheral neuropathy, pancreatitis, lipodystrophy, fatigue, abdominal pain
Didanosine	Pancreatitis, lactic acidosis, peripheral neuropathy, abdominal pain
Abacavir	Severe hypersensitivity reactions
Lamivudine	Usually no side effects
Nevirapine	Liver toxicity, fever, mild or severe skin rashes, fatigue
Efavirenz	Central nervous system symptoms(confusion, headache,
Tenofovir	Gastrointestinal(GI) symptoms, rash, renal complications
Saquinavir	GI intolerance, diarrhoea, lipodystrophy, headache, rash, fever, dizziness
Ritonavir	Lipodystrophy, pancreatitis, hepatitis, skin sensitivity, circum-oral paraesthesia
Indinavir	Lipodystrophy, renal stones, jaundice, muscle pain, GI intolerance, rash, blurred vision
Atazanavir	Jaundice, nausea, diarrhoea, headache, hyperbilirubinaemia
Lopinavir	Lipodystrophy, GI intolerance, diarrhoea, hyperglycaemia, hyperlipidaemia

[Adapted from: Guidelines for Antiretroviral Therapy in Zimbabwe, May 2010]

The development of complications in patients on HAART can lead to clinically significant diseases and can also lead to non-adherence to antiretroviral drugs [6]. Health care providers need to understand how to diagnose and manage these complications in order to provide optimal long-term care to their patients with HIV infection. When a complication has developed it is advisable to withdraw the drug and carry out some laboratory tests to assess the extent of the complication. In most cases the patient is switched to an alternative first-line therapy. The second-line therapy is only used when all the available first-line therapies have been exhausted.

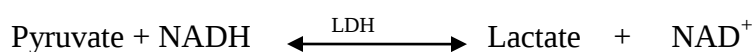
ARV side effects can be divided into four grades depending on the extent of the complication. Grade 1 side effects are those in which there is transient or mild discomfort and in such cases no medical intervention is required so the regimen is not changed. Grade 2 side effects include those in which there is mild to moderate limitation to activity and minimal medical intervention is required. In Grade 3 side effects the limitation in activity is severe and medical therapy is required. The Grade 4 side effects are life-threatening and significant medical intervention is required.

As shown in Table 1, lactic acidosis is a complication of stavudine which is present in one of the main first-line regimens still available in Zimbabwe.

1.6 HYPERLACTATEMIA AND LACTIC ACIDOSIS DURING ANTIRETROVIRAL THERAPY

1.6.1 PHYSIOLOGY OF LACTATE

Lactate is a 3 carbon intermediary of carbohydrate metabolism which is formed from pyruvate by the reversible action of lactate dehydrogenase [7]. This reaction occurs as shown in the equation below.



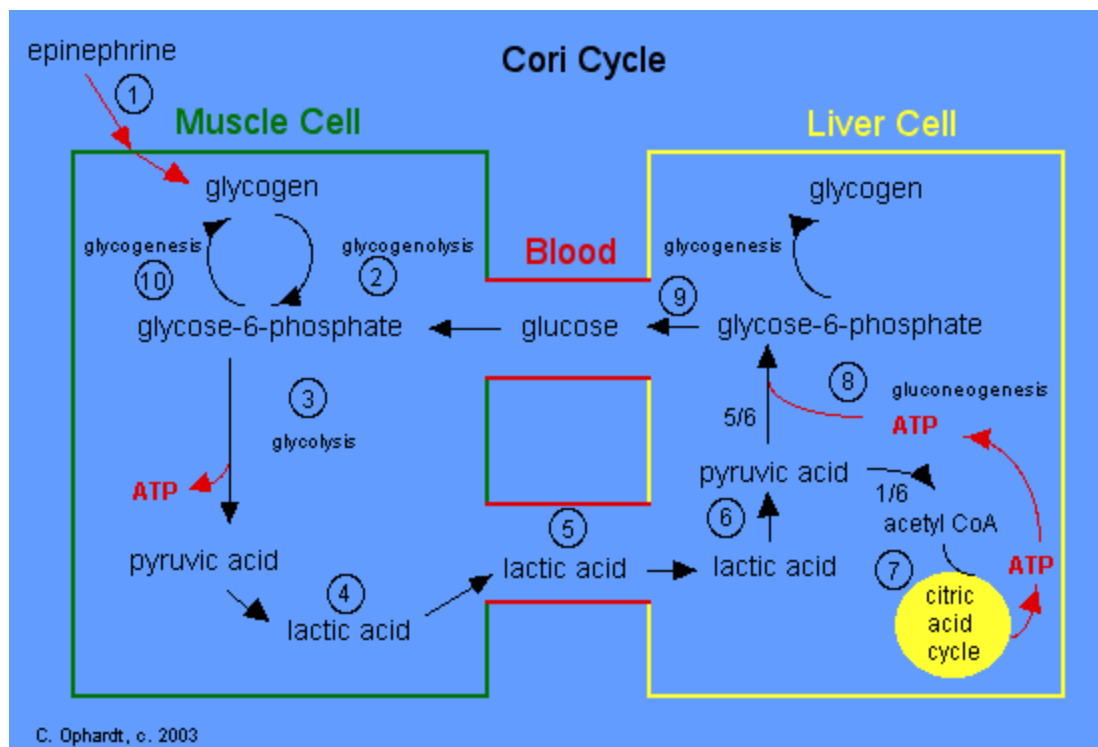
Lactate, the product of anaerobic glucose metabolism, is generated from pyruvate with lactate dehydrogenase as a catalyst. Pyruvate is normally aerobically metabolized to carbon dioxide (CO₂) and water (H₂O) in the mitochondrion during the process of gluconeogenesis.

Additionally, pyruvate is in a state of equilibrium with lactate such that under certain conditions the equilibrium can shift towards the overproduction of lactate. Lactic acidosis results from an increase in blood lactate levels when lactate production exceeds consumption and body buffer systems become overburdened. This occurs when tissue oxygenation is inadequate to meet metabolic requirements as a result of either hypoperfusion or hypoxia.

Lactate is cleared from blood, primarily by the liver, with the kidneys (10-20%) and skeletal muscles to a lesser degree [8]. Lactate exists in 2 forms, the L-lactate and D-lactate. L-lactate is the most commonly measured level, as it is the only form produced in human metabolism [8]. Its excess represents increased anaerobic metabolism due to tissue hypoperfusion. D-lactate is a by-product of bacterial metabolism and may accumulate in patients with short-gut syndrome or in those with a history of gastric bypass or small-bowel resection.

Under normal circumstances, L-lactate is produced in skeletal muscle, brain and erythrocytes and metabolized in the liver and kidney. The recycling of lactate is referred to as The Cori Cycle.

THE CORI CYCLE



[Adapted from: www.elmhurst.edu/615coricycle.html]

Fig 3: The Cori Cycle

The Cori cycle, named after its discoverers, Carl Cori and Gerty Cori, refers to the pathway whereby glycolysis in the muscle produces lactate, which is released into the bloodstream, transported to the liver and converted back to glucose for re-use in the muscle.

High levels of lactate may be produced during vigorous exercise. This occurs when energy requirements exceed oxygen supply and is known as oxygen debt. The lactate is rapidly cleared once the muscular activity ceases both by renal and hepatic clearance and by aerobic metabolism in the muscles. The half-life of serum lactate is about 60 minutes in these circumstances [8].

1.6.2 PATHOLOGY OF LACTATE OVERPRODUCTION

Pathological lactate production occurs when there is inadequate tissue oxygen supply or if there is defective pyruvate clearance or increased pyruvate production. Hypoxia is by far the most common cause of lactic acidosis and can occur with shock from any cause. Other factors which may cause tissue hypoxia include severe asthma, severe anaemia, regional hypoperfusion and carbon monoxide poisoning. The rate of lactate recovery after shock is much slower than after exercise with an average clearance half-life of about 18 hours [9]. There are quite a number of mechanisms which cause accumulation of lactate. Some of these mechanisms include the one which causes a blockage in the further metabolism of pyruvate, e.g. cyanide, nucleoside analogues, salicylates or mitochondrial cytopathies affecting the electron transport chain, thiamine deficiency which reduces the activity of pyruvate dehydrogenase, and defects of gluconeogenic enzymes due to inborn errors or alcohol effects [9]. Other mechanisms are those that lead to increased production of pyruvate, e.g. increased glycolysis with adrenaline treatment, and protein breakdown increasing the conversion of alanine to pyruvate in sepsis. The elevated pyruvate levels which arise from the above mechanisms then equilibrate via LDH to produce increased lactate levels.

In the case of hypoxia, or other causes of inhibition of the electron transport chain, NADH accumulates and the supply of NAD is depleted. This pushes the balance between pyruvate and lactate dramatically in favour of lactate thus further exacerbating its production in these circumstances.

Hyperlactatemia is defined as a serum lactate level of greater than 2.5 mmol/L [10]. The reference range for serum lactate is 0.5-2.5mmol/L. If the level exceeds 5mmol/L then lactic acidosis develops in which the blood pH goes below 7.35. This condition is fatal if not treated immediately [9].

In normal conditions, the majority of cellular energy needs are satisfied via metabolism of pyruvate in Krebs cycle in the mitochondria which requires oxygen and water [8]. An additional small quantity of ATP is derived by the cytosolic (anaerobic) cleavage of pyruvate thereby producing lactate and water as by-products. Approximately 1400mmol of lactate is produced daily [11]. Of note, there is no net production of hydrogen ions in this process.

Hyperlactatemia occurs when either increased production, increased cellular release of lactate or decreased utilization of lactate occurs and is not, therefore, always accompanied by or a prelude to acidosis [9].

1.6.3 LACTIC ACIDOSIS

Classically, lactic acidosis was described and classified by Cohen and Woods into 2 categories namely Type A and Type B with the main differentiating point being the adequacy of tissue

oxygen delivery. In both types, the fundamental problem is the inability of the mitochondria to deal with the amount of pyruvate with which they are presented [8].

(i). Type A lactic acidosis

This is lactic acidosis in which there is decreased tissue ATP in the setting of poor tissue perfusion or oxygenation. In this type of lactic acidosis there is clinical evidence of inadequate tissue oxygen delivery. This is the most common clinical situation. The inadequate oxygen supply slows mitochondrial metabolism and pyruvate is converted to lactate. This type of lactic acidosis occurs in the following conditions: anaerobic muscular activity (e.g. sprinting), tissue hypoperfusion (e.g. acute heart failure) and reduced tissue oxygen delivery or utilization (e.g. severe anaemia).

(ii) Type B lactic acidosis

Type B lactic acidosis refers to situations in which there is no clinical evidence of reduction in tissue oxygen delivery such as those ones seen in Type A. In this type of lactic acidosis, carbohydrate metabolism is disordered for some reason and excess lactic acid is formed.

Type B is divided into 3 subtypes based on underlying etiology.

Type B1 occurs in association with systemic disease such as renal and hepatic failure, diabetes and malignancy.

Type B2 is caused by several classes of drugs such as stavudine, salicylates, isoniazid and zidovudine.

Type B3 is due to inborn errors of metabolism e.g. congenital forms of lactic acidosis with various enzyme defects such as pyruvate dehydrogenase deficiency.

The onset of lactic acidosis may be either abrupt or insidious. Initial symptoms often include nausea, vomiting and abdominal pain although in more insidious cases fatigue and weight loss may predominate [11]. A tender enlarged liver may be palpable. Subsequently, shortness of breath, tachypnea, hyperventilation, liver or renal failure, clotting abnormalities, seizures, cardiac arrhythmia and death ensue. Although the condition is easily confirmed and quantified by measuring the serum lactate level, other biochemical abnormalities include acidosis with $\text{pH} < 7.35$, low bicarbonate, widened anion gap and elevated lactate dehydrogenase. Histologic examination of the liver may reveal diffuse microvesicular steatosis with slightly enlarged mitochondria.

1.6.4 ANTIRETROVIRAL DRUGS AND HYPERLACTATEMIA

Lactic acidosis represents a rare complication of HAART but a common challenge in critical care medicine [12]. Lactic acidosis with or without hepatic steatosis is the most common serious presentation of NRTI toxicity since it has been reported during therapy with all NRTI [15].

The source of H^+ which is key to the development of acidosis is the hydrolysis of ATP to ADP. In normal circumstances this H^+ is rapidly reutilized in the mitochondria-located oxidative phosphorylation process. Thus for acidosis to occur, oxidative metabolism must be impaired [15]. In the case of lactic acidosis, the acidosis is accompanied by raised lactate (hyperlactatemia). The clearance of lactate through its conversion back to pyruvate hence back to

glucose (gluconeogenesis) in the Cori cycle is ATP dependent. If the cell is energy deficient this conversion cannot occur thereby leading to accumulation of lactate.

A variety of NRTIs including stavudine, zidovudine and didanosine have been associated with hyperlactatemia because of their potential for mitochondrial toxic effects [17]. The pharmacologically active triphosphates moieties of NRTIs act as substrates for human mitochondrial DNA polymerase γ , thereby having the potential for incorporation into mitochondrial DNA (mtDNA) [15]. On the other hand, mitochondrial exonuclease is inefficient at removing these nucleoside triphosphates. The combination of nucleoside incorporation and inadequate removal disrupts mtDNA synthesis thereby resulting in the arresting of mtDNA-encoded protein synthesis. This causes the disruption of oxidative phosphorylation thus leading to accumulation of lactate.

Stavudine, a nucleoside analogue of thymidine is phosphorylated by cellular kinases to the active metabolite, stavudine triphosphate [17]. Stavudine triphosphate inhibits the activity of HIV-1 reverse transcriptase by competing with the natural substrate thymidine triphosphate and by causing DNA chain termination following its incorporation into viral DNA. Stavudine triphosphate inhibits cellular DNA polymerases β and γ and markedly reduces the synthesis of mitochondrial DNA.

Clinically, mitochondrial toxicity pertaining to serum lactate imbalance manifests in 1 of 3 forms, the most severe of which is a syndrome of fulminant lactic acidosis associated with gastrointestinal complaints and respiratory distress [20]. Although reports of lactic acidosis consistently feature stavudine as a component of the causative antiretroviral regimen, there is

insufficient evidence at present to quantify the risk of lactic acidosis among the various NRTIs [19].

Lactic acidosis has been reported in persons receiving both single and dual NRTI regimens. However, hyperlactatemia does not inevitably lead to acidosis [19]. Recent surveys of individuals on NRTI therapy indicate hyperlactatemia to be a relatively common event occurring in 10-20% of individuals whereas acidosis remains rare at less than 0.5% of treated individuals [17]. Lactic acidosis is rarely seen in individuals with serum lactate levels below 5 mmol/l. Elevated serum lactate levels may be related to sample collection errors such as tourniquet use, delayed sample processing or recent patient activity.

Patients treated for HIV infection may have increased cellular energy requirements. Elevated basal lipolysis, increased fat turnover and elevated endogenous glucose production and turnover have been reported in patients on HAART [19]. In such patients adipocytes and myocytes may release lactate into the systemic circulation more readily. Patients on antiretrovirals for prolonged periods commonly have metabolic disturbances including hyperinsulinaemia and glucose intolerance with hyperglycaemia. Both hyperinsulinaemia and hyperglycaemia stimulate lactate release from adipocytes in vivo.

Quite a number of studies on the effect of stavudine on serum lactate levels have been carried out in other countries. In Toronto at St Michael's Hospital in 2003 Tony Antoniou, Thea Weisdorf and Kevin Gough carried out a case study of one HIV-positive patient who was started on stavudine, nevirapine and didanosine 6 months after the failure of his initial regimen of zidovudine, lamivudine and nelfinavir [16]. 5 months after the initiation of this second regimen the serum lactate level was 3.7 mmol/l (Ref range: 0.5-2.5 mmol/l). The following week the

lactate level was 6.1 mmol/l. The regimen was discontinued and after one month the serum lactate level was 2.0 mmol/l and the symptoms of lactic acidosis disappeared. In this study only one patient was involved therefore it's difficult to draw some concrete conclusions from these results hence there is need for studies involving a large sample.

A study involving a larger sample was carried out in 2000 in Sweden by Boubaker K, Flepp M, Sudre P, et al [20]. It was a cross sectional study aimed at determining the prevalence, clinical presentation and risk factors for hyperlactatemia among 880 patients receiving antiretroviral therapy in the Swiss HIV Cohort Study. Seventy three (73) patients presented with an increase in serum lactate levels. For 9 patients, the lactate elevation was severe. The number of patients who developed hyperlactatemia was higher in those ones who were receiving stavudine-containing regimens than in those receiving zidovudine based regimens. It was also discovered that the magnitude of the elevation increased with increasing time of receiving stavudine. Although the sample size was good, a cross sectional study would not be appropriate since there is need to know the baseline serum lactate level before commencing the antiretroviral therapy. Better results can be obtained from a good prospective cohort study.

In Washington DC, a case study on lactic acidosis and hepatic steatosis associated with the use of stavudine was carried out at a community hospital [17]. In the study, 4 HIV-positive patients who were taking stavudine presented with lactic acidosis and elevated levels of aminotransferases. All patients had high levels of serum lactate and histologic findings on their liver and muscle biopsies were consistent with mitochondrial injury which is associated with NRTI-induced lactic acidosis. Once again the sample size was not adequate to draw some concrete conclusions on the association of stavudine with lactic acidosis.

1.7 SCOPE OF THE STUDY

The stavudine-containing first-line regimen is still being used on a large scale in Zimbabwe despite calls by WHO to phase out Stavudine and replace it with other NRTIs [2].

The question is, does this mean that the Triviro (stavudine, lamivudine and nevirapine) antiretroviral therapy being used in Zimbabwe does not cause any hyperlactatemia or is it that the dosage of 30mg of stavudine, 150mg of lamivudine and 200mg of nevirapine in each Triviro tablet being taken by HIV-positive patients in Zimbabwe is not high enough to cause symptomatic hyperlactatemia?. Currently no studies have been carried out in Zimbabwe to find out the effect of stavudine-containing antiretroviral therapy on serum lactate levels. Thus this study was set to find out the impact on serum lactate levels of the stavudine-containing ARV first-line regimen being used in Zimbabwe.

1.8 AIMS OF THE STUDY

To determine the effect of stavudine, lamivudine and nevirapine treatment (marketed as Triviro) on serum lactate levels in HIV positive adults aged between 20 and 60 years attending BRIDH (Beatrice Road Infectious Diseases Hospital) and WIDH (Wilkins Infectious Disease Hospital) Opportunistic Infections clinics in Harare.

1.9 OBJECTIVES OF THE STUDY

1. To evaluate the extent of the safety of continual use of stavudine-containing antiretroviral drugs for HIV treatment in Zimbabwe.
2. To determine the risk factors associated with the development of hyperlactatemia in patients on stavudine-containing antiretroviral drugs in Zimbabwe.

CHAPTER 2: METHODOLOGY AND MATERIALS

2.1 STUDY DESIGN

This was a prospective cohort study in which 180 HIV-positive adult patients who were about to be initiated on HAART receiving STALANEV(stavudine, lamivudine and nevirapine) at BRIDH (Beatrice Road Infectious Diseases Hospital) and WIDH (Wilkins Infectious Diseases Hospital) OI clinics were enrolled into the study.

BRIDH and WIDH are City of Harare-owned hospitals whose core business is to treat infectious diseases such as TB, diarrhoeal diseases and HIV. An OI clinic is located in each hospital and it deals with HIV and opportunistic infections associated with HIV. BRIDH OIC serves patients from the southern and western suburbs of Harare while WIDH OIC caters for patients from the northern and eastern suburbs. The annual patient load of each clinic is almost the same and is around 6 000. The OICs are manned by 1 medical doctor, 1 sister in charge, 5 nurses, 4 counselors, 1 clerk and 2 clinical attendants.

Before a patient is commenced on ARVs, intensive counseling is done so that he/she is educated on importance of drug adherence and is strongly urged to adhere to the review dates given by the nurses. If a patient misses the review date, recounseling is done before being issued another supply and it's a tedious process which patients do not like to undergo frequently. All these measures are done so as to ensure that patients are followed at regular intervals.

After enrolling at these two clinics, patients are monitored on monthly basis for 6 months and after the medical personnel is satisfied that the patients are stable, they are transferred to local clinics for drug collections.

Ninety (90) patients were enrolled from each clinic using the convenient sampling method. The required sample size was attained over a period of two weeks.

Blood was collected from these patients and their serum lactate levels was measured over a period of 4 months at 2 months intervals i.e. just before HAART initiation(baseline), after 2 months and 4 months on HAART.

2.2 STUDY POPULATION

Equal numbers of the study participants were enrolled from WIDH and BRIDH OI clinics (each clinic supplied 90 participants). A sample size of 160 was calculated using the following formula which is used to calculate sample size in a longitudinal analysis involving repeated measurements from a subject and also basing on literature where other longitudinal studies which have been done have shown that the mean change in serum lactate levels due to antiretrovirals is about 1.2mmol/l. The final sample size used was 180 so as to cater for drop outs.

$$N = \frac{2\theta^2(1-\rho)(Z_{\alpha/2} + Z_{\beta})^2}{ms_x^2d^2}$$

Where

m = number of repeats per person

N = Sample size

d =smallest difference of clinical importance

ρ = correlation between repeats

$$s_x^2 = \frac{\sum_{j=1}^m (x_j - \bar{x})^2}{m}$$

θ^2 = covariance

The inclusion criteria for the study were as follows:

1. HIV-positive
2. HAART naive at the time of recruitment
3. Eligible for initiation on STALANEV (stavudine, lamivudine and nevirapine)
4. Aged between 20-60 years
5. To be enrolled for HAART at BRIDH or WIDH OI clinic
6. Have consented to participate in the study
7. A permanent resident of Harare-so as to be easily followed up
8. Have a life expectancy of at least 4 months based on clinical assessment-so as to last the 4-months follow-up period for the study.

The patient was excluded from the study if he/she had the following characteristics

1. outside the 20-60 years age group
2. Was on anti-TB treatment, hypertensive drugs, hypoglycemics or any long-term drug treatment.
3. Hyperlactatemia at baseline.

The study was approved by The College of Health Sciences Joint Research Ethics Committee.

Permission to use patients from BRIDH and WIDH OI clinics and also to run the samples at

WIDH Laboratory was sort from The City of Harare Ethics Committee. All the patients gave

written informed consent and provided their demographic and health status data on a questionnaire which was provided.

2.3 SAMPLE COLLECTION AND ANALYSIS

Sample collection was done by an informed and qualified phlebotomist. A maximum volume of 3 millilitres of blood was drawn from each participant at each visit using vacutainer needles and then collected in fluoride tubes then immediately placed on ice. The use of a tourniquet was avoided in most patients but for those samples which were collected at 4 months, the tourniquet was frequently used since the veins were constricted due to the cold weather. In order to avoid false elevations of serum lactate, the samples were centrifuged and plasma was separated from the blood cells within 30 minutes from time of collection. The samples were processed in the laboratory situated within WIDH premises. As for the samples from BRIDH OI clinic, the plasma was separated at BRIDH laboratory, placed on ice in a cooler box and immediately transported to WIDH Laboratory for processing. Those samples which were not processed on the day of their collection were stored at -85°C in a freezer until their time of analysis. It is documented that serum lactate is stable for at most 3 months when stored at this temperature.

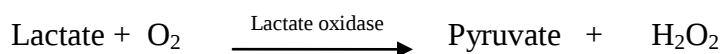
The samples were collected after ensuring that the patient had rested for at least 20 minutes so as to avoid elevated lactate levels due to exercise.

2.4 SERUM LACTATE DETERMINATION

The serum lactate analysis was performed on Selectra E analyzer using an ABX Pentra Lactic Acid commercial kit supplied by HORIBA ABX. The analyzer was calibrated as per manufacturer's specifications using the ABX Pentra Multicalibrator and the internal quality control was done using the ABX Pentra Normal control and ABX Pentra Pathologic Control as recommended by the manufacturer and in accordance with the standard operating procedure of the WIDH City Health Laboratory.

2.5 PRINCIPLE OF SERUM LACTATE DETERMINATION

The kit uses the Trinder method which is an enzymatic colorimetric assay. Lactate oxidase triggers the release of hydrogen peroxide, which reacts with 4-aminoantipyrine and ESPAS to form a coloured complex, Quinoneimine in the presence of peroxidase. The intensity of the colour formed is measured at 505nm and is proportional to the amount of lactate present in the sample.



(ESPAS = N-ethyl-N-sulfopropyl-m-anisidine)

CHAPTER 3: RESULTS AND STATISTICAL ANALYSIS

Although the calculated sample size was 160, a total of 180 adult participants were recruited into the study to cater for drop outs. Ninety (90) participants were recruited from Beatrice Road Infectious Diseases Hospital Opportunistic Infections Clinic (BRIDH OIC) and the other 90 were from Wilkins Infectious Diseases Hospital Opportunistic Infections Clinic (WIDH OIC). The baseline characteristics of the study population are summarized in Table 2 below.

Table 2: Baseline characteristics of the study population who initiated ART at WIDH OIC and BRIDH OIC

Characteristic	N=180
Age (yrs) [median (IQR)]	35 (28-43)
Gender	
Female	113 (63.1%)
Male	67 (36.9%)
HIV WHO staging	
II	23 (12.9%)
III	154 (85.5%)
IV	3 (1.7%)
CD4 cell count (cell/ μ L)	
<200	177 (98.3%)
200-350	3 (1.7%)
Mean Serum lactate (median IQR)	1.6 mmol/l (1.3-1.9 mmol/l)

Note: Data are n (%), unless otherwise stated.

The CD4 cell counts were only measured at zero months. It was difficult to measure CD4 cell counts at 2 and 4 months after initiation since the policy of BRIDH and WIDH OI clinics is that CD4 cell counts are repeated after 6 months on HAART.

The age distribution of the participants is shown in Figure 4 below.

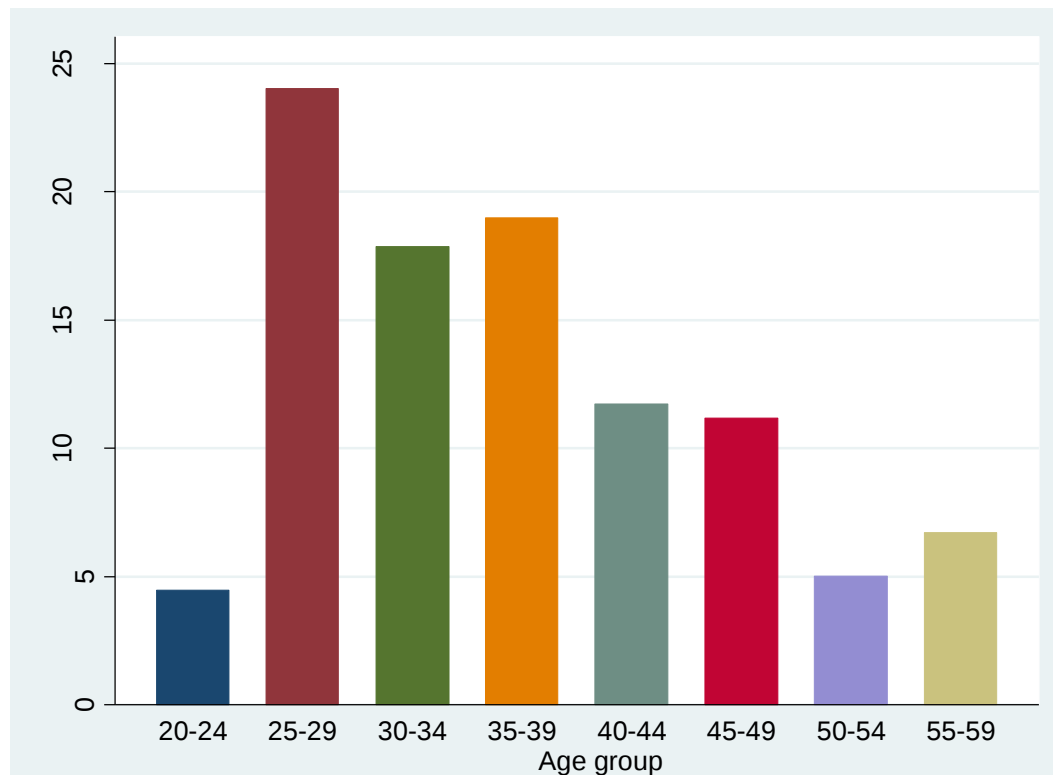


Figure 4: Age of participants in the study

The study participants mainly constituted of young adults as evidenced by the mean age of 35 years. Most of the participants were in the age group 25 to 39 years. There were few participants in the ages 50 to 59 years.

Female participants were more than their male counterparts in the age groups 20 to 54 years as shown in Figure 5 overleaf. As for the age group 55 to 59 years the male participants were more than the female counterparts.

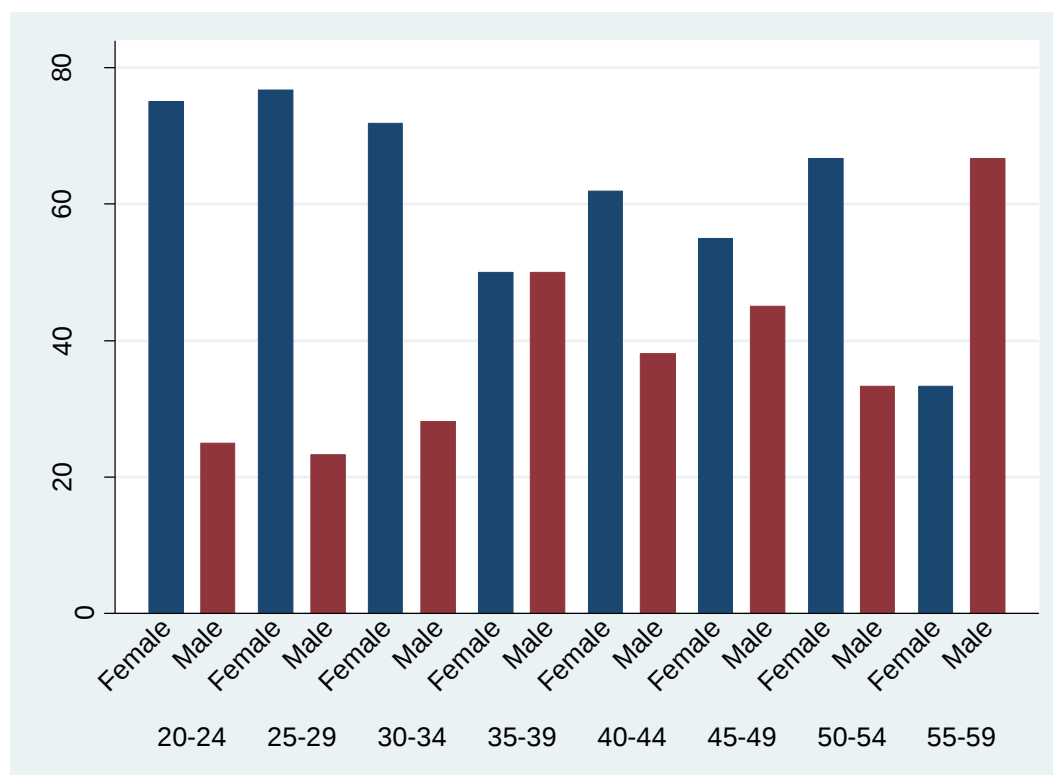


Figure 5: Age and sex of study participants

As for the whole group the total number of females was higher than that of males as shown in Table 2 on page 27. The data is showing that that the number of females was almost double the number of males.

Most of the participants were in the HIV WHO stage III as shown in Table 2 on page 17. None of the participants were in the HIV WHO stage I and only a small number was in the HIV WHO stage IV.

The baseline CD4 counts of most of the participants were in the range 100 to 200 cells/ μ l as shown in Figure 6 overleaf. Only a very small proportion was above 200 cell/ μ l.

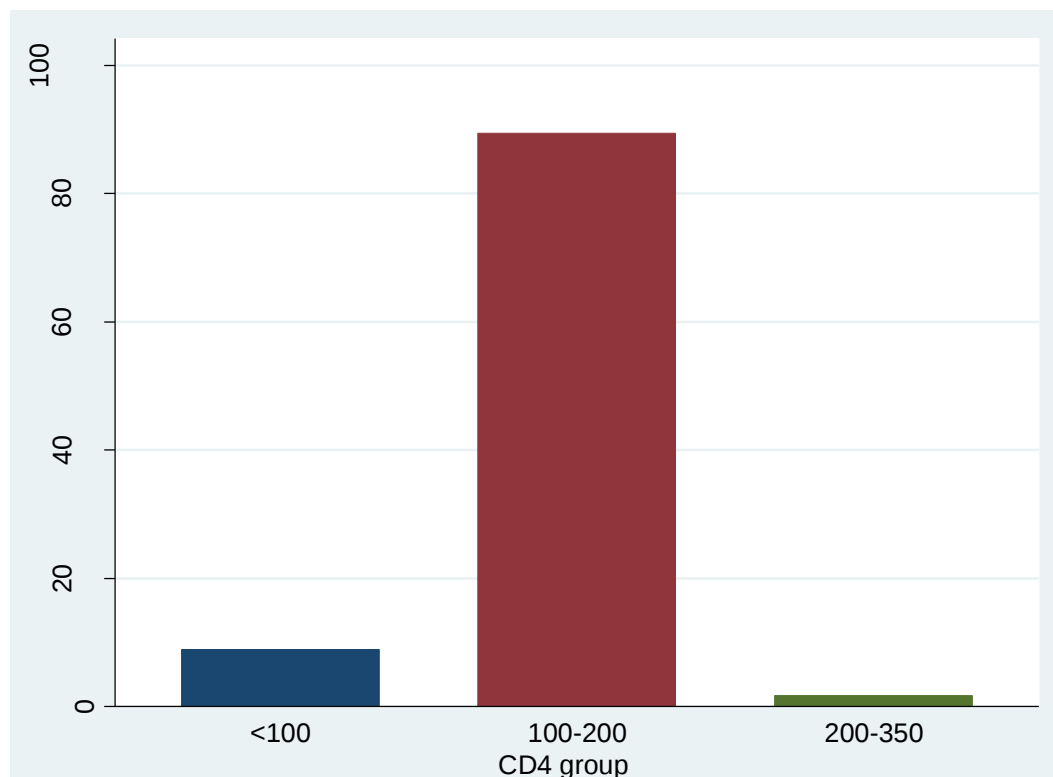


Figure 6: Baseline CD4 counts of the participants

The individual graphs of each patient's serum lactate levels over the four months follow up period are shown in Figure 7 overleaf. The individual graphs of each patient shown reveal that most of the patients' lactate levels were increasing throughout the 4 months study period. Only a few participants had serum lactate levels which decreased from baseline to 2 months after ART initiation.

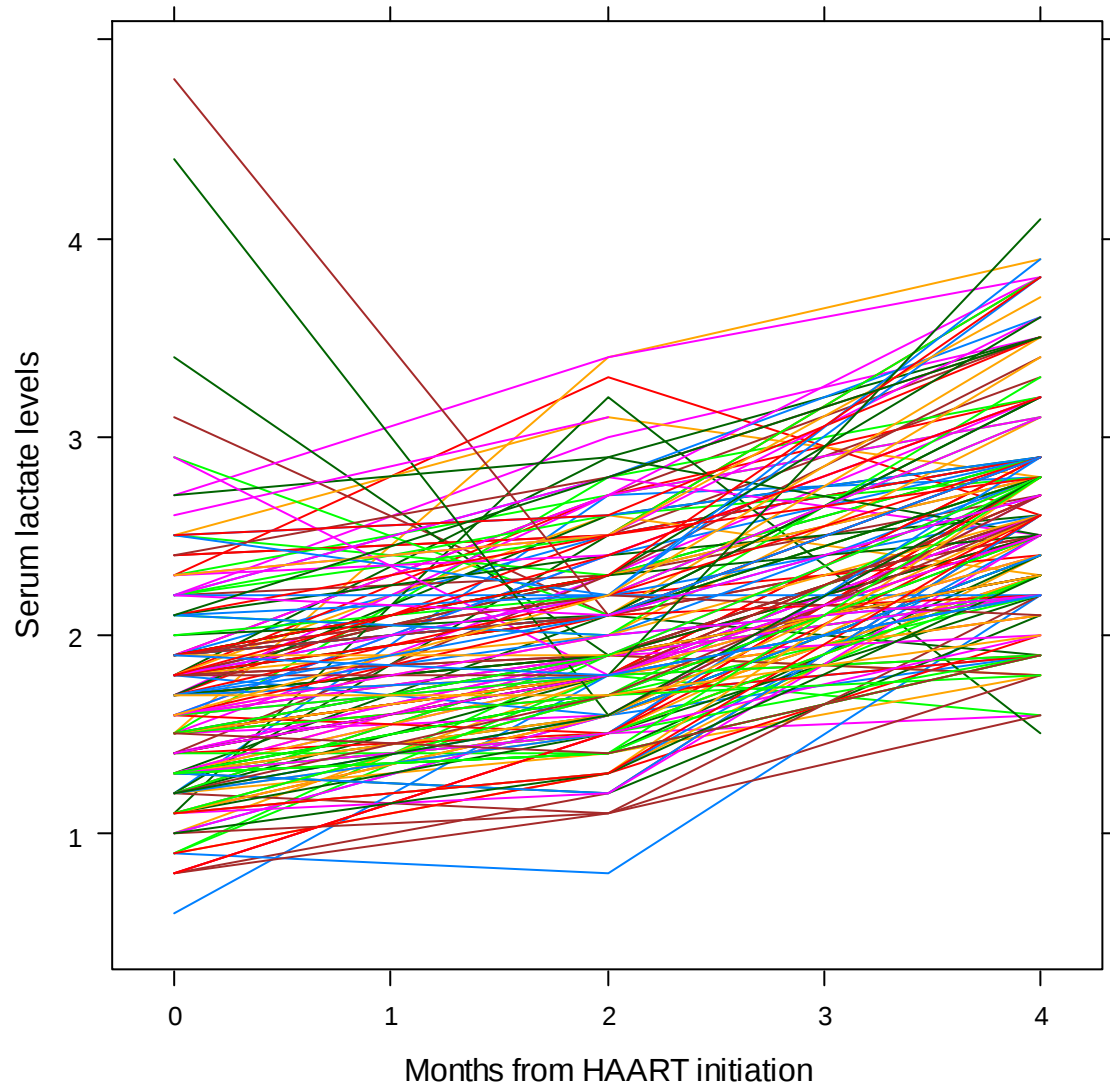


Figure 7: Serum lactate levels of all study participants from baseline to 4 months after HAART initiation

.The average percentage changes in serum lactate levels of the participants over the 4 months period are tabulated in Table 3 below. The change between the 0 and 2 months samples was 20% and this was lower than the change between 2 and 4 months which was 33%. A huge change of 59% between the baseline and the 4 months samples was obtained.

Table 3: Mean % Changes in serum lactate levels over the 4 months period

PERIOD	MEAN % CHANGE IN SERUM LACTATE LEVELS
0-2 MONTHS	20
2-4 MONTHS	33
0-4 MONTHS	59

The mean serum lactate levels of the patients were plotted in Figure 8 overleaf. As seen from the graph it is evident that there was a general increase in serum lactate levels from 0 months to 4 months but the increase from 0 months to 2 months was less than that one from 2 months to 4 months. There was a sharp rise in the mean serum lactate levels from 2 months to 4 months. The baseline and the 2 months mean serum lactate levels were within the reference range (0.5 mmol/l-2.5 mmol/l). However, the mean serum lactate levels for the 4 months samples was 2.7 mmol/l which was slightly above the reference range.

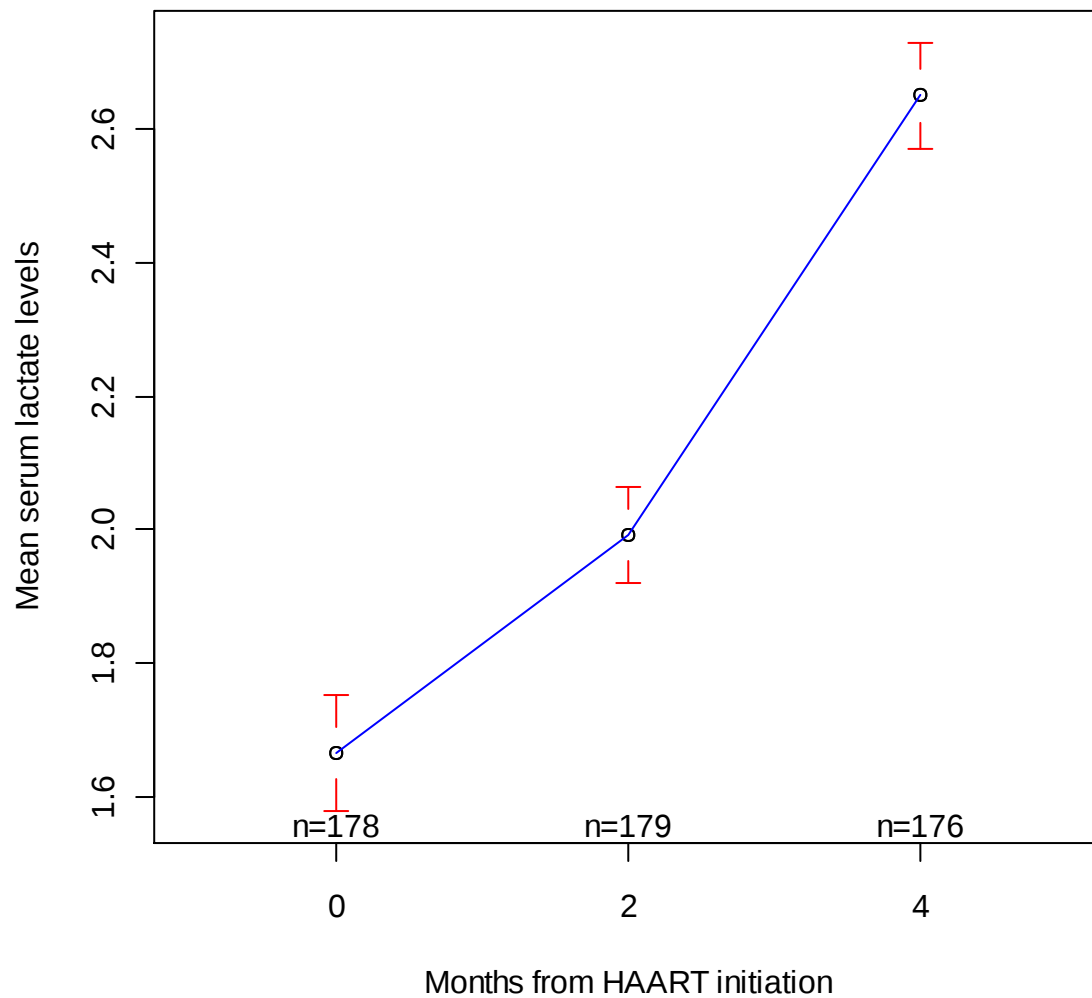


Figure 8: Mean serum lactate profile of the participants over the 4 months period

Table 4 overleaf shows the serum lactate levels of participants at the end of the 4 months follow-up period.

Table 4: Serum lactate status of participants at the end of the 4 months follow-up period

SERUM LACTATE LEVEL (mmol/l)	No OF PARTICIPANTS	% OF PARTICIPANTS
< 2.5	81	46.0
2.6-3.5	84	47.7
3.6-3.9	11	6.3
>3.9	0	0

Data in the table is revealing that 96(54.3%) participants had serum lactate levels above the reference range after the 4 months follow-up period. Out of the 96 participants, 12 (6.8%) had serum lactate levels which were above 3.5 mmol/l. These 12 patients were referred to the clinicians for further management and their serum lactate levels was monitored before changing the HAART regimen. The serum lactate levels of 81(45.8%) participants remained within the normal range although there was an increase over the 4 months follow-up period. There were no participants who developed serum lactate levels above 3.9 mmol/l.

Table 5 overleaf shows the different categories of the serum lactate levels and their classification.

Table 5: Categories and classification of serum lactate levels

SERUM LACTATE LEVEL (mmol/l)	CLASSIFICATION
< 2.5	Normal
2.6-3.5	Hyperlactatemia
3.6-4.9	Mild lactic acidosis
>5.0	Severe lactic acidosis

Basing on these categories and classifications, we can therefore represent the proportions of the serum lactate levels of participants at the end of the 4 months follow-up period as a graph in Figure 9 overleaf. The graph is showing that 81 (45.8%) participants remained normal, 84 (47.5%) developed hyperlactatemia, 12 (6.8%) developed mild lactic acidosis and none developed severe lactic acidosis over the 4 months follow-up period.

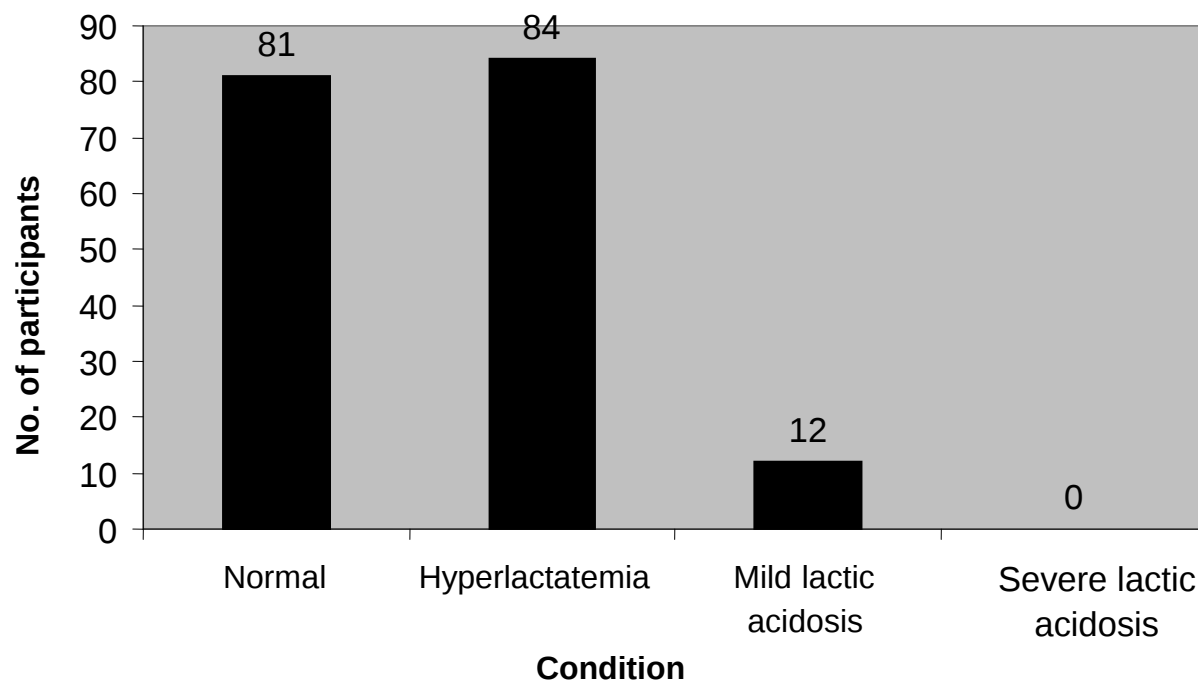


Figure 9: Serum lactate status of participants at the end of the 4 months follow-up period

Although the results of the study have shown that STALANEV treatment have an effect on serum lactate levels, a univariate analysis of risk factors associated with hyperlactatemia was done and the results are shown in Table 6 overleaf.

Table 6: Univariate analysis of risk factors for hyperlactatemia in patients on ART

Factor	Effect (se)	P-Value	95% CI
Age (yrs)	-0.0029 (0.003)	0.39	-0.01-0.004
Gender	-0.011 (0.068)	0.88	-0.14-0.12
HIV WHO staging	+0.18 (0.89)	0.043	0.01-0.35
CD4 cell count (cells/ μ l)	-0.005 (0.001)	<0.001	-0.01 -0.002

In a univariate analysis, if $p < 0.05$ it implies that the factor has a significant effect. Age, gender, HIV WHO staging and baseline CD4 counts were the only factors which were analyzed since that was the only data which was collected at baseline. If funds were available other factors would have been analyzed.

As seen from Table 6, age and gender have no effect on serum lactate levels since their p-values are greater than 0.05. HIV WHO staging and CD4 cell count have an effect on the development of hyperlactatemia since their p-values are less than 0.05.

CHAPTER 4: DISCUSSION

The results of the study are indicating that STALANEV treatment is causing an increase in serum lactate levels (hyperlactatemia). Although the study was done over a short period of time, information from the data has shown that the effect might be more apparent as the treatment period increases. It was observed that from baseline to 2 months there was a 20% increase in levels of lactic acid and a 59% increase from baseline to 4 month. This indicates that the increase is likely to be a lot more had the study period been longer.

Although 81 (46.0%) participants had lactate levels remaining within the normal range (0.5-2.5 mmol/l), the levels increased in 77 of these participants over the 4 months period. Three participants had decreased lactate levels and one had lactate levels which remained unchanged. The decrease of lactate levels in the three participants could be attributed to haemolysis induced during baseline sample collection or to lack of proper rest of the patients before the baseline collection. These problems were mainly encountered during baseline collections since this was the first time to collect samples for lactate levels determination. There was lack of insight of sample collection techniques. However, the problems were rectified in the second and third sample collection sessions.

Hyperlactatemia developed in 95 (54.0%) participants, with 11 (6.3%) developing mild asymptomatic lactic acidosis by the end of the 4 months period. None of the participants developed severe lactic acidosis. This was maybe due to the short period of follow up accorded to the participants since it has been documented that prolonged exposure to NRTIs especially stavudine is associated with adverse effects related to mitochondrial toxicity [19].

A true picture was likely going to be obtained if the study was done for a period of between 6 and 12 months. This was not possible due to the short period of time available for the submitting of the project and the long time it took to get the project approved by the City of Harare Ethics Committee.

The results of the study have not shown any relationship between hyperlactatemia and age or gender of the patient. There are indications that HIV WHO stage and CD4 cell count have a bearing on the development of hyperlactatemia in HIV-positive patients on stavudine-containing ART regimens. Patients in HIV WHO stages 3 and 4 and those with low CD4 counts are showing higher chances of developing hyperlactatemia.

RECOMMENDATIONS

Basing on the outcome of this study, it can be recommended that a slightly longer follow up period on lactate levels of patients on stavudine containing HAART would be of value. Should the lactate levels continue to rise, the treatment regimen should be stopped and an alternative one be instituted.

Note, the 2009 WHO recommendations proposed that countries progressively phase out the use of stavudine as a preferred first-line therapy and move to less toxic alternatives such as Zidovudine (AZT) and Tenofovir (TDF).

Although the Ministry of Health and Child Welfare has said that a systematic approach to the phasing out of stavudine will be implemented over time, it might be necessary to find ways of shortening the implementation time basing on the findings of this study.

The new regimens are available but on a smaller scale because currently the City of Harare OI clinics which are the biggest in the country are currently commencing patients on STALANEV unless the patient is co-infected with TB. They have limited stocks of TENOLAM (Tenofovir and Lamivudine) which is only being given to patients who have developed side effects of STALANEV.

Depending on symptoms of lactic acidosis the patient is presenting, the assessment of electrolytes and acid-base status of the patient might also be necessary. Histologic examination of a liver biopsy might also be of assistance in trying to investigate the cause of lactic acidosis. The presence of diffuse microvesicular steatosis with slightly enlarged mitochondria is typical of lactic acidosis due to NRTIs [19].

CONCLUSION

Hyperlactatemia, although frequently asymptomatic and transient is still an underestimated problem associated with antiretroviral therapy. The NRTIs which may cause hyperlactatemia are: Stavudine, Didanosine, Zidovudine and Zalcitabine. In Zimbabwe, stavudine and zidovudine are the ones which are currently in use in all public health institutions. This study has proved that the prolonged use of stavudine-containing HAART can lead to hyperlactatemia. Although the percentage of the participants who developed mild lactic acidosis was small, I think if time was permitting it was necessary to follow the patients for a longer period and see if there would be an increase in the proportion of the participants who will develop lactic acidosis.

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