PROFILING THE RISK FACTORS OF LACTIC ACIDOSIS IN HIV POSITIVE ADULT PATIENTS ON ANTI-RETROVIRAL TREATMENT IN SOUTH AFRICA IN THE PUBLIC SECTOR

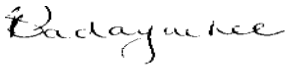
By: Neelaveni Padayachee

A research dissertation submitted to the Faculty of Health Sciences, University of Witwatersrand, Johannesburg, in fulfillment of the requirements for the degree of Master of Pharmacy

Johannesburg, 2011

DECLARATION

I Neelaveni Padayachee, declare that 'Profiling The Risk Factors of Lactic Acidosis In HIV Positive Adult Patients on Anti-retroviral Treatment In South Africa In The Public Sector' hereby submitted to the University of Witwatersrand for the degree of Master of Pharmacy has not previously been submitted by me for a degree at this or any other university; that it is my work in design and in implementation, and that all material included herein has been appropriately acknowledged.



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ABSTRACT

Background: *According to the 2010 edition of the UNAIDS* Report on the global AIDS epidemic, an estimated 320 000 (20%) fewer people died of AIDS-related causes in South Africa in 2009 than in 2004 due to the increase in availability of anti-retroviral medicines.(2) With this positive trend, the mindset should be shifted towards reducing adverse effects of ART. The need for permanent ART treatment and the significant increase in life expectancy have led to the observation of new, frequent, and sometimes severe drug-related adverse effects.(4) One of the most challenging and potentially dangerous side-effects is hyperlactataemia (Hlac) that may evolve to lactic acidosis (LA)(5) ART–associated Hlac may be asymptomatic, or symptomatic which in the extreme case can progress to life threatening acidosis. The latter, i.e. lactic acidosis is a fairly frequent and often misdiagnosed or under diagnosed and potentially fatal side effect of ARTs. (6)

Objectives: To explore the relationship between Hlac/LA and gender, weight, dosage CD4 and regimen alterations in HIV patients on ARTs and to compare the earlier regimens to the revised regimens as independent risk factors for Hlac and LA. Sample size would be based on the hypothesis that newer regimens would reduce the incidence of Hlac and LA.

Methods: A **Retrospective study** was conducted by reviewing 3 741 patient files from August 2004 to December 2007. This study was to assess the incidence and risk factors of Hlac/LA. Hlac was defined as a venous lactate measurement of ≥2.3mmol/L and LA was ≥5mmol/L. Immunological, virological, haemotological and biochemical results were recorded for all the patients. A second phase involved a **Prospective study**. Patients who were on treatment for >12 months were randomly selected from the queue at the clinic between the September 2008 and December 2009. Immunological, virological, haematological and biochemical information was recorded for all patients selected. Analysis involved descriptive statistics, comparison of means, frequency analysis and multivariate analysis.

Results: Two-hundred and thirty two patients were identified with elevated lactate levels in the **retrospective study**. The incidence was 6.2% in this population, with gastro-intestinal symptoms, peripheral neuropathy, abdominal tenderness, rash and upper respiratory tract infection being the significant symptoms. The major risk factor was a low CD4 count. The prospective study included 292 patients with 24.3% with Hlac/LA with peripheral neuropathy (*p* 0.209), gastrointestinal symptoms (nausea, vomiting) (*p* 0.148) and abdominal tenderness (*p* 0.214) were the most significant symptoms. In terms of the hypothesis that newer regimens would lower the incidence of elevated lactate levels by 50%, the observed incidence of 24.3% is no different from previously reported rates. This therefore shows that although regimen changes have been implemented the overall incidence of Hlac appears to be unchanged but the LA rate was found to be significantly lower than before, 6.8 cases per 1000 patient years vs ±19 cases per 1000 person years.(16) Gastro-intestinal symptoms but not peripheral

neuropathy; with low CD4 count, weight loss and low weight on entry were the significant risk factors, which is most likely representative of advanced disease. **Conclusions**: Although newer regimens have been introduced, Hlac/LA still exist. Healthworkers need to be on high alert for Hlac/LA particularly if a patient enters into the ART program with a low CD4 count and a low weight.

DEDICATION

First and foremost I would like to thank God for providing me the strength to see this dissertation to completion. I dedicate this thesis to my son, Shivashen W Padayachee, my reason and purpose in striving to be a better, more valuable member of society.

ACKNOWLEDGEMENTS

I am grateful and thankful to my husband, Lionel B Padayachee, who through the many challenging times gave me strength and belief in my capabilities.

I thank my parents for their unconditional support and their dogmatic approach in ensuring I fulfill this degree.

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I thank all the staff at the Tshwane District Health clinic, HIV division, particularly Dr Theresa Rossouw, for their assistance and their professional approach during the process of data collection.

I thank Roche for providing the handheld accutrend meter and test strips to successfully fulfill my study.

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NOMENCLATURE

3TC- Lamivudine
Acetyl CoA- Acetyl Coenzyme A
AIDS- Acquired Immune Deficiency Syndrome
ART- Anti-retroviral Therapy
ATP- Adenosine Triphosphate
AZT- Zidovudine
CD4- Lymphocyte Sub-Type cluster of differentiation 4
D4t- Stavudine
DDI- Didanosine
DNA- Deoxyribonucleic Acid
EFV- Efavirenz
HAART- Highly Active Anti-retroviral Therapy
HIV- Human Immunodeficiency Virus
Hlac- Hyperlactataemia
LA- Lactic Acidosis
mtDNA- Mitochondrial DNA
NAD- Nicotinamide Adenine Dinucleotide
NADH- Nicotinamide Adenine Dehydrogenase
NNRTI- Non Nucleoside Reverse Transcriptase Inhibitor
NRTI- Non Nucleoside Reverse Transcriptase Inhibitor
NVP- Nevirapine
OXPHOS- Oxidative Phosphorylation
PIs- Protease Inhibitors
UNAID- Joint United Nation Program on HIV/AIDS
URTI- Upper Respiratory Tract Infection
WHO- World Health Organization

CHAPTER 1

1.INTRODUCTION AND BACKGROUND

1.1INTRODUCTION

South Africa has the sixth highest prevalence of Human Immunodeficiency Virus (HIV) infection in the world, with 10.6% of the population estimated to be infected. The total number of people living with HIV is estimated to be 5.21million. Approximately one-fifth of South African women in the reproductive age are HIV positive.(1) According to the 2010 edition of the UNAIDS *Report* on the global AIDS epidemic, an estimated 320 000 (20%) fewer people died of AIDS-related causes in South Africa in 2009 than in 2004 due to the increase in availability of anti-retroviral medicines.(2) With this positive trend of improved drug treatment and fewer AIDS-related deaths, the mindset should be shifted towards reducing adverse effects of anti-retroviral agents and improving the quality of life of HIV-infected people. Anti-retroviral therapies *(ARTs) have* introduced the possibility of sustained suppression of HIV replication, recovery of the immune system and a substantial decrease in the frequency of opportunistic infections.(3) However, the need for permanent anti-retroviral treatment and the significant increase in life expectancy have led to the observation of new, frequent, and sometimes severe drug-related adverse effects.(4) One of the most challenging and dangerous side-effects is hyperlactataemia (Hlac) that may evolve to lactic acidosis (LA), particularly with the **N**ucleoside **R**everse **T**ranscriptase **I**nhibitors (NRTI).(5) ART–associated Hlac may be asymptomatic, or symptomatic which in the extreme case can progress to life threatening acidosis. The latter, i.e. LA is a fairly frequent and often misdiagnosed or under-diagnosed and potentially fatal side effect of ARTs. (6)

Table 1.1:**Categories and Examples of Anti-Retroviral Drugs (7)**

Nucleoside Reverse Transcriptase Inhibitors (NRTI) and analogue mimicked	Non-nucleoside Reverse Transcriptase Inhibitors (NNRTI)	Protease Inhibitors (PI)
Stavudine (d4T) Thymidine	Efavirenz	Indinavir
Zidovudine (AZT) Thymidine	Nevirapine	Ritonavir
Lamivudine (3TC) Non-Thymidine		Lopinavir
Abacavir (ABC) Non-Thymidine		
Didanosine (DDI) Non-Thymidine		
Tenofovir Non-Thymidine		

***Thymidine analogues** (structurally related to thymidine, a DNA building block)

****Non-thymidine analogues** (structurally related to other building blocks such as adenosine, cytosine, and guanosine).

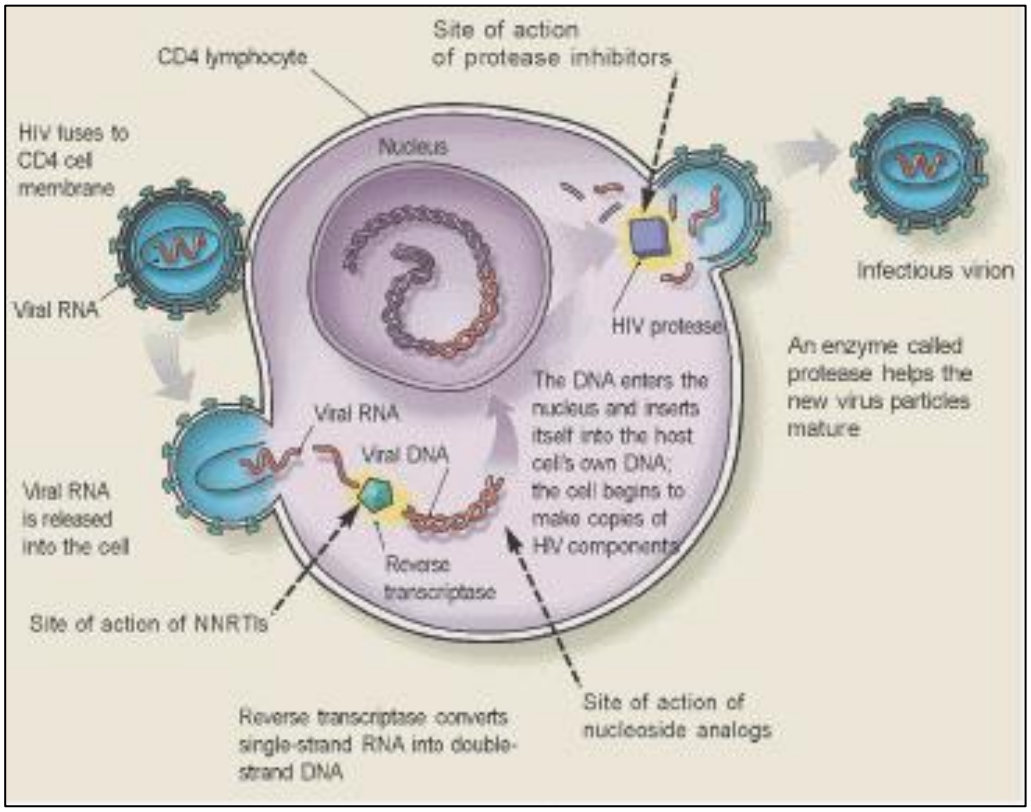


Figure 1.1:**Entry of HIV into Cell and Mechanism of Replication** [8]

As shown in Figure 1.1 the reverse transcriptase enzyme is essential for completion of the early stages of HIV replication, and the protease enzyme is required for the assembly and maturation of fully infectious viral progeny.[9] Nucleoside analogues bear a structural resemblance to the natural building blocks of DNA: the nucleosides adenosine and guanosine, thymidine and cytidine. Nucleoside analogues are triphosphorylated within the cell, and some undergo further modifications (didanosine, for example, is converted into its active moiety, 2',3'-dideoxyadenosine-5'-triphosphate). Nucleotide analogues resemble monophosphorylated nucleosides, and therefore require only two additional phosphorylations to become active inhibitors of DNA synthesis. Reverse transcriptase fails to distinguish the phosphorylated NRTIs from their natural counterparts, and the enzyme attempts to use the drugs in the synthesis of viral DNA. When an NRTI is incorporated into a strand of DNA being synthesized, the addition of further nucleotides is prevented and a full-length copy of the viral DNA is not produced. NNRTIs also inhibit the synthesis of viral DNA, but rather than act as false analogues, the NNRTIs bind to reverse transcriptase and inhibit the enzymes activity. Protease inhibitors bind to the active site of the viral protease enzyme, preventing the processing of viral proteins into

functional conformations. Viral particles are still produced when the protease is inhibited, but these particles are not effective at infecting new cells.[10]

Modern convention is to use **H**ighly **A**ctive **A**nti-retroviral **T**herapy (HAART) which usually contains two NRTIs combined with a **P**rotease **I**nhibitor (PI) or a **N**on-**N**ucleoside **T**ransciptase **I**nhibitor (NNRTI).[11] The South African national HIV treatment guidelines recommend stavudine (d4T), lamivudine (3TC), and efavirenz (EFV;regimen 1a) or nevirapine (NVP;regimen 1b) as an initial regimen, and didanosine (DDI), zidovudine (AZT) and lopinavir/ritonavir (LPV/R) as the subsequent treatment regimen (regimen 2). Agents included in regimen 2 are available for substitution following adverse events to agents in regimen 1, or for treatment failure following regimen 1 exposure. [12] Combination ART has been the standard of care for more than a decade, but ART combinations are not randomly allocated; rather they follow biological rationale and clinical experience, making it difficult over different time periods to assess any long term adverse effects of an individual drug, or changes in vivo intracellular processes such as lactate metabolism and mitochondrial function. More recent recommendations favour regimens based on abacavir or tenofovir over ones containing the thymidine analogues, on the basis of a lower risk of adverse events. However, the cost and availability of such alternatives make this difficult to implement on a large scale. In the context of expanding access to anti-retroviral therapy, particularly in resource-limited countries, NRTIs and particularly thymidine analogues (i.e. zidovudine and stavudine) are the cornerstone of first-line ART regimens.[13]

Almost all studies to date suggest that dideoxynucleosides, in particular stavudine, are associated with the development of Hlac and severe LA.[13,14-16] In 2005, Calza et al reported that most cases of LA during anti-retroviral treatment were associated with stavudine and didanosine therapy. This observation is in agreement with the relative affinities of various nucleoside analogues for mitochondrial DNA polymerase, and their relative potencies with regard to the inhibitory effects on mitochondrial DNA (mtDNa) replication in vitro.

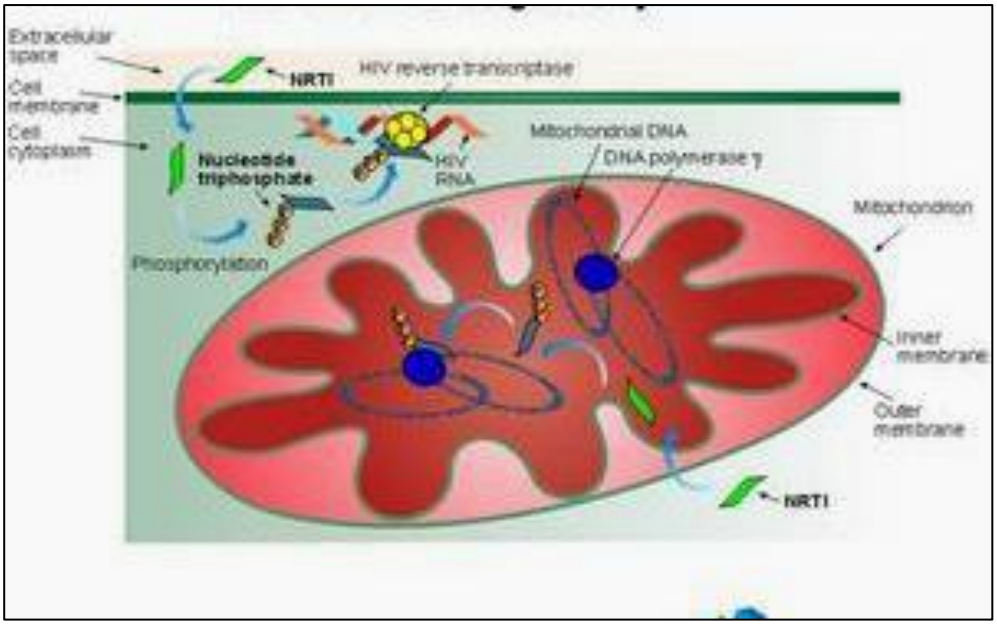


Figure 1.2:Mitochondrial Drug Toxicity [17]

The relative potency of these agents in reducing mtDNA content has repeatedly been found to be: zalcitabine>didanosine>stavudine>lamivudine>zidovudine>abacavir. Abacavir and tenofovir have been found to have a very low affinity for mitochondrial DNA polymerase, and a very low potency in inhibiting the mtDNA production (Figure 1.2). Non-nucleoside reverse-transcriptase inhibitors show a very low affinity for mitochondrial DNA polymerase, so it has been suggested that combinations of NNRTIs and PIs could be a safe treatment regimen for patients with previous Hlac.[18]

In a host of studies over the past decade a few risk factors and associations have been clearly established. Hyperlactataemia/Lactic acidosis have been shown to be associated with female gender, advanced immunosuppression, excellent compliance, duration of treatment of more than 6 months, chronic muscle or kidney disease, chronic hepatitis B or C infection, low CD4 count before starting a regimen containing NRTI, and possibly with ethnicity.[3,13,15,16] Patients ≥75kg at ART initiation with weight gain of ≥6kg in the first 3 months on therapy, and with peripheriphal neuropathy, were most likely to present with symptomatic Hlac and LA. On the other hand, patients of low body weight and with high serum lactate levels are at a higher risk of dying. [19, 20] Age can also be an independent predictor, with older patients being more likely to present with Hlac. Because NRTIs are known to be potential substrates of polymerase gamma, inhibition of this polymerase decreases mitochondrial DNA concentrations and thus the synthesis of mitochondrial enzyme protein subunits encoded by mitochondrial DNA. Alterations in mitochondrial function have been described during aging. This may explain why older

patients with physiologic alterations in their mitochondrial capacities are more likely to have higher lactate concentrations with NRTIs than younger patients.[21]

Table 1.2:**Most commonly Reported Risk Factors for Hyperlactataemia**[3,13,16,22]

- High Body Mass Index (BMI)
- Female
- Pregnancy
- Underlying Liver Disease
- Older Age
- Combination of Stavudine or Didanosine or Stavudine alone.
- Excellent Compliance
- Duration of Treatment of more than 6 months
- Chronic muscle or Kidney Disease
- Low CD4
- Advanced HIV-1-induced Immunosuppression

1.2 **PATHOPHYSIOLOGY**

Lactate, one of the components of the respiratory chain, is produced during the glycolytic pathway as a product of transformation of pyruvate. Intracellular processes are depicted in Fig 1.3. When the oxygen supply of tissues is sufficient, the metabolism of pyruvate is primarily oxidative with no intracellular accumulation of lactate. However, impairment of oxygen supply or cytoplasmic accumulation of pyruvate may result in increased lactate formation.[23]

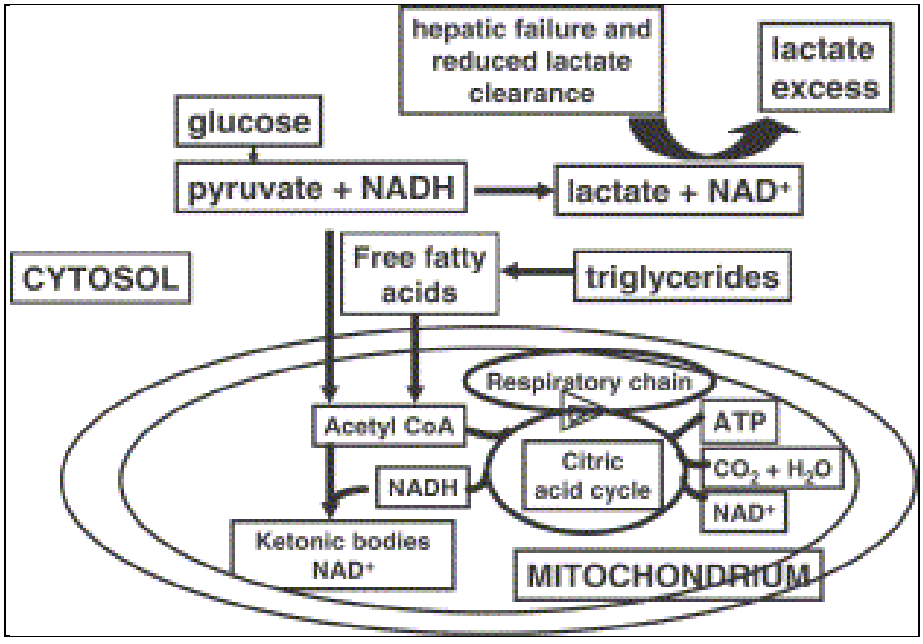
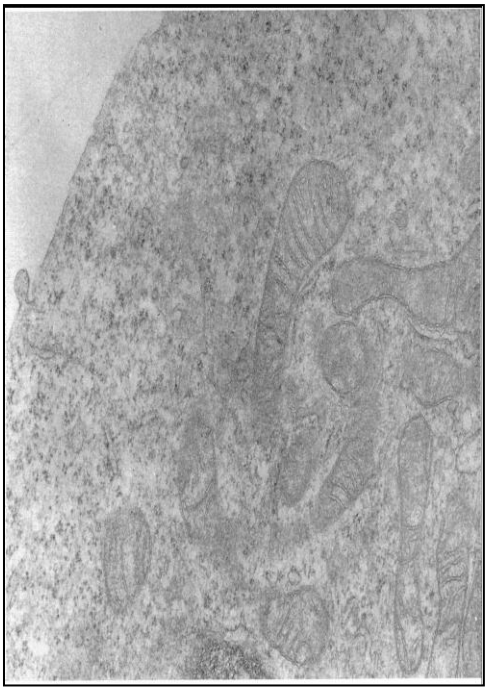


Figure 1.3:Intracellular Energy Processes [18]

Under aerobic conditions, glycolysis feeds into the citric acid cycle and the electron transport chain where most of the free energy in glucose is harvested. If oxidative phosphorylation is interrupted because of respiratory chain enzyme depletion, ATP synthesis decreases and the NADH/NAD+ ratio rises, leading to the reduction of pyruvate by NADH to form lactate, which is the end-point of cytoplasmic glucose metabolism (Figure 1.3). Normally, lactate concentration in venous blood reflects the equilibrium maintained between the rate of lactate production, and that of lactate utilization and elimination by liver and renal cortex. However, under various conditions of lactate excess, hepatic and renal clearance of lactic acid may be augmented, and other organs that are usually lactate producers, such as skeletal muscle, can become great lactate consumers. NRTI-related LA is almost always associated with impaired liver function manifested as massive hepatic steatosis or frank hepatic failure. Under these circumstances the liver has reduced lactate clearance capacity and also becomes a net lactate producer.[18]

Given the similarities in function between HIV reverse transcriptase and human DNA polymerases it is not surprising that nucleoside analogues are competitive inhibitors of human DNA polymerases. The affinity of these agents appears to be greatest for mitochondrial DNA polymerase. Their use, therefore, could lead to depletion of mitochondrial DNA and hence diminish cellular respiratory function.[4,24] In vitro, NRTIs have been shown to damage and deplete mtDNA, alter mitochondrial morphology and decrease cell viability. Cultured human cells exposed to varying concentrations of NRTIs have been shown to decrease mtDNA content quite rapidly, and significant changes in mtDNA have been followed by altered mitochondrial morphology and diminished cell growth (Figures 1.4; 1.5) Coté et al have studied changes in mtDNA relative to nuclear DNA in peripheral blood cells of patients with symptomatic, NRTI-induced Hlac. Increased serum lactate levels were associated with marked reductions in the ratios of mitochondrial to nuclear DNA which, during therapy, averaged 43% lower than those of HIV-infected, asymptomatic subjects not previously treated with anti-retroviral drugs. With drug use the decline in mtDNA preceded the increase in venous lactic acid levels, while on the other hand there was a statistically significant increase in the ratios of mitochondrial to nuclear DNA after the discontinuation of anti-retroviral treatment.[4]



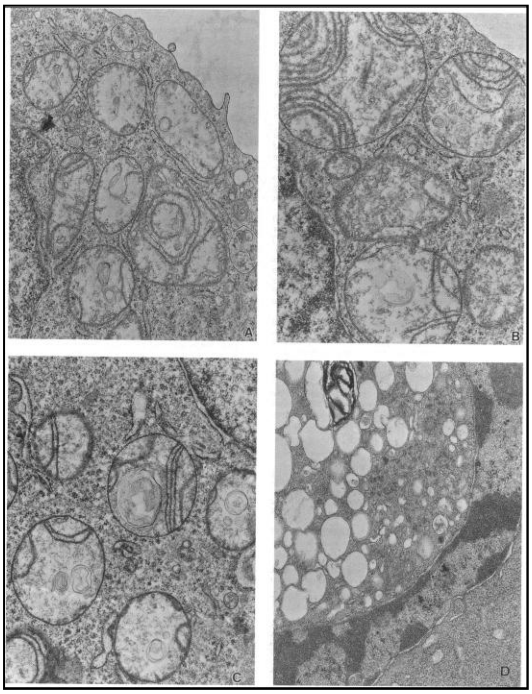


Figure 1.4 Figure 1.5

Figure 1.4: Normal Mitochondria in Response to ART

Figure 1.5:Progressively Abnormal Mitochondria in Response to ART[(5)]

Mitochondria treated with DDI and d4t showing swelling, reduced cristae and an increase in the number of cytoplasmic vacuole. [(5)]

1.3**CLASSIFICATION**

Hlac may be asymptomatic, or present with combinations of weight loss, nausea, vomiting and diarrhoea. Lactic acidosis is often difficult to diagnose because the presenting symptoms (e.g. nausea, emesis, abdominal pain, increased fatigue and unexpected weight loss) are non-specific and may be the result of drugs, the underlying disease or an intercurrent problem (Fig.1.6). On the other hand, pancreatitis, sepsis and certain pathological thermoregulatory states such as heat exhaustion can also cause serum lactate elevations.[(25)] Severe LA is typically symptomatic, with rapid or precipitous progress to classical features of metabolic acidosis (ph <7.35 and arterial bicarbonate <22mEq/L[(26)]), accompanied by hyperventilation, arrhythmias, and multiple organ failure.[(19)] Hlac in HIV is commonly categorized into one of three clinical and biochemical entities.[(16)]

Asymptomatic Hlac (also referred to as subclinical Hlac): Here the lactate level is mildly to moderately raised (2.5-5mmol/L) and the patient is asymptomatic. Hlac may be transient or

prolonged, and has not been found to predict the onset of symptomatic or severe LA. Therefore routinely screening asymptomatic patients on NRTIs is not considered beneficial. [3]

Symptomatic Hlac: In this category, patients again have mildly to moderately elevated lactate levels (2.5-5mmol/L), no acidosis, but are clinically symptomatic (Figure 1.6). This may be an early manifestation before the onset of acute, severe LA, and usually occurs in situations where tissues are well-perfused and buffering systems are able to prevent a fall in pH and development of metabolic acidosis. [3]

Lactic acidosis: The criteria used to diagnose LA include serum lactate ≥5mmol/l, bicarbonate level <20mmol/l, arterial pH <7.34 and anion gap >12. Patients in this category are symptomatic and the mortality rate is high (Figure 1.6). A serum lactate concentration of >10mmol/l is considered to be an independent predictor of mortality, and a level 15mmol/l is associated with >60% mortality. [3]

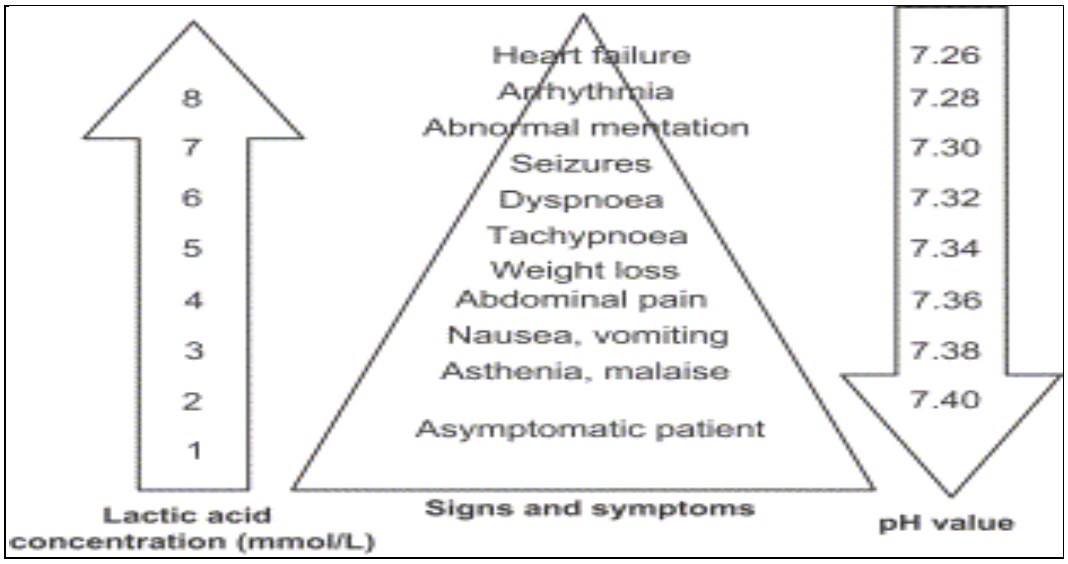


Figure 1.6:Clinical Manifestations of Hlac and LA, and Relationship to Arterial Blood pH Value[18]

Variations in the frequency of the categories of Hlac seen across studies or groups of patients may be attributed to differences in the definition of Hlac and/or variations in potential risk factors for Hlac such as ART regimen, patient-related factors and co-morbidities. Furthermore, venous lactate measurement is vulnerable to spurious increases, and the techniques employed to minimize falsely elevated results may vary from study to study.[6]

In 2008, Perez et al showed that in resource-poor settings it is difficult to definitively diagnose LA by laboratory measurement of lactate because of a lack of data related to the costs involved (equipment, transportation etc). In this regard, cost effective Point-Of-Care (POC) devices are

helpful in the measurement of lactate levels and have been validated. The Accutrend lactate meter is an appropriate device for screening patients on HAART with or without symptoms of Hlac/LA. The use of this device decreases analytical and intervention time, and may help to prevent further morbidity and mortality in patients on an NRTI (stavudine)-based regimen.[9] Point-Of-Care devices eliminate the need to transport blood specimens on ice and provide a significant advantage in the measurement of lactic acid.[27]

1.3 **PREVALENCE AND INCIDENCE**

Although symptomatic Hlac and metabolic acidosis are thought to be rare adverse effects, a recent study has shown that asymptomatic increased lactate concentration is a frequent event in patients receiving NRTIs, with an estimated prevalence range of 15% to 35%. Symptomatic Hlac and LA reportedly occur much less frequently, varying from 1.7 to 25.2 cases per 1000 person-years of treatment with NRTIs. However, there is substantial variation in the calculated frequency due to the variety of case definitions employed.[18]

A South African study in 2006 reported an incidence of 19 cases of LA (>5mmol/L) per 1 000 person-years of treatment. All patients were on two NRTIs (d4T and 3TC), with 86% on efavirenz (EFV), and 14% on nevirapine as the third (non-NRTI) drug.[16] In 2008, Fabian et al, reported an incidence of symptomatic severe Hlac (>5mmol/L) of 20.5 cases per 1000 person-years at Johannesburg Hospital, with 82.6% on stavudine, lamivudine and efavirenz, 10.4% on stavudine, lamivudine and nevirapine, 0.8% on zidovudine, didanosine and lopinavir/ritonavir, and 6.2% on various combinations.[15] In a similar study in Soweto, the overall incidence, where lactate of >4.5mmol/L was included, was 10.6 cases per 1000 patient-years of treatment. In this study, 66 of 67 patients were receiving stavudine and 5 patients were receiving didanosine.[14] A study at Grey's Hospital in western Kwa-zulu Natal in 2010, with all the patients being on stavudine, showed the overall incidence of LA (>5mmol/L) was 13.5 cases per 1000 patient years, and the overall incidence of Hlac (>2.2mmol/L) without acidosis was 31 cases per 1000 patient years.[12] These results are summarized in Table 1.3.

Table 1.3:Summary of Research Articles [12, 13, 15, 16, 18, 20, 21, 28]

Period	Location of Study	Incidence Rate	Lactate Definition	Symptoms	Risk Factors Found
1997-2004	London	No information given	Two consecutive lactates ≥5mmol/L	No information given	*exposure <12months *female gender d4T and didanosine exposure *age above 40years *advanced immunosuppression
1999-2000	Western Australia	None given	Hlac 2.8-4.1mmol/L and LA ≥5mmol/L	No information given	*d4T exposure *effect of d4T not confounded by longer NRTI history nor previous zidovudine exposure
2000-2002	France	None given	Hlac 2.25-5mmol/L LA >5mmol/L.	*weight loss from the initiation of the current regimen *gastrointestinal symptoms (vomiting,nausea, diarrhea) *respiratory symptoms (cough and dyspnea) *muscular symptoms (myalgia and cramps)	*older age *drug regimens with stavudine or a combination of stavudine-didanosine *use of buprenorphine
2004-2005	S. Africa	19 cases per 1000 person-years of treatment	≥ 5mmol/L= LA	*loss of appetite *abdominal pain *diarrhea *vomiting *shortness of breath	* d4T exposure *female *overweight
2004-2005	Italy	1.7 to 25.2 cases per 1000 person years	Hlac (2-5mmol/L) LA (>5mmol/L)	*asthenia, *malaise * nausea *vomiting * abdominal pain *weight loss, *tachypnoea, *dyspnoea * liver steatosis	*concurrent therapy on ddi and d4t
2003-2005	South Africa	None given	Hlac ≥5mmol/L	*weight loss of ≥2kg *rise in alanine aminotransferase * presence of one of three major symptoms (vomiting,nausea and abdominal pains) *peripheral neuropathy	*female sex *baseline weight between 60 and ≥75kg at ART initiation and gaining ≥6kg in the first 3 months
2004-2005	South Africa	20.5 cases per 1000 person-years of ART	LA /severe Hlac >5.0mmol/L	*non-specific gastrointestinal symptoms *weight loss *symptomatic neuropathy	*female
2004-2006	South Africa	13.5 cases per 1000 patient years.	Hlac >2.2mmol/L and LA >5mmol/L	*loss of weight *abdominal pain *loss of appetite *parasthesia	*baseline CD4 count <10cells/mm^3 at HAART initiation.

1.4 BACKGROUND TO THE RESEARCH

During a period of employment as the principal pharmacist at the Tshwane District Hospital from 2005 to 2006, the constant recognition of patients presenting with and dying with/from elevated lactate at the Tshwane District Hospital sparked an interest in categorizing the risk factors for Hlac. The lack of knowledge within the team of health workers and the despondency of the patients generated the interest, enthusiasm and determination to complete the research. Although certain protocols were in place for recognition and possible prevention, Hlac was a common presentation. The practitioners changed stavudine from 40mg to 30mg, and replaced stavudine with zidovudine if a patient presented with symptomatic Hlac. Certain trends and associations were becoming noticeable, for example the use of stavudine in overweight women. Questions arose such as whether Hlac presented because of the anti-retrovirals, and/or what conditions could aggravate the condition. Questions also arose as to whether weight, age and race affected Hlac. Thus, this research aimed to explore the frequency of and risk factors for Hlac/LA with the later treatment protocols. This might not only assist the patients, but empower health workers to identify and then mitigate the risk factors for the condition, thereby providing a better quality of life for the already-compromised patients.

1.5. AIM

The **Aim** of the study was to determine the risk factors associated with lactic acidosis in adult HIV patients on ARVs in a public sector hospital in Gauteng.

1.6 OBJECTIVES

- To explore the relationship between Hlac/LA and gender, weight, CD4 status, dosage and regimen alterations in HIV patients on ARTs.
- To compare the earlier regimens to the revised regimens as independent risk factors for Hlac and LA.

CHAPTER 2

2.1**METHODOLOGY**

2.1.1**Study Design**

There were two components to the study:

A **Retrospective** analysis of patient records from the Tshwane District Hospital, and a subsequent **Prospective** study of ART-naïve patients attending the same clinic.

2.2.1.1Retrospective Review

The retrospective study was to assess the incidence of and some risk factors for Hlac/LA in patients on ARTs. Data were obtained from hospital records of ART-treated HIV-positive patients who had been diagnosed with symptomatic Hlac or LA between August 2004 and December 2007. Hlac was defined as a venous lactate measurement of ≥2.3mmol/L because the hospital protocol at the time classified 2.3mmol/L and greater as Hlac. Levels ≥5mmol/L were classified as LA.

The inclusion criteria were as follows:

1. Male or female patients'≥18 years of age
2. HIV positive and treated with anti-retrovirals for longer than 3 months.

The collection protocol at the time is detailed below:

- For lactate measurement the specimen was drawn from an un-cuffed arm.
- It was then collected in a fluoride tube.
- Thereafter, the tube was put on ice immediately and sent to the laboratory within 4 hours.
- All the patients were well hydrated and did not exercise for 24 hours before sampling.[3]

Immunological, virological, haemotological and biochemical results were recorded for patients who had been on treatment for more than 3 months. Data collected included age, weight, gender, race, CD4, viral load, lactate levels and clinical symptoms. It took approximately 11 months from 2008 May to March 2009 to review the patient files which were accessed at least 3 times in a week, with each file requiring one hour. For each patient the following data were recorded by the researcher:

Table 2.1:**Nature of Data Recorded and Reasons**

Data recorded	Rationale
Demographics (age, gender, race, weight)	Identify risk factors
CD4, Viral load	Severity / stage of disease
Drug regimen and duration of treatment	Impact of these factors
Other diseases	Co-existing/contributing factors
Clinical signs/symptoms	Manifestations of lactate elevation

Information was recorded and analyzed from the initiation of patient on ARTs until the date of collection of the data. A total of 232 patients were included in the retrospective study.

The anti-retroviral guidelines produced by the Department of Health and in use by the clinic at that time stated that Hlac and LA should not be tested randomly but rather only on suspicion e.g. if the patient lost weight abruptly, had gastrointestinal symptoms or unexplained abdominal discomfort. However, due to the frequency of patients presenting with Hlac, Tshwane District Hospital changed the protocol from testing on suspicion to also testing asymptomatic patients. While the above strategy would provide some information in the areas detailed in Table 2.1, the non-systematic nature of the data collection that evolved during the review made it unlikely that completely accurate incidence rates would be measured.

2.2.1.2Prospective Review

In the prospective study, which was conducted between September 2008 and December 2009, HIV patients who had been on treatment for longer than 12 months were selected. The study was also performed at the Tshwane District Hospital. Convenience sampling took place from the queue at the clinic and patients were eligible, for lactate testing provided that they had not exercised or exerted themselves strenuously that morning. The inclusion criteria were as follows:

1. Patients'≥18 years of age attending the clinic at Tshwane District Hospital
2. Treatment with anti-retrovirals for ≥ 12 months

Given the accuracy and cost effectiveness of a point-of-care device (see page 13, Chapter 1), this was the method used to measure lactate levels in this study.[27] The device provides lactate results within 60 seconds of placing a drop of whole blood on the test strip, and is powered by three 1.5-volt batteries. The measuring range for whole blood is 0.8-22mmol/L and for plasma is 0.7-27mmol/L. Use of the device eliminates the need for centrifugation and specialized

laboratory equipment, requires a very small quantity of blood, and eliminates the need to transport blood specimens on ice.

The collection protocol performed by a trained nurse included the following:

- The patient was at rest for longer than 15 minutes and had not performed any exercise.
- Venipuncture was undertaken without a tourniquet.[29]

Immunological, virological, haemotological and biochemical results were recorded by the researcher for patients who had been on treatment for more than 12 months. Data collected included age, weight, gender, race, CD4, viral load, lactate levels and clinical symptoms as recorded in the patient files on the day of testing. Each patient took approximately 20 minutes to assess provided all their information was available. If a patient's file was inaccessible, that patient was excluded from the study. A total of 292 patients were sampled in the prospective study. Using the same lactate classification as for the retrospective study, lactate levels were recorded and patients categorised as asymptomatic, symptomatic or LA.

The data capturing for both the retrospective study and the prospective study was performed by the researcher. Verification of the data collected was ensured by randomly reviewing files twice to ensure the correct information was collated.

2.3.1 **Ethics Clearance**

Record review in the retrospective study did not require patient consent since all data were anonymous. In the prospective study, patients signed consent before data and blood collection were performed.

The study was approved by the Human Research Ethics Committee (medical). Clearance certificate number M071133.

2.3.2 **Statistics**

Analysis included simple descriptive statistics and comparison of means by t-testing. Frequency analysis was by Chi-square or Fisher exact test. Where appropriate, simple correlations were sought. Regression analysis was utilised to explore relationships between multiple independent variables and lactic acid levels. All data were analysed using Statistica Version 9.1(StatSoft Inc, USA). Significance was routinely accepted with p-values <0.05.

The sample size for analysis in the retrospective study included 3741 subjects while sample size for the prospective study was according to the formula described by Casagrande et al.[38] Based on the statistics of previous studies which showed that up to 15-35% of patients treated with ART had elevated lactate, it was hypothesized that in this study the new ART regimens would reduce by approximately 50%. At this rate the sample size required to give a p value(α) of 0.02 with a power (β) of 05% is 232.

CHAPTER 3

3.**RESULTS**

3.1**RETROSPECTIVE STUDY**

3.1.1**Descriptive Analysis**

A total of 3741 patient files were retrieved and reviewed covering the period from August 2004 until December 2007. As explained in Chapter 2, at some point the clinic policy changed from one of lactate testing only in the presence of symptoms, to the more frequent testing of patients receiving anti-retroviral treatment. This obviously resulted in a subset of the sample being in the asymptomatic category. Composite results showed that 232 (6.2%) patients presented with elevated lactate values, which may well be an under-representation for reasons described above. Within this group of 232 patients, 161 were symptomatic, representing 4.3% of the total sample. Characteristics of the group with elevated lactate levels are summarized in Table 3.1.

Table 3.1:Details of Patients in Retrospective Group with Elevated Lactate Levels

	All Patients	**Female**	**Male**	**p-value Male vs Female**
Total Number	N=232	N=183	N=49	
Average Age (years ±s.d.)	42.6(±8.7)	42.7 (±9.0)	42.1 (±6.0)	NS
Weight at ±1 year of treatment (Kg±s.d.)	67.0(±11.9)	67.1(±12.0)	66.6(±11.0)	NS
Race				
Black	231 (99.6%)	182 (78.5%)	49(21.2%)	
Coloured	1 (0.4%)	1 (0.43%)	0 (0%)	
Regimen				}0.058
1a1	90(38.8%)	70(38.3%)	20(40.8%)	
1a2	100(43.1%)	73(39.9%)	27(55.2%)	
1b1	18(7.8%)	17(9.3%)	1 (2.0%)	
1b2	13(5.6%)	13(7.1%)	0	
Varied	11(4.7%)	10(5.5%)	1(2.0%)	
CD4 (cells/µL±s.d.)	117(±80.4)	118(±76.0)	114(±93.0)	NS
Highest Lactate Value (mmol/L ±s.d.)	4.3(±1.8)	4.4(±2.0)	3.8(±1.0)	0.04
Average time taken to increased lactate presentation (months±s.d.)	12.0 (±5.9)	11.7 (±6.0)	13.4 (±6.0)	0.06
Elevated Lactate Groups				
Asymptomatic (≥2.3mmol/L)	71(30.6%)	52(28.4%)	19(38.8%)	}0.021
Symptomatic(≥2.3- 5mmol/L)	158(68.1%)	130(71.0%)	28(57.1%)	
Lactic acidosis (≥5mmol/L)	3(1.3%)	1(0.6%)	2(4.1%)	

Based on known adverse effects and relative toxicity, regimens were categorized in increasing order as: 1a1=3TC, d4t30mg, efavirenz **1a2=**3TC,d4t40mg, efavirenz **1b1=**3TC,d4t30mg ,nevirapine **1b2=**3TC,d4t40mg,nevirapine

Overall 6.2% of the population sampled had elevated lactate levels. Females tended to present earlier and had higher peak values. No absolute differences in weight were observed between males and females. However, it is necessary to compare the average weights of males and females in this study to average weights of similarly aged subjects in the general population. According to the African Project on Genes in Hypertension as carried out by the Cardiovascular Patho-physiology and Genomics Research Unit, University of the Witwatersrand, average weight for females is 81.6kg and for males is 73kg.[39] Comparing weights of the study group indicates that males were 91% of average weight for age versus females who were even lower at 82% of average weight. In other words, both males and females in the study group were nutritionally compromised, with females being more so than males. This suggests that patients who present to the clinic are at an advanced stage of the disease. CD4 counts were extremely low for both male and females. Regimens were similar for males and females, with a tendency towards more females receiving regimen 1a2. Analysis of the three categories of lactate elevation indicated that the majority of patients (68.1%) were in the symptomatic group and there were more symptomatic females. This is the result expected from the sampling strategy at the hospital at the time and is almost certainly an under-representation of asymptomatic patients.

3.1.2 **Symptoms versus Highest Lactate Value**

Table 3.2:Correlations between Symptoms and Highest Lactate Value

Symptom	**Correlation Coefficient**	**p-Value <0.05**
Peripheral neuropathy	0.209	√
GIT symptoms (nausea and vomiting)	0.148	√
Abdominal tenderness	0.214	√

Peripheral neuropathy, gastrointestinal symptoms and abdominal tenderness were the only symptoms that were shown to be associated with high lactate values. Fatigue, diarrhoea, rash, upper respiratory tract infection, lipodystrophy, heartburn, breast tenderness and conjunctivitis did not show any significant relationship with higher lactate values. While not a symptom but rather a sign of disease severity, the CD4 count was found to be negatively correlated with lactate values (-0.258; $p<0.05$), indicating that lower CD4 counts were related to higher lactate values.

3.1.3 Symptoms versus Regimen

Table 3.3:Correlation between Symptoms and Relative Toxicities of Drug Regimens

Symptom	Correlation Coefficient	p-Value <0.05
Rash	0.209	√
Upper Respiratory Tract Infection (URTI)	0.159	√

As per the sub-text below Table 3.1, regimens were categorized according to well-recognized adverse effects and toxicity. On this basis, rash and URTI were more frequent in subjects exposed to the more toxic regimens. Fatigue, peripheral oedema, nausea, vomiting, abdominal tenderness, diarrhoea, lipodystrophy, heartburn, breast tenderness and conjunctivitis did not show any significant relationship to regimen.

3.1.4 Multivariate Analysis

Table 3.4:Factors Related to Peak Lactate

Determinant	t value	p-Value
Average time taken to increased lactate presentation (months)	-2.88	0.005
CD4(cells/µL)	-2.33	0.022

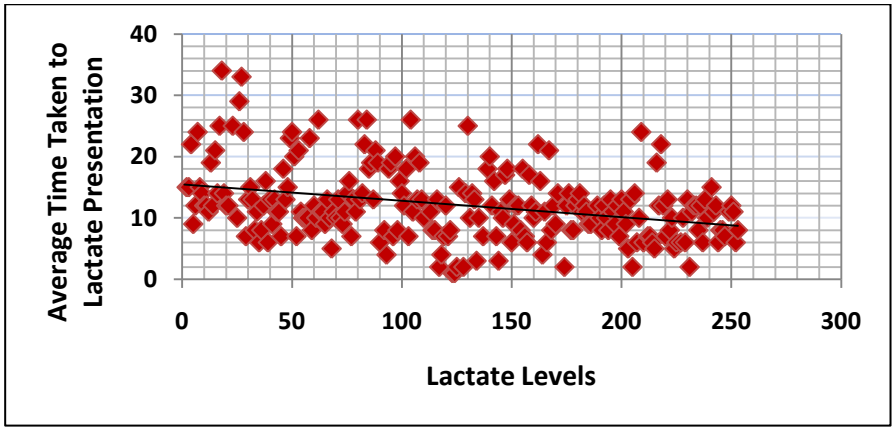


Figure 3.1: Relationship of Average Time (months) Taken to Increased Lactate Presentation from Initiation on ART with Elevated Lactate Levels

Multivariate analysis taking into account gender, age, average time to increased lactate presentation, weight at ±1 year of treatment and weight loss revealed that only shorter time to elevated lactate and lower CD4 count were predictive of peak lactate levels. These two factors

accounted for 10.8% of the variance in elevated lactate. Table 3.4 and Figure 3.1.shows the weak but significant inverse relationships.

In summary, 6.2% of the group studied had elevated lactate levels. Some 4.3% were categorized as having symptomatic Hlac or LA and 0.1% as LA. The group of subjects was significantly below average weight for age and CD4 counts were below 200 for both males and females. There was an inverse correlation between CD4 count and peak lactate level. Lactate values correlated with symptoms of peripheral neuropathy and gastrointestinal signs and symptoms, whereas drug toxicity correlated with symptoms of rash and presentation with upper respiratory tract infection. Multivariate analysis showed only that elevated lactate was related to low CD4 count, and that an early rise in lactate was associated with a peak that was higher. The latter findings suggest that it is essentially the severity of the underlying HIV infection that is a determinant of lactate elevation.

3.2**PROSPECTIVE STUDY**

3.2.1**Descriptive Statistics**

Table 3.5:**Details of Patients in Prospective Group**

	All Patients	**Female**	**Male**	**p-value Male vs Female**
Total Number	N=292	N=212	N=80	
Age(years±s.d.)	38.1(±9.4)	37.7(±9.6)	39.3(±8.7)	NS
Weight(Kg±s.d)	66.5(±14.5)	65.6(±14.8)	68.8(±13.5)	NS
Weight Loss(Kg±s.d)	6.3(±4.2)	6.6(±4.2)	5.5(±4.3)	NS
Race				
Black (%)	286 (98%)	208(73%)	78 (27%)	
Coloured (%)	6 (2%)	4 (67%)	2 (33%)	
Regimen				}NS
1 (%)	181 (61.9%)	127(59.9%)	54(67.5%)	
2 (%)	95 (32.5%)	72(34.0%)	23(28.8%)	
3 (%)	4 (1.4%)	3(1.4%)	2(2.5%)	
4 (%)	12 (4.2%)	9(4.3%)	1(1.2%)	
CD4(cells/µL±s.d)	295(±175.8)	315(±182.8)	241.9(±143.8)	0.015
Total number with elevated lactate(mmol/L±s.d)	71(24.3%)	54(25.5%)	17(21.3%)	NS
Elevated Lactate Groups				}NS
Asymptomatic(≥2.3mmol/L)	43(60%)	33(61%)	10(59%)	
Symptomatic(≥2.3mmol/L-5mmol/L)	26(37%)	20(37%)	6(35%)	
Lactic acidosis(≥5mmol/L)	2(3%)	1(2%)	1(6%)	

Based on known adverse effects and relative toxicity, regimens were categorized in increasing order as: 1= 3tc,d4t30mg, efavirenz /nevirapine **2**=azt,3tc,/ efavirenz /nevirapine **3**=abacavir,3tc,efavirenz /nevirapine **4**=tenofovir,3tc,efavirenz/nevirapine

Overall 24.3% had elevated lactate levels on routine testing, with 60% in the asymptomatic group and 40% being symptomatic or with LA. The CD4 counts were significantly lower for males in this sample. The majority of the subjects (62%) were being treated with regimen 1 and there were no differences in treatment regimens between males and females. As with the retrospective study, mean weight for both males and females was significantly below that of age-matched subjects in the general population.[39]

3.2.2 Symptoms versus Lactate

Table 3.6:**Correlation between Symptoms and Lactate Measurements**

Symptom	Correlation Coefficient	p-value p<0.05
Diarrhoea	0.12	√
Nausea	0.13	√

Diarrhoea and nausea were the only symptoms that were shown to be associated with high lactate values. Fatigue, peripheral neuropathy, vomiting, abdominal tenderness, rash, upper respiratory tract infection, lipodystrophy, heartburn, conjunctivitis, breast tenderness and pancreatitis did not show any significant relationship with higher lactate values.

3.2.3 Symptoms versus Regimen

Table 3.7:**Correlation between Symptoms and Relative Toxicities of Drug Regimens**

Symptom	Correlation Coefficient	p-value <0.05
Diarrhoea	0.101	√
Abdominal tenderness	0.054	√

As per subtext below Table 3.5, regimens were categorized according to known adverse effects and toxicity. Based on this, diarhoea and abdominal tenderness were increasing as toxicity increased. Fatigue, peripheral neuropathy, vomiting, abdominal tenderness, rash, upper respiratory tract infection, lipodystrophy, heartburn, conjunctivitis, breast tenderness and pancreatitis did not show any significant relationship with treatment regimen.

3.2.4 **Multivariate Analysis**

Table 3.8:Factors Related to Lactate Values

Determinant	t value	p-value
Weight	-2.47	0.01
CD4 cells/µL	-2.29	0.02
Weight Loss	3.47	0.005

Multivariate analysis taking into account race, gender, age and regimen at high lactate level revealed that only low weight, low CD4 and weight loss were predictive of peak lactate levels. These three factors accounted for 6.2% of the variance in elevated lactate

3.2.5 **Comparisons between Subjects with High versus Low Lactate Levels**

Table 3.9: **Comparisons between Patients with Lactate Levels Above and Below 2.3mmol/L**

	Elevated Lactate	Normal Lactate	p-value
Viral Load(copies/ml±s.d.)	3259710.0(±395020)	81958.1(±133559)	0.014
CD4(cells/µL±s.d)	248.9(±192.6)	305.7(±170.3)	0.031
Weight(Kg±s.d.)	62.9(±15.2)	67.3(±14.3)	0.049

This analysis showed that weight and CD4 count were lower in those with elevated lactate levels and viral loads were higher.

In summary, 24.3% of the group studied had elevated lactate levels and 9.6% of those had symptomatic Hlac or LA. As with the retrospective group, the subjects were significantly below average weight for age. The mean CD4 count averaged around 300 cells/µl but was lower for males than for females. Female patients predominated but lactate levels were elevated in proportion to the genders enrolled.

Lactate levels correlated with gastrointestinal symptoms, as did toxicity of the drug regimens. Multivariate analysis showed that lower weight, weight loss and CD4 count correlated with higher lactate levels, again suggesting that severity of the underlying HIV infection is a determinant of lactate elevation. This is consistent with the finding of significantly higher viral loads in the group with elevated lactate levels.

3.2.6 Review of Retrospective and Prospective Studies

Table 3.10:Comparisons of Subjects in Retrospective and Prospective Studies

	Retrospective	Prospective	P-value
Total number	232	292	
Female	183(78.9%)	212(72.6%)	}NS
Male	49(21.1%)	80(27.4%)	
Age	42.6±8.7	38.1±9.4	P<0.001
CD4(cells/µL)	117(±80.4)	295(±175.8)	P<0.001
Weight(kg)	67.0(±11.9)	66.5(±14.5)	NS
Incidence	232(6.2%)	71(24.32%)	P<0.001
Asymptomatic	71(30.6%)	43(60.6%)	}P<0.001
Symptomatic	158(68.1%)	26(36.6%)	
Lactate>5mmol/L	3(1.3%)	2(2.8%)	
Regimens(*)			
A	90	181	
B	100	Not applicable	
C	18	Not applicable	
D	13	Not applicable	
E	Not applicable	95	
F	Not applicable	4	
G	Not applicable	12	
Symptoms	**Significantly represented**	**Significantly represented**	
Peripheral neuropathy	√	-	
GIT symptoms	√	√	
Abdominal tenderness	√	-	
Rash	√	-	
Upper respiratory tract infection	√	-	
Determinants of High lactate			
Average time to increased lactate presentation (months)	√	Not measured	
Low CD4	√	√	
Weight loss	Not measured	√	
Low weight on entry	Not specifically measured	√	

***A** = d4t30mg,3TC, efavirenz**B** = d4t40mg,3TC, efavirenz

C = 3TC, d4t30mg, nevirapine**D** = 3TC,d4t40mg, nevirapine

E = AZT, 3TC, Nevirapine/ efavirenz**F** = abacavir,3TC, efavirenz /nevirapine

G = Tenofovir, 3TC, efavirenz /Nevirapine

Male:Female ratios were similar in both the retrospective and prospective groups. The age groups are significantly different when comparing the two studies: subjects presented when they were older in the retrospective study at ±43 years compared to the prospective study were the average age was around 38 years. The CD4 counts were significantly higher in the prospective study, while the average weights in both the retrospective and prospective studies were similar but low when compared to the population average weight for age.

Incidence rates of all the categories of Hlac (2.3mmol/L- 5mmol/L) were higher in prospective study, but as previously discussed, for the retrospective study the sampling strategy was expected to deliver a falsely low rate of Hlac.

As per study design, the regimens differed between the two study groups, with the less toxic more recent regimens being found in the prospective group. Gastrointestinal symptoms were a common presentation in both the retrospective and the prospective groups even with the newer and supposedly less-toxic regimens. However, peripheral neuropathy, a relatively common problem with older regimens was not observed.

Although several other factors contributed to elevated lactate levels, low CD4 count was the common determinant of elevated lactate levels in both the retrospective and prospective groups, as were other manifestations of advanced disease.

CHAPTER 4

4.**DISCUSSION**

Anti-retrovirals have revolutionized the treatment of HIV/AIDS, providing better quality of life. However, the use of these drugs is associated with adverse effects which reduces adherence and decreases patient morale. Asymptomatic and symptomatic Hlac and LA are part of the spectrum of mitochondrial toxicity associated with NRTI therapy.[15] Mitochondria are small intracellular organelles that are located in the cellular cytoplasm. All cells except erythrocytes contain hundreds to thousands of mitochondria. Mitochondria have a double lipid membrane that is folded into numerous cristae surrounding the matrix space. This matrix contains copies of the mtDNA genome, which encodes subunits of four of the five complexes of the oxidative phosphorylation (OXPHOS) system located in the inner membrane of the mitochondrion. This OXPHOS system is responsible for providing most of the energy to cells.[42] The active intracellular anabolites of NRTIs block viral replication by competing with cellular nucleotides for incorporation into proviral DNA and are relatively specific for HIV reverse transcriptase. The ability of NRTIs to inhibit human mtDNA is associated with toxic manifestations that include peripheral neuropathy, hepatic steatosis, lipoatrophy, and LA syndrome.[12]

4.1**RETROSPECTIVE STUDY**

The retrospective group showed an incidence of Hlac of 6.2% in the total population of 3741 patient files reviewed. Seventy-one subjects (30.6%) were asymptomatic, 158 (68.1%) were symptomatic and 1.3% had LA. However, this is not a true reflection of the patients with elevated lactate due to the particular process of testing in the retrospective study. The lack of knowledge of the health workers in respect of the condition, the clinic's protocol, testing and ART guidelines which stipulated that patients should only be tested upon presentation of certain symptoms which led to an under-representation of the true incidence of Hlac.[42] On the other hand, the increase in frequency and mortality of patients presenting with Hlac and LA prompted the Tshwane district clinic healthworkers in 2005/2006 to test more regularly for elevated lactate levels, particularly, if patient fell into what was at the time considered to be the high risk profile i.e. female gender, obesity, prolonged ART, excellent adherence, chronic renal failure and pregnancy.[42]

Amongst the 3741 patient files reviewed, 67% were female, but while female gender did not emerge as a determinant of Hlac in this study, women also had significantly higher lactate levels compared to the males (4.4 vs 3.8mmol/L; *p* 0.04). This is in line with other studies. [12, 14, 25] Females also tended to present with elevated lactate levels at an earlier stage (11.7 months vs

13.4 months; *p* 0.06). Overall, this is in line with previous studies that have shown that on average, patients present with Hlac after 6-18 months of HAART.[12, 14, 16, 20] Seventy-two percent of females fell into the symptomatic Hlac or LA group which was significantly higher than among males (*p* 0.021). This further illustrates that Hlac and LA affect females to a greater extent. Although there were no significant weight differences between the females and males, it must be noted that the weights were below those of age-matched subjects in a comparable population, suggestive that subjects in this study are at an advanced stage of disease and/or suffering from co-morbidity such as tuberculosis. It has been shown that many factors, either individually or in combination, could contribute to females having different responses in terms of Hlac. These include better adherence, more women attending HIV clinics or hormonal factors.[11] Based on the above it is reasonable to propose that healthcare workers should regard women as being at greater risk for manifestations of Hlac and LA and should be prepared to test early for the condition.

Regimens tended to differ between the males and females (*p* 0.058) and females (37.1%) were on regimens with stavudine compared to males (11.6%) with 1a2 (stavudine 40mg, 3TC and efavirenz) combination being the most prescribed. Stavudine has been reported in several studies as showing strong associations with Hlac.[12, 13, 14, 16, 21, 39]

Peripheral neuropathy, gastrointestinal symptoms (nausea, vomiting) and abdominal tenderness were the most significant symptoms associated with elevated lactate in this study. These symptoms have been described in several studies, and if present should indicate the need to test the patient for elevated lactate levels.[11,12,15,20,28,35]

In this study, multivariate analysis showed that elevated lactate was related to lower CD4 count, and that an earlier rise in lactate was associated with a higher peak lactate value. Perez and the International Lactic Acidosis Group have shown that patients with advanced progression of the disease (low CD4) are at much higher risk of developing serious NRTI-induced mitochondrial dysfunction.[12,13]

4.2 **PROSPECTIVE STUDY**

Amongst the 292 patients sampled, larger proportions were female (72.6%). The prospective study was done in late 2008, and the trend of more women getting tested and evaluated still existed.

In the prospective group, as expected and as per the objective of the study, the regimens varied significantly from the retrospective study. This change was partly due to recognition by the Tshwane District Hospitals' healthcare workers of the increasing morbidity of patients with Hlac, but also due to the changing national policy guidelines. Sixty-two percent of patients were on stavudine 30mg, 3TC or efavirenz/nevirapine and 32.5% were on zidovudine, 3TC and efavirenz/ nevirapine. The removal of stavudine 40mg, the switch to stavudine 30mg and the introduction of patients onto zidovudine showed fewer side effects. In 2011, Matthews et al, showed that switching from stavudine to zidovudine in patients at risk for Hlac was beneficial in terms of Hlac and LA.[45]

In the prospective study, the symptoms that correlated best with elevated lactate levels were gastrointestinal i.e. nausea and diarrhea. These symptoms were slightly less severe when compared to the symptoms that presented in the retrospective study significantly, and peripheral neuropathy was not observed. The decrease in level of severity of symptoms is likely related to the reduced toxicity of more-recent drug regimens.

Low weight, low CD4 counts and weight loss were significantly predictive of elevated lactate levels. When comparing viral loads, CD4 and weight between patients with elevated lactate and patients with normal lactate, viral loads were higher, CD4 counts lower, and weight was lower in patients with elevated lactate. Once again, the prospective study suggested that patients resort to treatment at an advanced stage of disease, also indicative that if patients' immunity is compromised they are more likely to present with elevated lactate levels. Therefore, in keeping with recent international trends, it is prudent to initiate on ARTs at higher CD4 counts than previously recommended.

4.3**COMPARISON OF THE RETROSPECTIVE AND PROSPECTIVE STUDIES**

Seventy-nine percent and 73% were female in the retrospective and prospective study respectively but it should be kept in mind that females predominated in both the retrospective and prospective studies.

The average ages of the retrospective and prospective groups were 42.6(±8.7) and 38.1(±9.4) years (p<0.001). This difference could be due to the change in mindset of the population at risk, and a reflection of the progress made in South Africa in creating awareness and in addressing the HIV epidemic.

CD4 counts were significantly higher in the prospective group (295±175.8 vs 117±80.4; *p*<0.001). As mentioned before, this is likely the result of patients presenting earlier to be tested and evaluated compared to the retrospective group. Although the CD4 is higher in the prospective group, the value is still at the lower end of normal and as per current guidelines patients should be started on ART at an earlier stage in the disease. So, despite evidence that patients present for treatment at an earlier age, and treatment is initiated with higher CD4 counts, South African patients still appear to be presenting with advanced disease and possibly also with co-morbidity.

In this study, the rate of LA, when converted to a rate per 1000 patient year is 6.8 cases. This is significantly below the 13-20 per 1000 cited in Table 1.3 which summarises a number of studies. However, in terms of the hypothesis that newer regimens would lower the incidence of elevated lactate levels by 50%, the observed incidence of 24.3% is no different from previously reported rates. This therefore shows that although regimen changes have been implemented, the overall incidence of Hlac is still elevated but the LA rate is significantly lower than before. Removal of stavudine completely from the South African ART 2010 guidelines and the use of tenofovir as the only first line regimen would possibly increase patient quality of life and decrease the incidence of symptomatic Hlac and LA.

4.4**CONCLUSION**

In this study, the key findings were that the overall incidence of Hlac appears to be unchanged with newer drug regimens, but LA occurs less frequently. Gastro-intestinal symptoms and signs are still observed but peripheral neuropathy does not appear to be a problem. Advanced disease as manifested by low weight, weight loss and low CD4 counts appear to place patients at a particular risk for the development of Hlac. Healthworkers need to be aware of these risk factors in order to monitor possible progression to Hlac and LA, and should recognize that Hlac/LA are likely to affect females to a greater extent than males.

4.5**LIMITATIONS**

In the retrospective study, the pattern of testing for Hlac was erratic and no proper clinic protocol was used and therefore Hlac was almost certainly underrepresented. Although, point-

of-care measurement devices have been validated, the lower LA rates found in this study might be spurious.

4.6 **RECOMMENDATIONS**

Although regimen changes have been implemented, the more toxic regimens (stavudine, didanosine) should be removed from the South African treatment guidelines. Patients who have a low weight upon entry into the ART program should be closely monitored for Hlac. Patients who have sudden weight loss and unexplained GIT symptoms should be tested for Hlac. Handheld lactate meters should be formally assessed against conventional laboratory methods and if validated as in other studies should become a norm in every resource limited clinic to monitor for the development of Hlac and LA.[27]

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APPENDIX A

- **Retrospective Study**

RETROSPECTIVE STUDY

Race* **1 - White** **2 - Indian** **3 - Coloured** **4 - Black**
Gender** **1 - Female** **First lactate v** **2 - Male**
Asympt **(2.3-5)= 1** **Sympt=(2.3-5)=2** **LA=>5 =3**

Number	Patient Number	Race A-Asian B-Black C-Coloured W-White	Gender M-male F-female	Age in years	Weight kg	CD4	Viral Load	Length of Time on Treatment Months	Lactate Level (mmol/L)	Time on Txt when Lactate Occurred Months

1A-STAVUDINE+LAMIVUDINE+EFAVIRENZ
1B-STAVUDINE+LAMIVUDINE+NEVIRAPINE
2- KALETRA+ZIDOVUDINE+DIDANOSINE

Retrospective Study Table cont. page 2

Initial regimen 1A 1B or 2	**Was the Stavudine Dose Changed from 30mg to 40mg**	**New Regimen**	**Weight Loss (kg)**	**GIT symptoms Diarrhea, Vomiting, Nausea, Stomach Cramps**	**Cholesterol Levels mmol/L**	**Peripheral Neuropathy Y-Yes N-No**	**Hepatitis Type A,B or C**	**TB Type Y- Yes N-No**

Retrospective Study Table cont. page 3

lipodystropy Y-Yes N-No	Insulin Levels mmol/L	Other diseases	Chronic conditions e.g. diabetes	Other diseases e.g. malignancies	Other Drugs e.g. Fluconazole	Other Symptoms

APPENDIX B

- **Prospective Study**

PROSPECTIVE STUDY

Gender	**Race**	**Regimen**
Female 1	1 white	1= 3tc,d4t30mg,efv/nvp
Male 2	2 Indian	2= azt,3tc,efv/nvp
	3 coloured	3=abc,3tc,efv/nvp
	4 black	4=tenofovir,3tc,efv/nvp

Number	**Patient Number**	**Race A-ASIAN B-BLACK C-COLOURED W-WHITE**	**Gender M-MALE F-FEMALE**	**Age in years**	**Height cm**	**Weight kg**	**CD4 count cells/mm^3**	**Viral Load**	**Lactate Before txt mmol/ L**	**Lactate Level (mmol/L)**	**Time on Txt when lactate occurred**

Prospective Study Table cont. page 2

Creatine Clearance mL/min	**Transaminase Levels u/L**	**Initial regimen 1A 1B or 2 or other**	**Was the d4t Regimen Changed from 30mg to 40mg**	**New Regimen**	**Weight Loss kg**	**GIT Symptoms Diarrhea, Vomiting, Nausea, Stomach Cramps**	**Cholesterol Levels mmol/L**	**Peripheral Neuropathy Yes or No**

1A-STAVUDINE+LAMIVUDINE+EFAVIRENZ
1B-STAVUDINE+LAMIVUDINE+NEVIRAPINE
2- KALETRA+ZIDOVUDINE+DIDANOSINE

Prospective Study Table cont. page 3

Lipodystropy Yes or No	**Insulin Levels**	**Other Diseases**	**Chronic Conditions e.g. Diabetes**	**Other Diseases e.g. Malignancies**	**Other Drugs e.g. Fluconazole**	**Other Symptoms**	

APPENDIX C

- **Informed Consent**
- **Patient Informed Consent**

INFORMED CONSENT

Hello,

I am Veni Padayachee, a researcher in the Department of Pharmacy at Wits Medical School. I am interested in finding out more about the risk factors of a condition called lactic acidosis in patients on anti-retroviral drugs. Lactic acid is found in your body when you exercise and sometimes when you take certain medicines .If the lactic acid is not used up then it stays in your blood. A person who has too much of this can have weight loss, nausea (feel like vomiting), vomiting, tiredness and other symptoms that can be sometimes serious. There are scientific studies that indicate that patients on ARVs can have this problem

I would like to study which patients are at risk for this problem so that we can better prevent this problem. I am inviting you to take part in this research study. Before you decide, it is important for you to understand why the research is being done and what it involves. Please read the following information carefully and discuss it with your friends or someone you trust. Please do not hesitate to ask the doctor or nurse or me if there is something you don't understand or you need more information on. If you are unhappy about the study you don't have to take part in the study. The decision is entirely yours.
It does not affect your care at this clinic.

WHY ARE WE DOING THIS STUDY?

The study will look at the different aspects that could increase the amount of lactate in your body. Anti-retrovirals have shown to increase the life span of individuals who are HIV positive. But, the same anti-retrovirals can cause unpleasant effects, like lactic acidosis. We would like to find out more about what causes these unpleasant effects. We would like to know how these drugs affect you. We will check if it makes you tired or sick, nauseous or gives you diarrhea. The Human Research Ethics Committee has reviewed this study to ensure your rights are protected at all times.

IF YOU TAKE PART IN THIS STUDY WHAT WILL HAPPEN?

If you decide to take part in this study, we will need a small amount of blood at 3 and 5 months. We will prick your finger with a needle and then put the blood onto a test strip and into a machine. Your name and other details will not be used in this study. Your identification will be a code. This code will be able to identify you as the patient and can only be accessed by the researcher. This list will be kept locked in

an office.We will need your height and weight before and during the study. Other information will also be asked like if you take other medication (from the doctor or if you buy medication from the pharmacy) or if you take traditional medication. Once again, all this information is kept confidential and no one but the researcher can link this information to you.

If you do not understand anything or if you need more information, please don't hesitate to ask. You can, at any time decide to withdraw from the study. If you decide to take part in this study, we will ask you to sign a consent form indicating that you understand what the study is about.

WITHDRAWAL FROM THE STUDY

Your participation in the study is up to you. You can decide not to continue in the study at any time without any reason. If you decide to stop participating in the study it will not affect your future treatment in any way.

COMPENSATION AND EXPECTED BENEFITS

There will be no financial gain or any other direct benefits if you take part in the study. The medical information gained from the study will help doctors, pharmacists and nurses to be able to diagnose lactic acidosis easily, and to help to prevent this problem in you land other patients.

CONFIDENTIALITY

We will need to look at your hospital and clinic records. We need to check details of illnesses you have had previously and some personal information like your age, weight and height. Your medical data and the results of this study will be recorded. By signing the informed consent you are giving us right to access such information. If the results of this research are to be published your personal information and your identity will not be revealed. Your HIV status will not be revealed to anyone without your consent.

CONTACT NUMBERS

Any symptoms, adverse events or injuries occurring during the course of the trial must be reported without delay to the study coordinator (Mrs Veni Padayachee)/ nurse or counselor

The contact detail for the study coordinator is:

Mrs Veni Padayachee
Department of Pharmacy and Pharmacology
Faculty of Health Sciences
Wits Medical school
University of Witwatersrand
7 York Road
Parktown,Johannesburg
Neelaveni.padayachee@wits.ac.za
Telephone Number: 0117172269 or 0117172552 (Secretary-leave a message)

Contact details for the Supervisors:

Ms Shirona Naidoo
Department of Pharmacy and Pharmacology
Faculty of Health Sciences
Wits Medical school
University of Witwatersrand
7 York Road
Parktown, Johannesburg
Telephone Number: 0117172268

Prof Alan Rothberg
School of Therapeutic Sciences
Wits Medical school
University of Witwatersrand
7 York Road
Parktown,Johannesburg
Telephone Number: 0117172064

If you are concerned with the conduct of the study or you feel that your rights are being abused as a participant you are free to contact the chair of the human research committee.

Contact details for the Human Research Ethics Committee (Medical)

Professor PE Cleaton-Jones (Chairman)
Faculty of Health Sciences
Wits Medical School.
University of Witwatersrand
7 York Road. Parktown, Johannesburg
Telephone Number: 0117172130

Ms Anisa Keshav (Administrative Officer)
Senate House
University Of Witwatersrand
Jorrisen Street, Braamfontein. Johannesburg
Telephone Number: 011717 1234

PATIENT INFORMED CONSENT FORM

The risk factors of lactic acidosis in HIV positive patients receiving Anti-retroviral Therapy

I have fully understood the above information about this study, which I have read or which has been read or translated to me. I understand what will be required of me if I take part in the study.

My questions concerning this study have been answered by **…….**
(**Name of study staff member**)

I agree to take part in this study: **Yes / No (answer to be circled**)

I understand that I may withdraw from the study at any time without giving a reason and without affecting my normal care and management.

Yes/No (**answer to be circled)**

Participants signature **……**. Date **……..**
Participants name **……**

If the information sheet and consent form were translated or explained to the participant please enter the name of the translator here and their signature:

Translator signature …**……** Date **……..**
Translator name **……..**

If the participant gave verbal consent, please enter the name of the person who witnessed the consent here and their signature:

Witness signature **……** Date **……**
Witness name **……**

Name and signature of investigator or designated investigator taking consent

Investigator signature **……** Date**……**
Investigator name**……**

APPENDIX D

ATT Dr M. Mosoane 011 9201195

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Padayachee

CLEARANCE CERTIFICATE **PROTOCOL NUMBER M071133**

PROJECT Profiling the Risk Factors of Lactic Acidosis in HIV Positive Adult Patients on Antiretroviral Treatment in South Africa in the Public Sector (RE-SUBMISSION)

INVESTIGATORS Ms N Padayachee

DEPARTMENT Pharmacy & Pharmacology

DATE CONSIDERED 07.11.30

DECISION OF THE COMMITTEE* APPROVED UNCONDITIONALLY
It is unlikely that there will have been 300 cases of lactic acidosis as such, in this regard the Committee is uncertain how the study will fulfil the investigators aim

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE 07.12.07 **CHAIRPERSON** .....
(Professors PE Cleaton-Jones, A Dhai, M Vorster, C Feldman, A Woodiwiss)

*Guidelines for written 'informed consent' attached where applicable

cc: Supervisor : S Naidoo

DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10005, 10th Floor, Senate House, University.
I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. **I agree to a completion of a yearly progress report.**