THE PREVALENCE OF HYPERLACTATEMIA IN ADULT PATIENTS ON ANTI-RETROVIRAL THERAPY PROGRAMME IN A PUBLIC SECTOR CLINIC IN FREE STATE PROVINCE.

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DECLARATION

I, Ralph Nhiwatiwa, declare that this research is my own work. It is being submitted for the degree of Master of Science in Medicine in the branch of Pharmacotherapy, at the University of the Witwatersrand, Johannesburg.

It has not been submitted before for any degree or examination at this or any other university

Ralph Nhiwatiwa

19th day of April 2010

DEDICATION

In memory of all my patients who died of HIV/AIDS between 1st January 1989 and this day.

ABSTRACT

The national programme of expanded access to antiretroviral therapy in the South African public health sector has resulted in hundreds of thousands of South Africans being subjected to prolonged therapy with the risk of adverse drug effects. Among the most common adverse effects are metabolic disorders one of which is mitochondrial toxicity. Mitochondrial toxicity may manifest as hyperlactatemia.

The study was designed to determine the frequency with which hyperlactatemia occurs in HIV – infected adults on long-term antiretroviral therapy (ART). The objective was to determine the proportion of patients with blood lactate levels that exceed a predetermined cut-off level and to attempt to relate hyperlactatemia to a set of factors namely, gender, age, obesity, symptoms, type of ART regime and duration of ART use.

The study was conducted at an ART clinic in the provincial state hospital of Bongani in the town of Welkom in Free State. The target population was male and female adult patients (18 years and above) on ART for a duration of 1 year or longer. Participants were selected by a random sampling of hospital case file numbers using random table numbers.

The patients answered a set of 7 questions on symptoms, underwent weight and height measurements before having blood drawn for lactate assays Blood specimens for lactate assays were processed at the local National Health laboratory.

Hyperlactatemia was picked up in 33.4% of the patients. Most cases were mild with few or no symptoms. The combination of efavirenz, stavudine and lamivudine (regime 1A) emerged as the strongest risk factor and predictor for hyperlactatemia.

The most significant finding was a higher than usual frequency of hyperlactatemia and the strong influence of the specific combination of efavirenz, stavudine and lamivudine

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Prof H. S. Schoeman for assistance with statistical calculations

My wife and family for encouragement.

ABBREVIATIONS

(1) ART – antiretroviral therapy

(2) ARV – antiretroviral drug

(3) BMI – body/mass index

(4) DNA – deoxyribose nucleic acid

(5) HIV – human immune deficiency virus

(6) H_2O_2 – hydrogen peroxide

(7) NRTI – nucleoside analogue reverse transcriptase inhibitor

(8) ULN – upper limit of normal

(9) b.d – twice a day

(10) nocte – at night

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1. INTRODUCTION

Over 700 500 South Africans were receiving antiretroviral drugs (ARV's) in the public health sector in December 2008. There are 216 treatment sites nationwide making it the largest state-driven ART programme in the world. This was a 53% increase on 2007 – 2008 period[1]

The first line ART regime recommended by the South African national ART guidelines is made up of two combinations of three drugs; regime 1A consisting of stavudine 30mg bd, lamivudine 150mg bd and efavirenz 600mg nocte. Regime 1B consisting of stavudine 30mg bd, lamivudine 150mg bd and nevirapine 200mg bd.[2]

The protocol on use of ARV's in the public health services recommends use of regime 1A in males and in women with no childbearing potential. The idea is to avoid the potential teratogenic effects of efavirenz. Regime 1B is recommended for women with childbearing potential.[2]

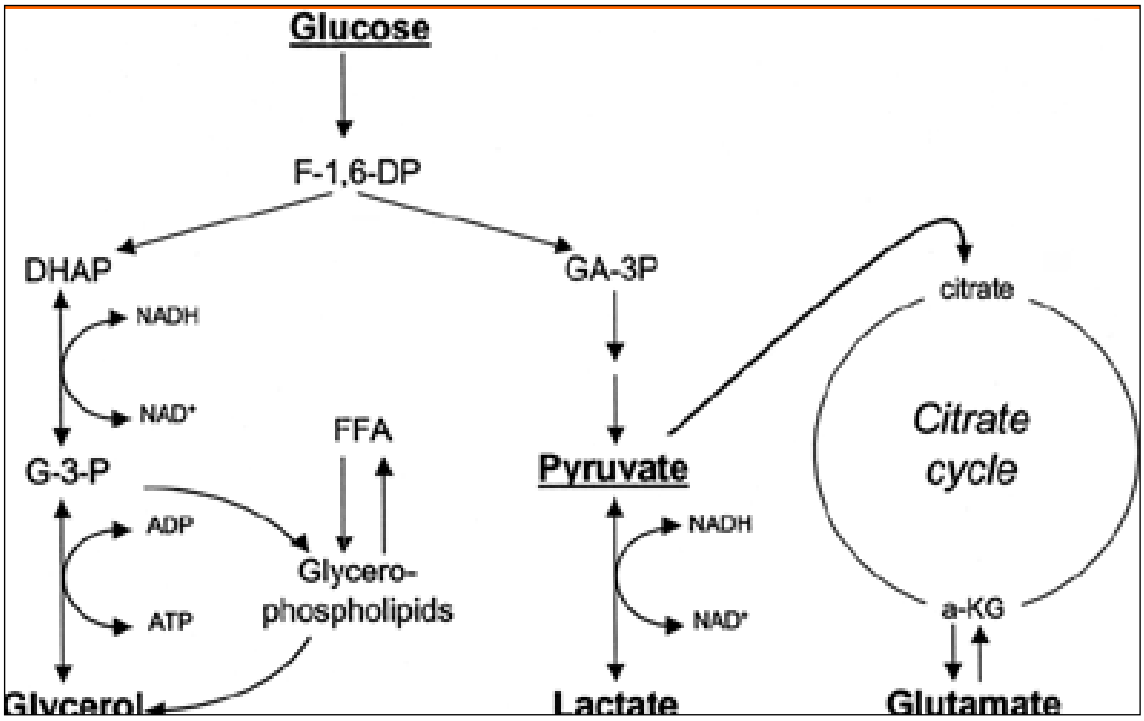
There are many potential threats to the programme, one of the most important being the propensity for drugs to induce adverse metabolic complications. Toxicity to the mitochondria is common and results in metabolic disorders such as neuropathies, lipoatrophy, lipodystrophy, pancreatitis, hepatic steatosis and lactic acidosis. Mitochondrial toxicity results in elevated blood lactate levels and for this reason, hyperlactatemia can be used to screen for mitochondrial toxicity. The Southern African HIV Clinicians Society defines hyperlactatemia as increased plasma lactate level ≥2.5 mmol/L.[3] Of the available classes of

ART's, the nucleoside analogue reverse transcriptase inhibitors (NRTI's) have demonstrated the strongest causal relationship with hyperlactatemia. Stavudine and didanosine are the most potent inducers of hyperlactatemia.[4] This class of drugs suppresses HIV replication by blocking reverse transcriptase, which is a viral RNA-dependent DNA polymerase enzyme.[4]

Nucleoside analogues have been shown to induce mitochondrial injury by way of inhibiting the host DNA polymerase gamma which is involved in mitochondrial DNA replication.[5]

Under normal circumstances, in aerobic tissues, the process of glycolysis produces pyruvate, which is an essential substrate to cellular energy production. Pyruvate is metabolized in the Krebs's cycle producing carbon dioxide and water in the mitochondria. In addition, smaller quantities of pyruvate are metabolized in the cytosol producing smaller quantities of ATP and having water and lactate as by-products (anaerobic metabolism)[6]. Figure 1 shows an outline of the biochemical pathways involved.

Figure 1 shows the biochemical pathways linking glycolysis to the Kreb's cycle and origins of cytosolic lactate



F-1,6 DP ….fructose 1,6-diphosphate

DHAP……dihydroxyacetone phosphate

G-3-P…….glyceraldehyde -3-phosphate

FFA……free fatty acid

GA-3P……glyceraldehydes-3-phosphate

NAD+……nicotinamide adenine dinucleotide (oxidized)

NADH…….nicotinamide adenine dinucleotide (reduced)

ADP……..adenine diphosphate

ATP……adenine triphosphate

a-KG……..alpha ketoglutarate

Approximately 1400 mmols of lactate are produced daily. In a resting individual the concentration of venous lactate reflects the equilibrium maintained between the rate of lactate production in metabolically active tissues and the rate of lactate utilization by the liver and renal cortex.[3] The liver uses approximately 30% of total lactate produced by the body. Moderate increases in lactate production result in compensatory increase in hepatic lactate clearance, but uptake by the liver is saturable at concentrations exceeding 2mmol/L. Inhibition of mitochondrial energy production by NRTI's means more cellular requirements are met by cytosolic metabolism of glucose leading to hyperlactatemia.[6]

Studies on frequency of hyperlactatemia in patients on ART have yielded wide-ranging results. A study on 4788 person-years in Zurich showed a prevalence of hyperlactatemia of 42.3% and severe hyperlactatemia of 3.1%. However this study took a cut-off blood lactate level of 2.4mmol/L and entered patients who were on didanosine and stavudine.[7] The Swiss

Cohort Study on adverse events undertook to determine hyperlactatemia in 880 patients. 8.5% of the patients had hyperlactatemia (1.1times ULN) and only 1% had moderate to severe hyperlactatemia. This study was carried out over three months and patients were on didanosine and stavudine.[8] Women on ART in Soweto, South Africa, were found to have a higher frequency of symptomatic hyperlactatemia and lactic acidosis than reported in western nations[9]. A study of symptomatic hyperlactatemia was carried out on 1735 patients at Chris Hani Baragwanath hospital, South Africa. A venous lactate level cut-off of 4.5mmol/L was used. The overall incidence of symptomatic hyperlactatemia was 20.2 cases per 1000 patient-years.[9] In Botswana, a study of adult patients on a public sector HAART programme showed that 2% of 650 subjects had moderate to severe symptomatic hyperlactatemia.[10] An observational case series study by Geddes et al in KwaZulu Natal estimated the incidence of lactic acidosis as 19 per 1000 person-years. However, all patients were female on Stavudine and Lamivudine, with median age of 36 years, median weight of 81kg and median duration of 7.5 months.[28]

Factors that are known to have strong association with hyperlactatemia in HIV medicine include the use of the drugs stavudine and didanosine. zidovudine and lamivudine have a lower risk. No significant demographic differences were demonstrated between patients with or without hyperlactatemia.[6] Other factors include female gender and having a BMI of greater than 25.[10] The Lactic Acidosis International Study Group released results of a multi-centre case control study on 110 patients in 2007. The study identified the major risk factors as exposure to the drugs didanosine and stavudine, age above 40 years (OR 2.6), female gender(OR 5.57) and advanced immune suppression.[30] This is consistent with

research reports from Stellenbosch University by Eshun-Wilson et al. By reviewing cases of symptomatic hyperlactatemia and lactic acidosis they identified the following as significant risk factors; female gender, duration of 6 months or longer on ART, chronic liver and kidney disease and the combination of stavudine and didanosine. Correlation with BMI was unproven.[31]

Cases of mild to moderate hyperlactatemia are often subclinical. Where symptoms develop, they tend to be non-specific and may not be distinguishable from symptoms of other conditions, which affect HIV-infected individuals. The most common symptoms are nausea, vomiting, diffuse abdominal pains, fatigue, weakness, weight loss and dyspnoea. The nature of these symptoms is such that the diagnosis of hyperlactatemia can be missed or delayed and thus the need for a high index of suspicion by clinicians. Symptomatic hyperlactatemia usually occurs after patients have been on treatment for several months (median 9 months).[11]

Hyperlactatemia can proceed further to the disorder of lactic acidosis, which is a life-threatening condition requiring hospitalization and treatment in an intensive care facility. The precise natural history of hyperlactatemia and its relationship to the risk of severe lactic acidosis is not known.

The literature reports different opinions on routine screening of lactate in patients on ART. Some studies have shown limited value in performing routine lactate assays in asymptomatic individuals.[12] However a 2 year follow up study on symptomatic patients and patients unexplained

symptoms while on HAART which included stavudine, hyperlactatemia was detected in 0.8% who underwent arterial blood lactates, functional respiratory tests, liver or muscle biopsies concluded that "potentially fatal adverse events occurring during antiretroviral treatment may be avoided by close monitoring of clinical signs and blood lactate levels".[13] The South African public health ART programme does not recommend routine lactate testing. The Southern African HIV Clinicians Society defines hyperlactatemia as a venous lactate level of 2.5 mmol/L and higher, mild hyperlactatemia as range 2.5 – 5mmol/L, moderately severe hyperlactatemia as range 5- 10mmol/L and severe hyperlactatemia as > 10 mmol/L. However the WHO HIV/AIDS Programme recommends routine blood lactate testing in symptomatic patients.[11,14]

Management of hyperlactatemia depends on the severity of symptoms and the judgment of the physician regarding the clinical significance of the lactate elevation. However, the International AIDS Society – USA Panel recommends withdrawal of antiretrovirals in all patients with lactate levels >10mmol/L and in all symptomatic subjects with lactate levels >5 mmol/L.[15] Asymptomatic mild hyperlactatemia does not require intervention as there is no conclusive evidence that the condition is a danger or predictive of more severe lactic acidosis in the short term.

2 AIM AND OBJECTIVES

2.1 The aim of the study was to determine the frequency with which the adverse effect of hyperlactatemia (which reflects mitochondrial toxicity) occurs in HIV-infected adults on the first line antiretroviral therapies in a public sector setting.

The main objective was to determine venous lactate assays in patients on two first line ART regimes. Secondary objectives were to attempt to relate hyperlactatemia to factors such as types of ART regimes, symptoms, gender, obesity and duration of ART use; and to review opinion on the practice of routine lactate testing.

2.2 The study aims to answer the following questions;

(1) What is the overall prevalence of hyperlactatemia in adult patients on the two first line ART regimes? How do the prevalences on patients on regime 1A compare with 1B?

(2) What is the distribution pattern of blood lactate levels in these populations of patients?

(3) What is the proportion of males to females in the population of adults on the two first line ART regimes?

(4) Does gender have an influence on the prevalence of hyperlactatemia?

(5) How is the distribution of patients influenced by the selection criteria of the national guidelines as outlined in the introduction?

(6) How does the prevalence of hyperlactatemia in female patients on regime 1A compare with the prevalence of hyperlactatemia in male patients on the same regime? (Regime 1B is essentially for female patients only and gender-based analysis is not possible).

(7) What is the age distribution of adult patients on first line ART and how does age influence the presence of hyperlactatemia?

(8) What is the distribution pattern of BMI's in this population of patients? Does BMI have an influence on prevalence of hyperlactatemia? Are there significant differences in the mean BMI's of groups of patients who are grouped according to their lactate levels?

(9) Is there a significant difference between the prevalence of hyperlactatemia between patients of normal body weight (BMI<25) and overweight patients (BMI>25).

(10) Does the duration of ART use influence the prevalence of hyperlactatemia?

(11) How often do symptoms of hyperlactatemia occur in patients on ART?

(12) Is there a relationship between severity of symptoms and severity of hyperlactatemia?

3 METHOD

3.1 Location of study;

The study was performed at an ART clinic in Bongani Hospital, which is a secondary level institute (i.e. referral but non-teaching hospital) in Welkom. The clinic forms part of the Free State provincial ARV centers serving the municipalities of Matjhabeng, Virginia, Odendaalsrus, Bothaville, Theunissen and Wesselsbron which have a combined population of over 800 000. The general population consists mostly of indigenous African working class. The ART clinic serves as a referral center for a group of primary level clinics, which are Matjhabeng clinic, Welkom city clinic, Phomolong clinic and Theunissen clinic.

3.2 The target population;

The study targeted adult patients who are on first line ART regimes for a period of one year or longer. Adult age definition was 18 years and older and both sexes were included. Selection was restricted to patients on first line regimes 1A and 1B.

The first line regimes are used as shown in table 1 below.

Table 1: **Drug composition of ARV regimes**

Regime	Drug	Strength	Dose
1A	Stavudine	30mg	1bd
	Lamivudine	150mg	1bd
	Efavirenz	600mg	1 nocte
1B	Stavudine	30mg	1bd
	Lamivudine	150mg	1bd
	Nevirapine	200mg	1bd

3.3 Study design;

The study was designed as a prospective observational study. Clinical records were used to obtain demographic data, information on type of ART regime, and duration of ART use.

A questionnaire was used to obtain information on symptoms and symptom scores. (Appendix D).

Venous blood samples were collected to provide data on blood lactate levels. Venesection was performed at the time of the study. No existing blood results were used.

3.4 Sample selection;

Study sample was selected through a simple random sampling of the clinic case file numbers, which have 5-6 digits. Computerized random numbers were generated from Data Desk programme and used for selection. Sample size calculation was made using estimation of proportions with formula $n=\pi(1-\pi)x(1.96/a)$ where "n" is sample size, "π" is proportion and "a" is degree of accuracy. Sample size estimation was 307.

Exclusion criteria were; age below 18, pregnancy and critical illness. Critical illness was defined as any medical condition warranting hospitalization. Critically ill patients were excluded

because blood lactate levels tend to be influenced by the illness rather than drug exposure.

3.5 Informed consent;

The first step involved providing all selected patients with verbal information about the purpose of the study, the potential benefits and risks. The patients' language of preference was used. The second step involved handing out written information sheets for patients to read. The third step involved signing the consent document by the patient (appendix B). The researcher and one clinic nurse facilitated the process.

3.6 Questionnaire;

Consenting patients were provided with questionnaires in the language of their choice. The patients were given time to read and respond to 7 questions on the questionnaire. Respondents indicated their answers by making a ring around either Y for yes or N for no. The number of Y's was summed and this was used as the symptom score which ranged from 0 to 7. The study was designed to capture the scores only and not the specific symptoms. The questionnaire was written in SeSotho, Xhosa, English and Afrikaans to cover most of the languages of the population of patients. The English version was written by the researcher and the three other translations were written by the assisting clinic nurse. (Appendix D)

3.7 Measurements;

Anthropometric measurements were performed by the researcher and the assisting clinic nurse. Patients' heights were measured using a Detecto physician's height measure(ProMed®) with subjects standing upright and head positioned in the Frankfort horizontal plane. Body weights were measured using a Detecto physicians scale (entered in kilograms, estimated accuracy of 85.1% under standard environmental conditions). Subjects were weighed without shoes and only wearing light clothing. Weights were recorded to nearest 0.1kg. Body/ mass indices were calculated using the formula BMI= body weight (kg)/height $(m)^2$ (equation 1,appendix E). The same equipment was used on all patients.

3.8 Phlebotomy procedure

Phlebotomies were performed for blood lactate levels. There were no existing blood lactate results at the time of the study.

Strict application of standard procedures of lactate specimen collection was done according to the guidelines of the USA Department of Health and Human services. The guidelines require tourniquet-free phlebotomy, no fist clenching, no tight clothing around arms and collection of approximately 4 milliliters blood sample into fluoride oxalate tubes. Specimen containers were placed on ice and transported to the laboratory within 20 minutes of collection.[16,17]

The Free State division of the National Health Laboratory Services, which is contracted to the hospital, handled the blood specimens.

The Abbot Diagnostics® Architect Ci8200 integrated processor was used to process the specimens. The processor is a product of Abbott Laboratories Company. (100 Abbott Park Road, Abbott Park, Illinois 60064-5500, USA.) The Ci8200 lactic acid assay procedure uses the reaction of conversion of lactic acid to pyruvate and hydrogen peroxide (H_2O_2) by lactate oxidase. The peroxidase then goes on to catalyse the oxidation of chromogen precursor by H_2O_2 to produce a coloured dye. The coloured dye results in increased absorbance at 548 nm, which is directly proportional to the lactic acid concentration in the sample. The accuracy of the process is dependent on specimen integrity and has an intra-day accuracy of 90.6 – 108%.

The results were initially entered onto the laboratory database before they were transferred to an Excel data sheet as shown in appendix C.

3.9 Clinical records;

Patients' clinical records were reviewed for information relating to age, type of ART regime and duration of treatment. All information was entered onto an Excel data sheet as shown in appendix C.

3.10 Statistical method;

The Excel data sheet contained the following characteristics; the hospital file number, age in years, gender, weight in kilograms, height in metres, BMI, regime types 1A/1B, duration of ART use in months, symptom scores and the blood lactate levels. All the statistical analyses were performed on SAS, release 9,1,3 which was run under Microsoft Windows Vista Business for a personal computer. The data was both discreet (for age, duration and symptom scores) and continuous numerical (for blood lactate levels and BMI). None of the data was censored i.e. no lactate levels beyond assay limits).The main statistical measurements were mean and p-values using the Fisher exact test, ANOVA and the T-test. Correlations of variables were done by the Pearson's correlation factor and calculation of predictor values by the Wald Chi squared regression analyses.

4.1 Questionnaire design

The questionnaire was designed to elicit the common symptoms of hyperlactatemia. The nature of the symptoms is non-specific for which reason the questionnaire is designed to pick up significant symptoms by the following methods;

(a) Building in a time period to the symptoms. Patients make monthly visits to their local clinics. Therefore the term "since your last visit" confines the symptoms to the preceding one month.

(b) Attempting to select out minor symptoms by adding phrases such as "requiring medical attention or medications, lasting more than 3 days, requiring you to stay in bed much of the time during daytime"

No such questionnaire has been used before. The language accuracy of the SeSotho and Xhosa questionnaires was checked and validated by the researcher(first language English) and the assisting clinic nurse, Professional nurse M. Lekhalanyane(first language Sotho, second Xhosa). The Afrikaans questionnaire required extra validation which was performed by Nurse R. Prinsloo (first language Afrikaans, second language English).

4.1 ENGLISH QUESTIONNAIRE

NO.	ENGLISH QUESTIONS		
1	Have you experienced nausea for which you required medical attention or medications since your last visit?	Y	N
2	Have you experienced any vomiting which lasted more than 3 days since your last visit?	Y	N
3	Have you experienced any abdominal pains requiring you to seek medical attention or to take medications since your last visit?	Y	N
4	Have you noticed weight loss over the last month?	Y	N
5	Have you suffered from diarrhea requiring you to seek medical attention or to take medications since your last visit?	Y	N
6	Do you ever feel so tired that you stay in bed much of the time during daytime?	Y	N
7	Have you experienced any feeling of shortness of breath or breathing difficulties that worried you since your last visit?	Y	N

4.2 XHOSA QUESTIONNAIRE

1	Oko u suke apha u ke wa khonyuluka ukuthu u sele amayeza okanye u bone u gqira?	Y	N
2	Uke wa hlanza (gaba) u kwe dhlula iintsuku ezintathu?	Y	N
3	Uke waba namahlaba e suswini ukuthi u sele amayeza okanye u ye kwa gqira	Y	N
4	U ke wa qaphela u kwehla kwe mzimba ku le nyanga i dlulileyo?	Y	N
5	U ke wa ba ne sisu e si hambisayo ukuthi u sele amayeza okanye u ye kwa gqira	Y	N
6	U no ku ziva u khathele ngamanye amaxhesha ukuthi u fune u ku lala nje?	Y	N
7	U ba ne phika okanye u be nobunzima e ku phefumleni u phelele ngu moya ngamane ama xhesha?	Y	N

4.3 SESOTHO QUESTIONNAIRE

No.	SOTHO DIPOTSO		
1	O kile oa ikutlwa o nyekelwa haholo hao o bileng wa hloka pheko ya meriana kapa ya bongaka?	Y	N
2	Haesale o qetela ho tla kwano phekolong, o kile wa ba le lehlatso le ka etsang matsatsi a mararo kapa hofeta?	Y	N
3	Haesale o qetela ho tla kwano phekolong, o kile wa longwa ke mala hao bileng wa hloka ho nwa meriana kapa ho bona ngaka?	Y	N
4	Na o hlokometse hore ka mohlomong oi ntse o fokola mmeleng kgweding ena?	Y	N
5	Na o kile wa ba le letshollo moo o kile wa nwa meriana teng?	Y	N
6	Na o ba le ho tepella hoo o sa batleng le ho tsoha hara motsheare?	Y	N
7	Na o nale ho fellwa ke moya ha o phefumoloha?	Y	N

4.4 AFRIKAANS QUESTIONNAIRE

1	Het u enige naarheid ondervind waarvoor u enige behandeling of medikasie ontvang het, sedert u laaste besoek?	Y	N
2	Het u enige braking ondevind wat vir langer as 3 dae aangehou het, sedert u laaste besoek?	Y	N
3	Het u enige abdominale pyn ondervind waarvoor u mediese behandeling of medikasie ontvang het, sedert u laaste besoek?	Y	N
4	Het u enige gewigs verlies waargeneem oor die laaste maand?	Y	N
5	Het u gesukkel met diaree waarvoor u mediese versorging noding gehad het of medikasie daarvoor geneem, sedert u laaste besoek?	Y	N
6	Voel u ooit so moeg dat u die meeste tyd deur die dag in die bed bly?	Y	N
7	Ondervind u einige kortasemigheid of benoudheid wat u bekommer, sedert die laaste besoek?	Y	N

5. RESULTS AND DATA

5.1 Over a period of 6 weeks, 350 patients were enrolled onto the study with 305 completing the study.45 patients were not considered due to missing data particularly missing laboratory lactate results. 1 female patient refused consent.

5.2 Overall prevalence of hyperlactatemia in both regimes.

This was calculated by grouping all blood lactate results and taking a predetermined cut-off level of 2.5mmol/L. The number of patients with blood lactate levels of ≥2.5mmol/L was expressed as a percentage of all the patients in the study.102 patients had hyperlactatemia which translated to an overall prevalence of 33.4%.This proportion calculation had a 95% confidence interval of 28.1-38.7%.

All the blood lactate results were then grouped into 4 categories (class intervals) to show the distribution of the patients according to the blood lactate results as shown in table 2 and figure 2. The range of lactate values was 0.5 – 8.1mmol/L. Approximately 90% of patients with hyperlactatemia fell in the mild category. Approximately 2.5% had moderate hyperlactatemia. No cases of severe hyperlactatemia were documented. The overall prevalence of hyperlactatemia in this study is higher than the previously recorded in other studies. A study by Minah John and colleagues showed hyperlactatemia prevalence of 5 in 516(2.8 – 4.0%) in

patients on stavudine and zidovudine. Severe hyperlactatemia was even less prevalent 2 in 516(1.8%).[18]

Table 2. Lactate level categories and number of patients per category

Lactate Level (mmol/L)	**Lactate classification**	**Number of patients**	**(percentage)**
< 2,5	Normal	203	(66.6%)
[2,5 – 5,0)	Mild	93	(30.5%)
[5,0 – 10,0)	Moderate	9	(2.9%)
≥ 10,0	Severe	0	(0%)
	Total	305	(100%)

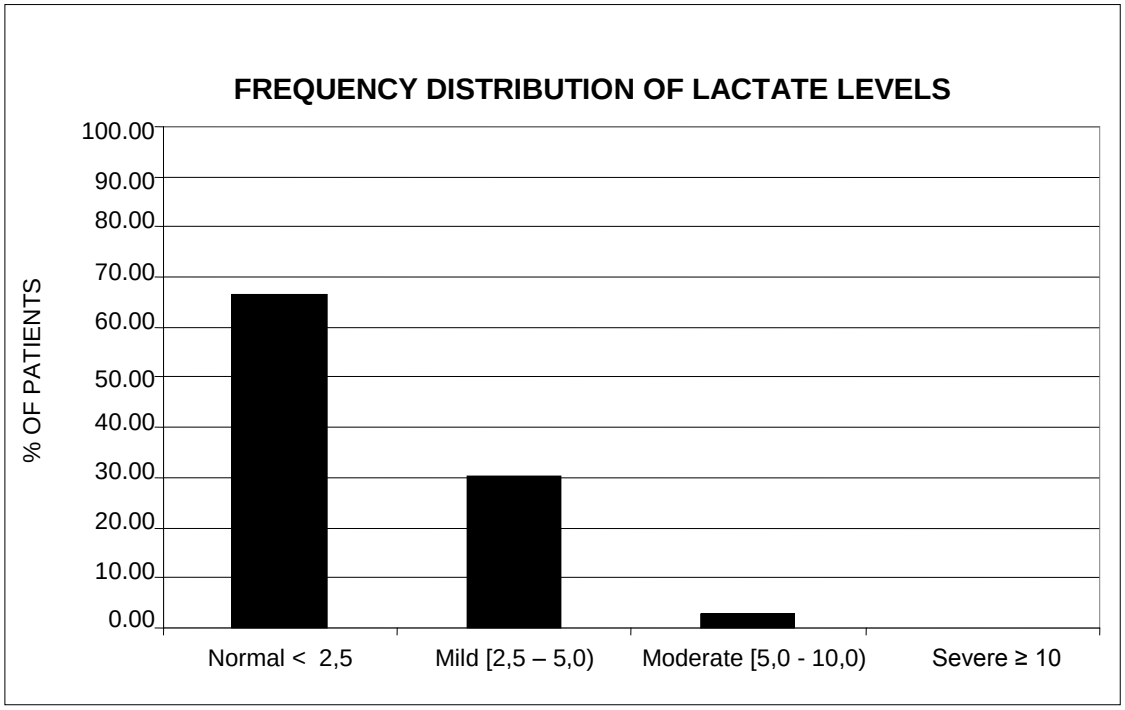


Figure 2. Percentage frequency distribution pattern of lactate levels in all patients.

5.3 Gender distribution

A total of 236 female patients and 69 male patients were in the study. Thus females constituted 77.7% and males 22.3% giving a female: male ratio of 3.5: 1.This is a reflection of the general population of patients attending ART clinics[8] Table 3 and figure 3 demonstrate the distributions.

Table 3. The proportion of males to females in all participants.

	Number of patients	Percentage of patients
Males	69	22.3%
Females	236	77.7%
Total	305	100%

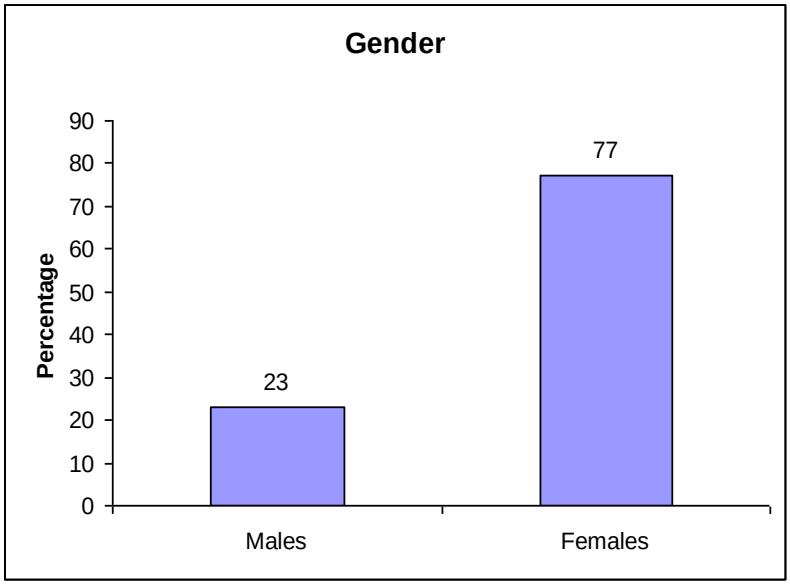


Figure 3 proportion of males to females

5.3.1 Relationship between the blood lactate levels and gender

The lactate results of all patients (both regimes) were analysed as females and males in each category of normal, mild, moderate and severe to make a comparison between the gender groups as shown in table 4 and figure 4. This shows a higher proportion of males with mild hyperlactatemia but statistical analysis showed that the distributions for males and females in each lactate level category and over the three categories do not differ significantly. Normal lactate p=0.191, mild hyperlactatemia p=0.181 and moderate hyperlactatemia p=1.00 by Fisher Exact test.

Logistic regression analysis was performed with lactate levels as dependent variables and gender as a predictor variable. Wald Chi-squared = 0.1451 and p=0.7032 meaning gender is not a predictor.

Table 4. The number and percentage of male and female patients in the three lactate level categories.

Lactate level	**Number (%) of patients**		
	Female	**Male**	**Total**
Normal	162 (68.6)	41 (59.4)	203 (66.6)
Mild	67 (28.4)	26 (37.7)	93 (30.5)
Moderate	7 (3.0)	2 (2.9)	9 (2.9)
Total	**236 (100)**	**69 (100)**	**305 (100)**

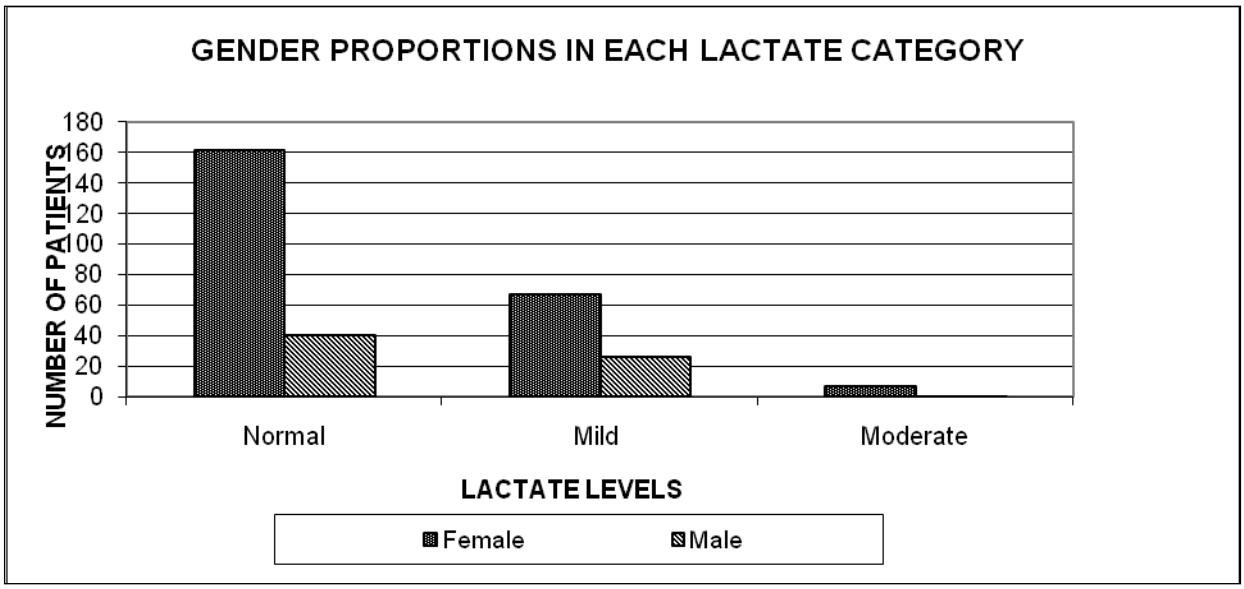


Figure 4.Comparison of gender proportions in the different lactate level categories.

5.4 The distribution of patients between the treatment regimes

All patients were categorized according to treatment regime as 1A or 1B. Table 5 shows this. 60% of patients were on regime 1A and 40% on regime 1B.Regime 1A had a male : female ratio of 1 : 1,6 while regime 1B had a male : female ratio of 1 : 39.The distribution was influenced by the protocol guidelines which exclude women with child-bearing potential from regime 1A due to potential Efavirenz teratogenicity. Only 3 males received regime 1B.

Table 5. The distribution of patients between the two ART regimes

Regime	Gender	Number of patients	Percentage of patients
1A	**Male**	72	23.6%
1A	**Female**	112	36.7%
1B	**Male**	3	0.01%
1B	**Female**	118	38.7%
Total		305	100%

5.4.1 The relationship between prevalence of hyperlactatemia and the type of treatment regime

The blood lactate results of patients in each regime were separately analyzed according to the categories of normal, mild, moderate and severe as shown in table 6 and figure 5.

Statistical analysis with the Fisher exact test showed a significant difference between the lactate level distributions for regimes 1A and 1B ($p < 0.001$).

Pairwise comparisons of the percentages for 1A and 1B in each hyperlactatemia category yielded the following results:

- The percentage patients with **normal lactate** levels on regime 1A (58.5%) differs significantly from the percentage patients with normal lactate levels on regime 1B (78.7%). (Fisher exact test, **$p < 0.001$).**

- The percentage patients with **mild hyperlactatemia** on regime 1A (38.2%) differs significantly from the percentage patients with mild hyperlactatemia on regime 1B (18.8%). (Fisher exact test, **$p < 0.001$)**.The percentage patients with **moderate hyperlactatemia** on regime 1A (3.3%) does not differ significantly from the percentage patients with moderate hyperlactatemia on regime 1B (2.5%). (Fisher exact test, **$p = 0.746$**).

Table 6. Comparison of lactate level categories between the two ART regimes.

Lactate level	**Number (%) of patients**		
	1A	**1B**	**Total**
Normal	107 (58.5)	96 (78.7)	203 (66.6)
Mild	70 (38.2)	23 (18.8)	93 (30.5)
Moderate	6 (3.3)	3 (2.5)	9 (2.9)
Total	**183 (100)**	**122 (100)**	**305 (100)**

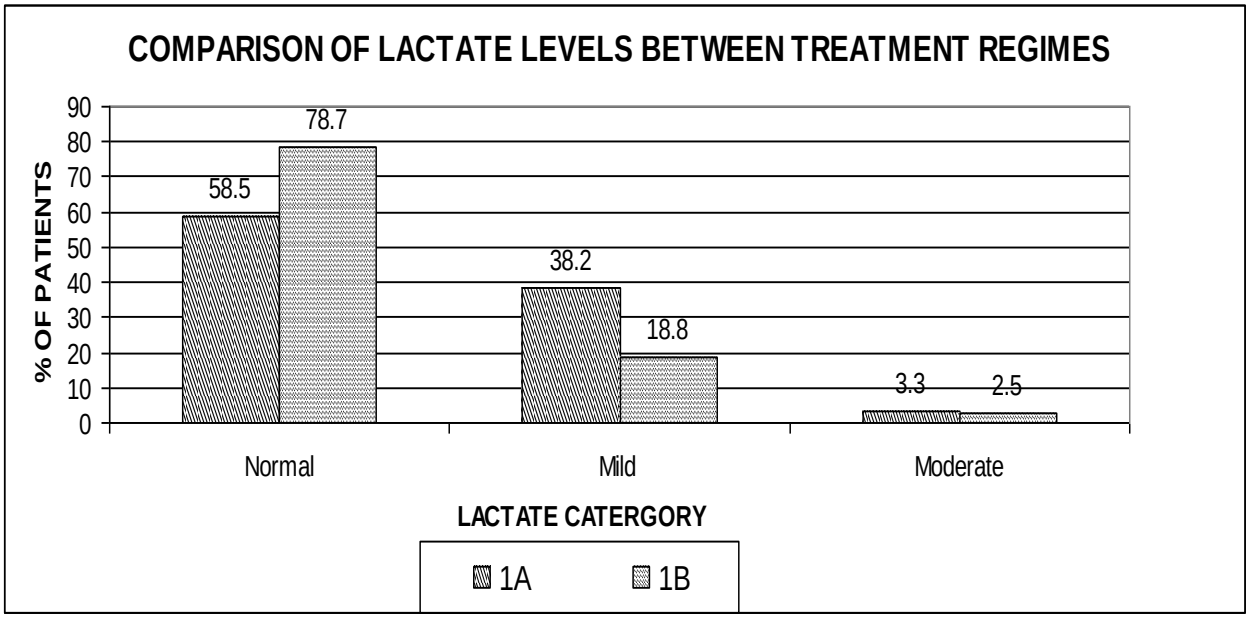


Figure 5. the prevalence of hyperlactatemia in patients on regimes 1A & 1B

5.4.2 The association between type of regime and hyperlactatemia

Taking a cut-off of 2.5mmol/L (which defines hyperlactatemia) a comparison of the proportions of patients with hyperlactatemia was made between the two regimes. The percentage of patients on Regime A with hyperlactatemia (41.5%) differs significantly from the percentage of patients on regime B with hyperlactatemia (21.3%) (Fisher Exact test, $p< 0.001$).This implies that regime 1A is comparatively a stronger risk factor for development of hyperlactatemia than regime 1B.The difference is particularly for patients with mild hyperlactatemia($p<0.001$).See table 7.

Use of regime 1A is also a significant predictor for hyperlactatemia as shown by a logistic regression analysis (Wald Chi-squared test, $p=0.009$). This is consistent with the findings of the Swiss HIV Cohort study in which significant risk factors were identified as regimes containing stavudine and didanosine (hazard ratio, 6.65) or efavirenz (hazard ratio, 2.85)[13] While the mechanism of NRTI-induced hyperlactatemia is well established, it is not clear how efavirenz causes hyperlactatemia. However recent in-vitro studies have suggested suppression of the lipogenic pathway by efavirenz as a possible explanation.[14]

Table 7. Association of ART regime and prevalence of hyperlactatemia

Lactate level mmol/L	Number of patients on 1A	Number of patients on 1B
< 2.5	107 (58.5%)	96 (78.7%)
≥ 2.5	76 (41.5%)	26 (21.3%)
TOTAL	183 (100%)	122 (100%)

5.4.3 Influence of gender on prevalence of hyperlactatemia.

To make a comparison of the prevalence of hyperlactatemia between the gender groups, patients on regime 1A were divided according to gender such that the results of blood lactate levels in each group could be analyzed according to the blood lactate categories of normal and elevated lactate. Only regime 1A was used as it has a more comparable gender distribution.

The prevalence of hyperlactatemia was 39% in females and 37.5% in males on regime 1A. The percentage normal lactates for males and females were compared by the Fisher's exact test and were found not to differ significantly ($p= 0.88$). In the same way a comparison of the percentages for hyperlactatemia was done with no significant difference ($p= 0.88$).

Gender comparison was done in regime 1A only because regime 1B is for female patients in childbearing age. The results suggest that the overall prevalence of hyperlactatemia was influenced by the distribution of patients between the regimes and not the gender.

The results are demonstrated in table 8 and figure 6 below.

Table 8. Comparison of prevalence of hyperlactatemia between males and females on regime 1A

LACTATE CATEGORY	**NUMBER OF MALES (36.9%)**	**NUMBER OF FEMALES (63.1%)**
NORMAL LACTATE <2.5 mmol/L	45 (62.5%)	75(61%)
HYPERLACTATEMIA ≥2.5mmol/L	27(37.5%)	48(39%)
TOTAL	72(100%)	123(100%)

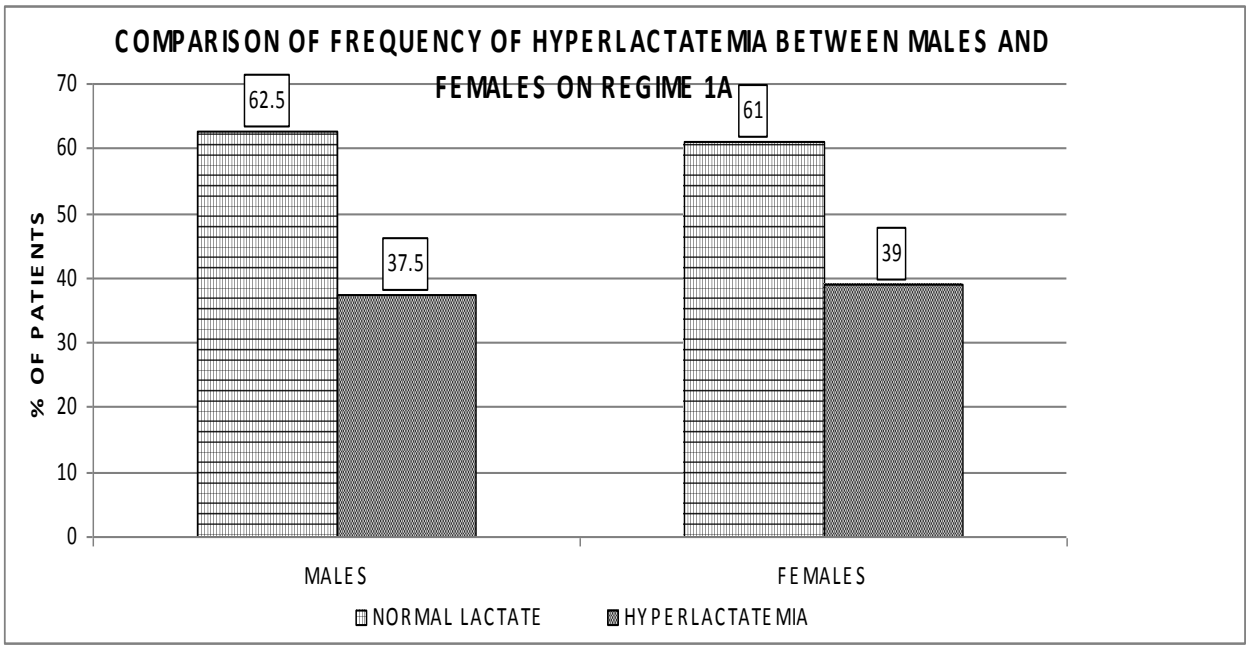


Figure 6. Comparison of prevalence of hyperlactatemia between males and females on regime 1A

5.5 The ages of the patients.

The frequency distribution of the ages of all participants was demonstrated by classifying all ages into 6 class intervals as determined by the total number of patients(305). Age range was 18 – 67 years with the age intervals, number and percentages of patients as shown in table 9 and figure7 below;

Table 9. The age distribution of patients

Age	Number of patients	Percentage of total number of patients.
18-24	5	1.6%
25-34	75	24.6%
35-44	127	41.6%
45-54	81	26.6%
55-64	16	5.3%
65-67	1	0.3%
TOTAL	305	100%

5.5.1 The influence of age on prevalence of hyperlactatemia.

All patients were grouped according to the lactate level categories and each category was subjected to statistical analysis of age. The average age increased from normal lactate to mild hyperlactatemia to moderate hyperlactatemia (39.46 to 41.65 to 43.44). However, the 3 mean values did not differ significantly (ANOVA, $p > 0.05$). However age analysis comparing the 2 categories of normal and high lactate showed a significantly higher mean age in the latter suggesting that age above 40 may be associated with hyperlactatemia $p=0.025$. A logistic regression analysis was performed with lactate categories(normal or high) as the dependent variables and age as a predictor variable for high lactate. See table 10.

Table 10 logistic regression analysis of age as a predictor of hyperlactatemia.

	High lactate	Normal lactate	P value
Number	102	203	
Mean	41.80	39.46	0.025
Standard deviation	8.14	8.78	
Minimum/maximum	25/59	21/67	
95% CI for mean	40.21-43.40	38.24-40.67	

This showed statistically significant difference between mean ages of patients with normal and high lactate levels (T-test, p=0.025)

Table 11 Analysis of age statistics in relation to categories of lactate levels.

	Age (years)				
	n	**Mean**	**SD**	**Minimum**	**Maximum**
Normal	203	39.46	8.78	21	67
Mild	93	41.65	8.05	25	59
Moderate	9	43.44	9.32	30	59
Overall	305	40.24	8.63	21	67

5.6 Body- mass index.

The frequency distribution of BMI`s of all participants was demonstrated by classifying all BMI`s into 5 WHO class intervals.[27] BMI ranged from 16.4 – 42.9kg/m^2 . The pattern of BMI distribution showed that most patients (77.6%) were normal or under weight.

BMI cluster	Frequency	Percentage
< 18.5	14	4.64%
18.5 – 24.9	155	51.32%
25 – 29.9	92	30.46%
30.0- 39.9	39	12.91%
≥ 40	2	0.66%
total	302	100%

(3 BMI recordings missing due to a recording error)

5.6.1 Relationship between BMI and blood lactate levels.

All patients were grouped according to blood lactate levels into the categories of normal, mild, moderate. Mean BMI of group was calculated and a comparison was made between the means as shown in figure 8.

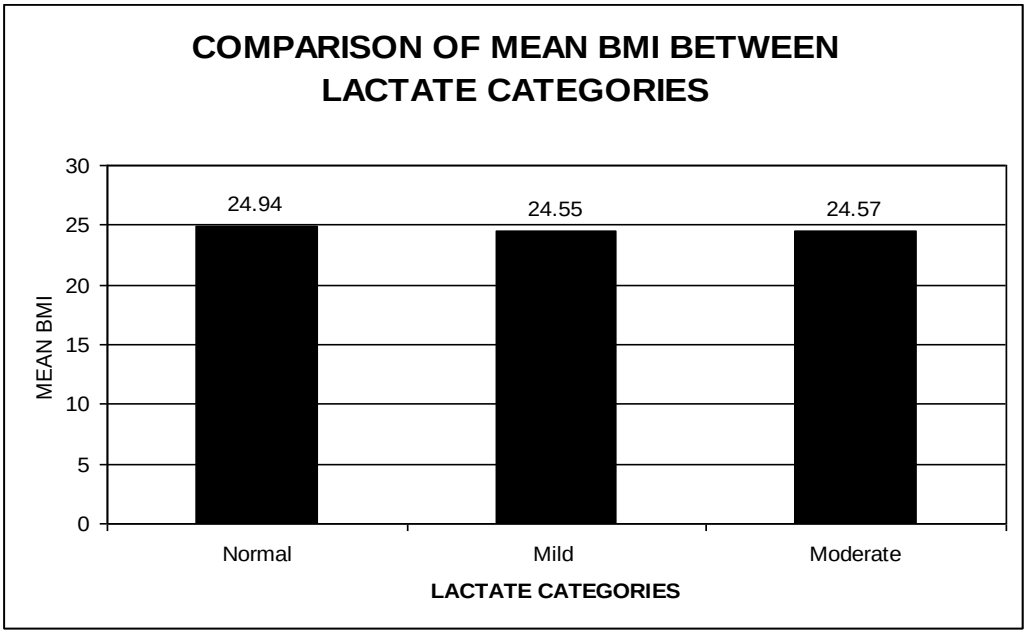


Figure 7. the mean BMI`s in 3 lactate level categories.

5.6.2 The influence of BMI of patients on the prevalence of hyperlactatemia,

To demonstrate the influence of BMI of patients on the prevalence of hyperlactatemia, the study lactate level categories were separately analyzed according to BMI statistics of mean, standard deviation, minimum and maximum. The three mean values for normal, mild and moderate were 24.94, 24.55 and 24.57 respectively, the differences did not reach statistical significance (ANOVA, $p > 0.05$). This is shown in table 12.

Table 12. An analysis of BMI statistics in relation to the categories of lactate levels.

Lactate level category	BMI (kg/m^2)				
	Number of patients	Mean	SD	Minimum	Maximum
Normal	200	24.94	5.25	15.98	42.90
Mild	93	24.55	3.98	16.40	34.00
Moderate	9	24.57	3.04	21.00	31.20
Overall	302	24.81	4.83	15.98	42.90

Several studies have demonstrated a positive association between obesity and hyperlactatemia but results of this study

do not show influence of BMI on hyperlactatemia. Abdominal obesity, which is best measured by magnetic resonance imaging of the abdominal fat, is more closely linked to hyperlactatemia. This may explain the absence of association between BMI (which does not take abdominal obesity into account) and hyperlactatemia in this study. Study from the Harvard research institute in Botswana, where 69% of the patients are female, showed a 2% prevalence of mild to moderate hyperlactatemia. Female gender and BMI > 25 were predictive of hyperlactatemia (p = 0-001).[10]

5.6.3 Relationship between BMI and prevalence of hyperlactatemia.

The purpose of this particular analysis was to make a comparison based on standard clinical BMI cut-off of 25 and standard lactate level cut-off of 2.5mmol/L as an attempt to demonstrate influence of BMI on frequency of hyperlactatemia. The frequency of hyperlactatemia (lactate ≥2,5mmol/L) in each BMI category is shown in figure 9.

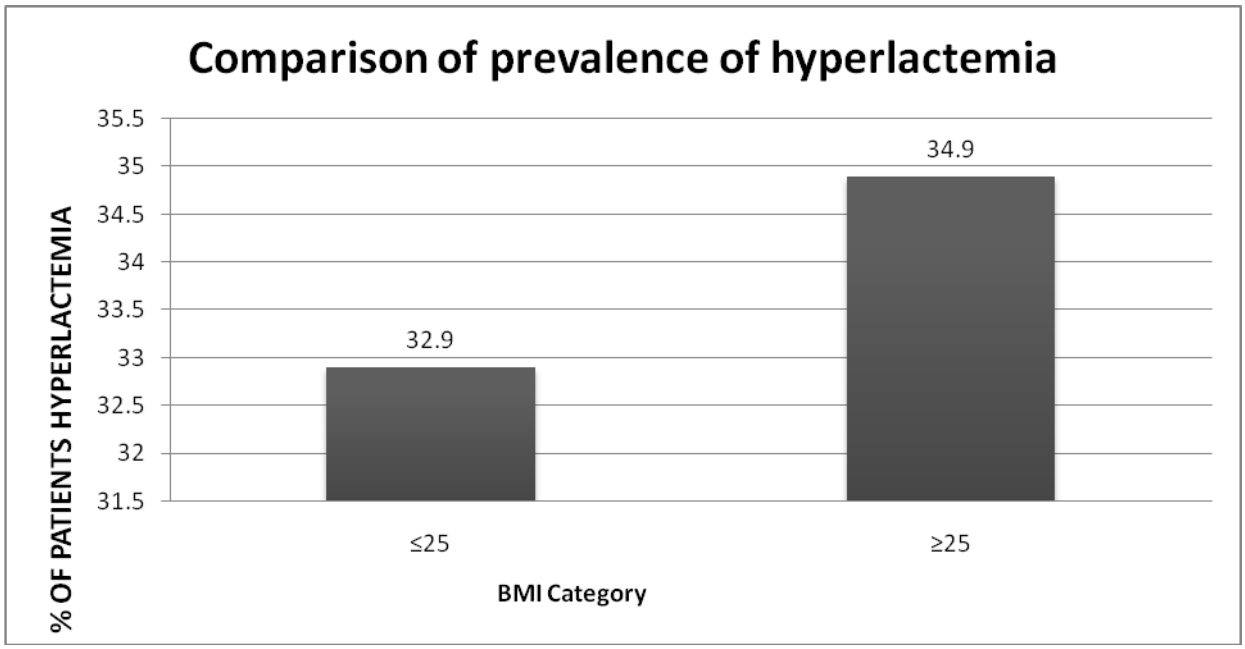


Figure 8. Comparison of prevalence of hyperlactatemia between two categories of patients with normal body weight and patients who are over weight.

Linear (straight line) regression analysis of lactate level (y) versus BMI (x) was performed as y= 2.49-0.01x. Coefficient of determination r^2=0.002=0.2% implying a lack of correlation.

Normal-weight patients had a hyperlactatemia prevalence of 32.9%. Over weight patients had a lactate prevalence of 34.9%. The two prevalences do not differ significantly.(Fisher exact test, p<0,806) The data shows a relative scarcity of patients with extreme obesity with only 2 cases with BMI ≥40. This may be a reflection of the general weight in this population of patients or it may be affected by the relative contraindication of stavudine in extreme obesity.

5.7 The duration of ART use.

To demonstrate the relationship between the duration of ART exposure and the prevalence of hyperlactatemia all patients were grouped according to blood lactate levels into the categories of normal, mild and moderate. The mean of duration of ART use in each category was calculated and a comparison made as shown in table 12 and figure 10. The three mean values for normal, mild and moderate (26.84, 25.47 and 26.67) do not differ significantly (ANOVA, $p > 0.05$).

Table 13. An analysis of the duration of ART use in relation to the categories of lactate levels

Hyperlactatemia	Duration (months)				
	Number of patients	Mean	SD	Minimum	Maximum
Normal	202	26.84	9.52	12	50

Mild	92	25.47	9.72	12	50
Moderate	9	26.67	7.33	12	36
Overall	303	26.42	9.52	12	50

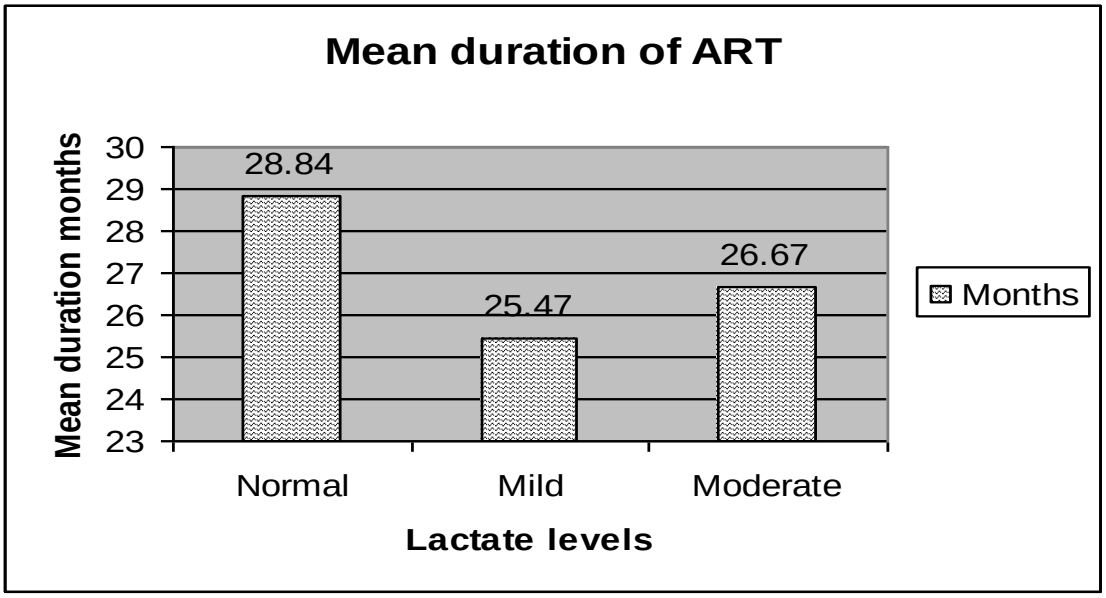


Figure 9. Mean duration of ART use in relation to the categories of lactate levels.

The duration of ART use, after one year, may not influence risk of hyperlactatemia although studies from California have demonstrated that duration of NRTI use is independently associated with higher lactate levels. In this study of 95 HIV infected patients a relationship was demonstrated between

cumulative exposure to NRTI's, insulin resistance and plasma lactate levels. However this study population was 90% male.[19]

5.8.1 Symptoms of hyperlactatemia.

All patients were classified according to symptom scores to demonstrate the occurrence of symptoms of hyperlactatemia.

54% of all participants had scores of 0 or 1. High symptom scores of 6 or 7 were recorded in 2.8% of participants (figure 11)

Symptom score	
0	73 (23.93%)
1	92 (30.16%)
2	56 (18.36%)
3	44 (14.43%)
4	21 (6.89%)

5 10 (3.28%)

6 6 (1.97%)

7 3 (0.98%)

Total 305 (100%)

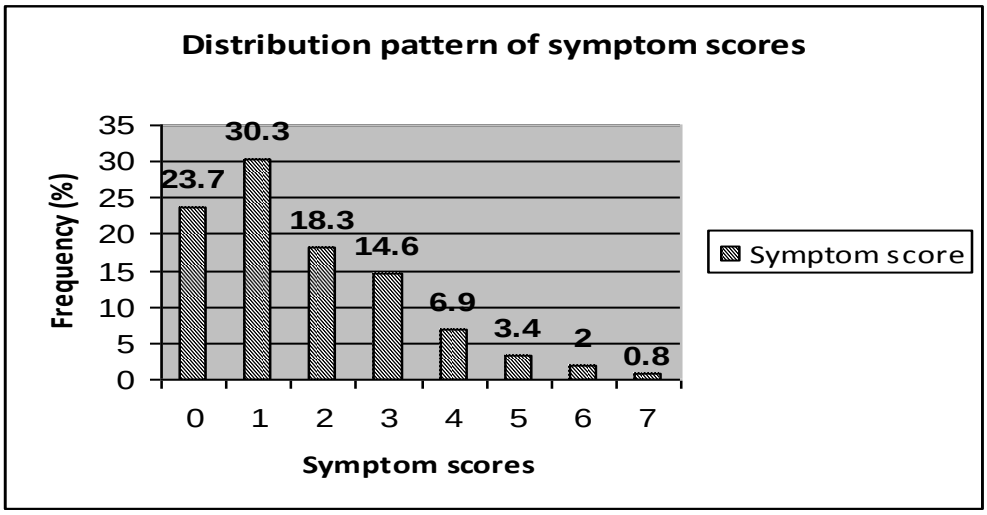


Figure 10. The distribution pattern of symptom scores of hyperlactatemia

5.8.2The relationship between severity of hyperlactatemia and severity of symptoms

To demonstrate this relationship, an analysis of symptom scores in each lactate level category of normal, mild and moderate was made as shown in table 12 and figure 12 below.

Table 14; Analysis of symptom scores in relation to categories of hyperlactatemia.

Hyperlactatemia	**Symptom score**				
	N	**Mean**	**SD**	**Minimum**	**Maximum**
Normal	203	1.70	1.52	0	7
Mild	93	1.75	1.69	0	7
Moderate	9	2.11	1.69	0	5

Overall	305	1.73	1.57	0	7

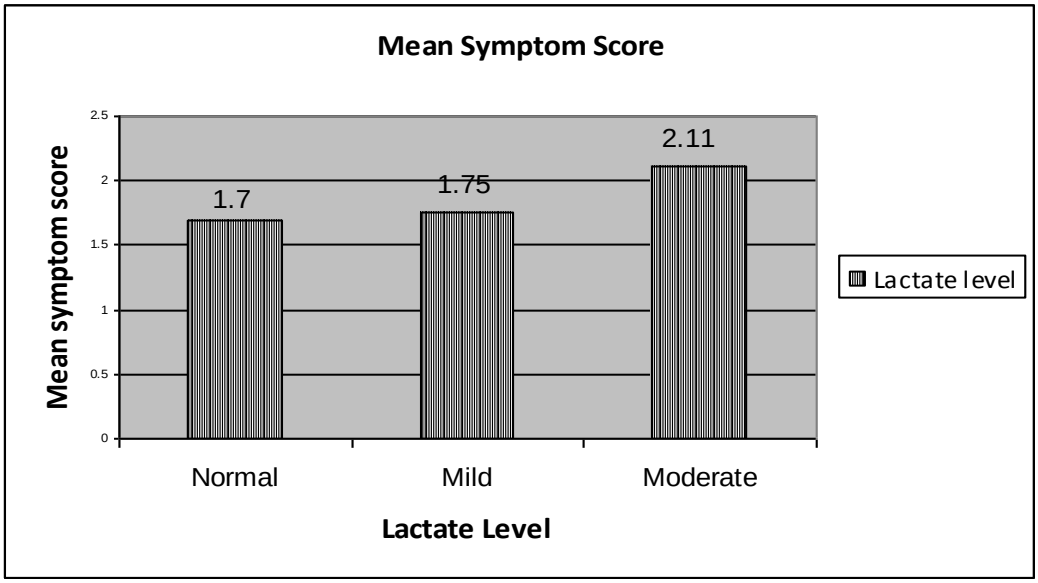


Figure 11; relationship between the lactate level categories and the mean symptom scores

The three mean values for normal, mild and moderate show a progressive rise (1.70, 1.75 an 2.11). The differences however do not reach statistical significance (ANOVA, $p > 0.05$).

Severity of symptoms has no relationship to lactate levels. This is consistent with other studies. One such study is the Canadian study on correlation of mild to moderate symptoms with lactate levels. In

284 individuals on NRTIs, hyperlactatemia occurred in 8% and a Spearman's correlation coefficient of 0.07 demonstrated absence of correlation between severity of symptoms and hyperlactatemia.[15]

Logistic regression was carried out to analyze the variables age, duration of treatment, BMI (= or <25 or >), symptom score, type of regime and gender to determine significance of these variables as predictors of hyperlactatemia (Wald chi-squared test). The only significant predictor was exposure to regime 1A.($p = 0.009$)

6. DISCUSSION

6.1 This was a study on HIV-infected adults on first line ART regimes for a period of one year or longer. In a sample of 305 patients, hyperlactatemia prevalence of 33.4% was found (95% CI of 28.1% - 38.7%). This implies a high level of drug-induced mitochondrial toxicity in this population of patients. The overall prevalence of hyperlactatemia in this study is higher than previously recorded in other studies.[10]

This difference may be resulting from the fact that this study focused on patients on ART for one year or longer with most patients being on regime 1A which contains the combination of the NRTI drug stavudine and the NNRTI drug efavirenz which are strong inducers of mitochondrial toxicity. Most cases of hyperlactatemia were however mild/moderate with few symptoms. Most patients in the study were female. Comparison of frequency of all levels of hyperlactatemia between the gender groups showed no significant difference and no predictor value. The gender distributions of patients between the treatment regimes was very skewed with only 3 males on regime 1B. For this reason gender comparisons could only be performed on patients on regime 1A.Research work in this field in other areas has shown higher prevalence in female patients.[10]

The use of the drug combination in regime 1A i.e. stavudine, lamivudine and efavirenz was shown to be strongest risk factor and has significant predictor value. The most significant statistical difference is shown in the level of mild hyperlactatemia.

The age distribution of patients showed a normal distribution pattern with a range of 18 – 67 years. An analysis of age statistics showed that the mean age increases with progression through the categories of normal lactate, mild hyperlactatemia to moderate hyperlactatemia but the differences were statistically insignificant. However when age is analysed in relation to the two broad categories of normal and hyperlactatemia, significantly higher mean age was shown in patients with hyperlactatemia implying that the age of a patient may influence the prevalence of hyperlactatemia. In a study from Botswana – Harvard School of Public Health, older age i.e. above 40 years was a significant risk for hyperlactatemia ($p=0.051$).[10]

There is no demonstrable influence of BMI on the prevalence of hyperlactatemia and linear regression fail to show correlation between BMI and the categories of normal and high lactate. This is similar to other studies in public institutes in South Africa.[20] This may be so because the actual link is through abdominal obesity which is best demonstrated by magnetic resonance imaging, a radiological technique that is not readily available in South African public institutes. A study by Wester and colleagues from Botswana painted a different picture. The study was carried out over 3 years with 111 participants, 69% were female, 31% male and took a blood lactate level cut-off of 4.4 mmol/l. The researchers demonstrated that an increase in BMI of 1 unit is associated with an increase in lactate level (adjusted hazard ratio=1.17 and $p<0.0001$)[29]

Prolonged use of ART does not increase the risk of developing hyperlactatemia as shown by an analysis of mean durations of ART use in relation to lactate level categories. This is different from studies reported from Canada.[19]

Symptoms of hyperlactatemia are uncommon and the severity of the symptoms is not related to levels of hyperlactatemia. Symptoms are not a reliable means of screening for hyperlactatemia.[21]

6.2 Limitations

For a study of this nature, the following limitations need to be acknowledged; the influence of other disorders which are commonly found in HIV infected individuals such as chronic viral hepatitis B and C and occult malignancies such as lymphomas and other physiological states e.g. impaired renal function with GFR < 70 mls/min which have an influence on venous lactate levels.[22] Some sampling errors which have been known to influence lactate results are patient activity before venesection, tourniquet use and delay in processing samples.[22] The relevant tests for some of the factors mentioned here were beyond the scope and affordability of the study.

The study makes an assumption of adequate adherence to ART by patients (which means taking 85% or more of doses).Verification of adherence is difficult in clinical practice.[23]

Asymptomatic hyperlactatemia may be transient which is the reason some guidelines recommend a series of venous lactate tests to verify hyperlactatemia but research suggests that random single tests as done in this study give an acceptable indication of low levels of mitochondrial DNA; nuclear DNA ratios reflecting mitochondrial toxicity. Alternative methods to assess mitochondrial depletion are available and include testing peripheral blood lymphocytes.[24]

The practical challenges of performing venous lactate sampling correctly in an outpatient clinic need to be acknowledged. The tourniquet- free procedure is difficult and delays sample collection.

While routine lactate testing in patients on ART remains controversial, it may have a place in selected high-risk groups on regime 1A.[25,26]

7. CONCLUSIONS

The first line ART regimes in the South African public health sector are associated with mitochondrial toxicity. By using hyperlactatemia as a screening test, this study has demonstrated that this side effect occurs in about 33% of adults on treatment for a minimum of one year. This is higher than previously reported.

The study identified use of regime 1A (stavudine, lamivudine and efavirenz) as a strong risk factor and predictor for hyperlactatemia compared to regime 1B (stavudine, lamivudine and nevirapine). Advancing age is associated with increasing risk of hyperlactatemia but it is not a predictor.

The gender, BMI of a patient and duration of use of ART do not have a significant influence on the prevalence of hyperlactatemia in this population of patients. The study results also show that clinical symptoms of hyperlactatemia are infrequent and when they occur they are usually mild and bear no relation to levels of venous lactate.

Basing on these study results, the following recommendations can be made in relation to clinical practice of HIV medicine; the potential for first line drugs to induce mitochondrial toxicity is significant and for this reason all patients on regime 1A and 1B must undergo routine clinical examinations with view to identifying clinical disorders related to mitochondrial toxicity e.g. lipoatrophy, lipodystrophy, neuropathy, pancreatitis and lactic acidosis. While performance of routine venous lactate assays in all patients is not recommended, the

results of this study suggest that older patients on regime 1A should be regarded as special risk category and lower thresholds of clinical suspicion must apply.

The study brings into perspective the magnitude of one of the important adverse effects of ART use i.e. mitochondrial toxicity. It is a clinical challenge that poses a potential frustration to the efforts of expand access to ART in the South African public health sector. The current practice of HIV medicine in the public health sector may have to adopt stricter measures to detect and monitor ARV drug toxicities for the purpose of generating valuable data to assist with strategic alterations to ART first line regimes in future and consideration of pharmacoepidemiological screening.

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APPENDIX A

PATIENT INFORMATION

Dear patient,

My name is Ralph Nhiwatiwa. I am a medical doctor currently studying at the University of the Witwatersrand. I would like to invite you to take part in a study on the medications you are using at present.

HIV medicines can bring about side effects one of which is called hyperlactatemia. Hyperlactatemia stands for the condition in which there is an accumulation of excess lactate in the body. Lactate is a by-product of energy-producing processes in the body. The process of accumulation is slow and if allowed to proceed unabated it can lead to acid building up in the blood. This is a serious condition called lactic acidosis. We try to prevent lactic acidosis by picking it up at the early stage of hyperlactatemia where treatment may be changed as a corrective measure. The purpose of this study is to see how common the condition of hyperlactatemia is you the patients who have taken ART for a long time. Further, the study may help to show whether certain drugs are particularly responsible and conditions such as obesity (too much body weight) may be playing a role in causing hyperlactatemia.

Do not hesitate to contact me should you need more information.

Thank you,

Ralph Nhiwatiwa tel.057-3571271, 082808598

APPENDIX B

CONSENT FORM

Dear patient,

You are kindly invited to participate in this study, which is explained in the information you have received. The study will be conducted by myself and is supervised by the University of the Witwatersrand Faculty of Health Sciences. It is sponsored by the conditional grant for HIV care for the Free State Department of Health.

You will be requested to sit and rest on the waiting room seats while you answer some questions on the questionnaire. Following that you will have your weight and height measured. Blood will be collected from a vein on your arm at the same time as your routine clinic tests. You may experience minor pain during needling. 4 milliliters of blood will be collected. There is no risk to your life.

Your names and personal information will not be attached to the tests and results. If you agree to participate you will retain your right to withdraw any time without change or compromise to the care you receive in the clinic.

I agree:

Signature of patient……Signature of researcher……

For further information contact R Nhiwatiwa on 0828085998

APPENDIX C

DATA ENTRY FORM

Number	
Gender M/F	
Age (Years)	
Regime 1A/1B	
Duration (Months)	
Weight (Kg)	
Height (M)	
BMI	
Symptom Score	
Lactate level	

APPENDIX D :QUESTIONNAIRE

NO.	ENGLISH QUESTIONS		
1	Have you experienced nausea for which you required medical attention or medications since your last visit?	Y	N
2	Have you experienced any vomiting which lasted more than 3 days since your last visit?	Y	N
3	Have you experienced any abdominal pains requiring you to seek medical attention or to take medications since your last visit?	Y	N
4	Have you noticed weight loss over the last month?	Y	N
5	Have you suffered from diarrhoea requiring you to seek medical attention or to take medications since your last visit?	Y	N
6	Do you ever feel so tired that you stay in bed much of the time during daytime?	Y	N
7	Have experienced any feeling of shortness of breath or breathing difficulties that worried you since your last visit?	Y	N

XHOSA QUESTIONNAIRE

No.	XHOSA IMIBUZO		
1	Oko u suke apha u ke wa khonyuluka ukuthu u sele amayeza okanye u bone u gqira?	Y	N
2	Uke wa hlanza (gaba) u kwe dhlula iintsuku ezintathu?	Y	N
3	Uke waba namahlaba e suswini ukuthi u sele amayeza okanye u ye kwa gqira	Y	N
4	U ke wa qaphela u kwehla kwe mzimba ku le nyanga i dlulileyo?	Y	N
5	U ke wa ba ne sisu e si hambisayo ukuthi u sele amayeza okanye u ye kwa gqira	Y	N
6	U no ku ziva u khathele ngamanye amaxhesha ukuthi u fune u ku lala nje?	Y	N
7	U ba ne phika okanye u be nobunzima e ku phefumleni u phelele ngu moya ngamane ama xhesha?	Y	N

AFRIKAANS QUESTIONNAIRE

1	Het u enige naarheid ondervind waarvoor u enige behandeling of medikasie ontvang het, sedert u laaste besoek?	Y	N
2	Het u enige braking ondevind wat vir langer as 3 dae aangehou het, sedert u laaste besoek?	Y	N
3	Het u enige abdominale pyn ondervind waarvoor u mediese behandeling of medikasie ontvang het, sedert u laaste besoek?	Y	N
4	Het u enige gewigs verlies waargeneem oor die laaste maand?	Y	N
5	Het u gesukkel met diaree waarvoor u mediese versorging noding gehad het of medikasie daarvoor geneem, sedert u laaste besoeke?	Y	N
6	Voel u ooit so moeg dat u die meeste tyd deur die dag in die bed bly?	Y	N
7	Ondervind u einige kort- asemheid of benoudheid wat u bekommer, sedert die laaste besoeke?	Y	N

No.	SOTHO DIPOTSO		
1	O kile a ikutlwa o nyekelwa haholo hao o bileng wa hloka pheko ya meriana kappa ya bongaka?	Y	N
2	Haesale o qetela ho tla kwano phekolong, o kile wa ba le lehlatso le ka etsang matsatsi a mararo kappa hofeta?	Y	N
3	Haesale o qetela ho tla kwano phekolong, o kile wa longwa ke mala hao bileng wa hloka ho nwa meriana kappa ho bona ngaka?	Y	N
4	Na o hlokometse hore ka mohlomong oi ntse o fokola mmeleng kgweding ena?	Y	N
5	Na o kile wa ba le letshollo moo o kile wa nwa meriana teng?	Y	N
6	Na o ba le ho tepella hoo o sa batleng le ho tsoha hara motsheare?	Y	N
7	Na o nale ho fellwa ke moya ha o phefumoloha?	Y	N

APPENDIX E

Equation 1

Body mass index = body weight in kg ÷ (height in meters)^2

APPENDIX F

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARENCE CERTIFICATE PROTOCOL NUMBER M080622

PROJECT: Frequency of hyperlactatemia in adult patients on antiretroviral therapy programme in a public sector clinic in Free State.

INVESTIGATOR: Dr R. Nhiwatiwa

DEPARTMENT: Pharmacy and pharmacology

DATE CONSIDERED: 27th June 2008

DECISION OF COMMITTEE: Approved unconditionally

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

DATE …… **CHAIRPERSON** ……

DECLARATION OF INVESTIGATORS(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 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.**