AN INVESTIGATION OF METABOLIC SIDE EFFECTS OF ANTIRETROVIRAL THERAPY USING LABORATORY BIOMARKERS IN HUMAN IMMUNODEFICIENCY VIRUS (HIV) INFECTED INDIVIDUALS

BY

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BY

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AUTHOR'S DECLARATION

This study represents the author's original work. It has not been submitted previously for any qualification to this or any other tertiary institution. Where use of work of others was made, such help has been duly acknowledged in the text.

The research described in this dissertation was carried out in the department of Biomedical and Clinical Technology, Faculty of Health Sciences, Durban University of Technology, Durban, South Africa; at uMlazi Medical Centre, V Section, Durban, South Africa and Global Clinical and Viral Laboratory, Amanzimtoti, Durban, South Africa.

It was supervised by Dr S Madurai (Global Clinical and Viral Laboratory) and Mrs P Pillay (Department of Biomedical and Clinical Technology, Durban).

SIGNED_____

Thandie Sylph Ndlovu

I, the undersigned approve the submission of this dissertation

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Dr S Madurai, PhD

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Mrs P Pillay, MPH

ABSTRACT

Introduction

Antiretroviral therapy (ART) was introduced because it has shown to reverse the Acquired Immunodeficiency syndrome (AIDS), by reducing the HIV replication, allowing the regeneration of the patient's immune system. ART is given to patients for the rest of their lives as part of HIV clinical care, but the use of ART has shown evidence of metabolic side effects which range from manageable to life threatening complications.

Aims and objectives of the study

The aim of the study was to investigate whether patients on ART developed metabolic side effects such as pancreatitis, dyslipidaemia and hepatotoxicity. These metabolic side effects were determined by laboratory testing of blood levels of specific biomarkers at stipulated intervals. Any significant change in the blood levels of these specific biomarkers was identified.

Methodology

The study included 92 patients who were already selected for the ART programme which is in accordance to the South African National Antiretroviral Therapy Guidelines of 2003 Laboratory blood analysis was conducted. The repeated measures analysis of variance (ANOVA) was used to compare changes in biomarkers over time. The severity of each side effect was assessed by grading each biomarker laboratory result through the use of an established toxicity grading table.

Results

It was found that the biomarker blood levels were not significantly altered within 12 months of ART, however, there was a gradual increase of most biomarker values, indicating that abnormalities may be detected after a longer period of treatment.

Conclusion

Within 12 months of treatment, life-threatening toxicities were not detected. It may be speculated that if ART is monitored correctly, life-threatening toxicities may be avoided in many patients.

DEDICATION

This thesis is dedicated to:

My late mother, Ntombili Elizabeth Ndlovu (MaHadebe). If it were not for your teachings, guidance, beliefs and unconditional love, I wouldn't be where I am today.

My sister, Teressa Ntokozo Hadebe, for your love and support.

My son, Sithabiso Mthokozisi Ndlovu and daughter, Yandisa Nelisa Ndlovu for your unconditional love and giving me the reason to persevere.

My Lord, Almighty God, who has carried me through trying and difficult times. I am humbled by Your mercy and love. Without You by my side, I would have given up hope and not completed this journey. All that I have accomplished, I owe it to You.

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RESEARCH DEFINITIONS

	DEFINITION
biomarker	laboratory measurement that reflects the activity of a disease process or damage to the body
side effects	reactions that happen when the drugs affect the body in ways other than those intended
Prevalence	the number of cases of a specific disease present in a given population at a certain time
Incidence	the rate at which the number of new cases of a specific disease occurduring a certain period
Pandemic	An outbreak of a disease that has spread through human populations worldwide
Epidemic	The occurrence of more cases of a disease than would be expected in a community or region during a given time period, affecting a large proportion of people

LIST OF ABBREVIATIONS

ABBREVIATION	DESCRPTION
AE	Adverse event / effect
AIDS	Acquired immunodeficiency syndrome
ALT	alanine aminotransferase
ANOVA	analysis of variance
ART	Antiretroviral therapy
ARVs	Antiretroviral drugs
AST	aspartate aminotransferase
CASCADE	Concerted Action on Seroconversion to AIDS and Death in Europe
CCR5	C-C chemokine receptor type 5
cDNA	copy DNA
CRFs	circulating recombinant forms
DAIDS	Division of AIDS
DHHS	Department of Health and Human services
DNA	deoxyribonucleic acid
FDA	United States Food and Drug Administration
GDP	gross domestic product
GRID	gay related immune deficiency

ABBREVIATION	DESCRPTION
HAART	Highly Active Antiretroviral Therapy
HIV	Human immunodeficiency virus
HIV/AIDS	Human immunodeficiency virus and Acquired immunodeficiency syndrome
HIV-1	Human immunodeficiency virus type 1
HIV-2	Human immunodeficiency virus type 2
HDL	high density lipoprotein
KZN	KwaZulu-Natal
MACS	Multicenter AIDS Cohort Study
MDR-TB	multi drug resistant tuberculosis
MRC	Medical Research Council
NNRTI	non-nucleoside- analogue
NRTI	nucleoside- analogue
PEPFAR	President's Emergency Plan for AIDS Relief
PHAC	Public Health Agency of Canada
PI	Protease inhibitor
PMTCT	prevention of mother-to-child transmission
RNA	ribonucleic acid
RTI	Reverse transcriptase inhibitor

ABBREVIATION	DESCRPTION
TB	Tuberculosis
ULN	Upper limit of normal
UNAIDS	Joint United Nations Programme on HIV/AIDS
WHO	World Health Organisation
XDR-TB	extensively drug resistant tuberculosis
<	less than
>	greater than

APPENDICES

THE PREFACE

The following chapters are presented in this research report:

Chapter 1: Introduction

This chapter provides the history of HIV and antiretroviral therapy. It also gives a brief overview of the challenges of antiretroviral therapy and side effects. The aims, objectives and potential benefits of the study are also explained.

Chapter 2: Review of Literature

This chapter includes current literature on ARVs. It covers the magnitude and epidemiology of HIV, and then focuses on ARVs, and the surrounding debates. Examples of ARV regimens in other developing countries are presented as well as the ART programme in South Africa. The issues surrounding the challenges with the ARV rollout are also discussed. Antiretroviral therapy side effects and challenges are also discussed.

Chapter 3: Methodology

This chapter focuses on the execution of the study and includes the protocol that was followed in order for the study to be conducted.

Chapter 4: Presentation of Data

The data collected in this study was analysed both quantitatively and qualitatively. Data is presented mainly in the form of frequency distribution tables and graphs.

Chapter 5: Discussion of Data

Key findings are discussed in this chapter. The main purpose of this chapter is to describe the effects of the ARVs as evaluated in terms of the objectives of this study. The findings are critiqued in light of similar studies and the strengths and weaknesses of the data are presented.

Chapter 6: Conclusions and Recommendations

This chapter draws conclusions from chapter 5 and recommendations are suggested regarding the way forward to improve the current position. Positive aspects of the study are reinforced and areas for improvement are noted.

CHAPTER ONE INTRODUCTION

1.1 STUDY BACKGOUND

It was estimated that there were between 74,000 and 120,000 people in South Africa living with Human immunodeficiency virus (HIV) (Pembrey, 2009). In 1999, the HIV prevalence among pregnant women was 22.4% (Pembrey, 2009). In 2005, the HIV prevalence among pregnant women was 30.2% (Department of Health, South Africa (DoH SA), 2008). According to the UNAIDS report on the global acquired immunodeficiency syndrome (AIDS) epidemic (2010), by the end of 2009 there were 22.5 million people living with HIV in sub-Saharan Africa and this is 68% of the global total as indicated in Figure 1.1. In 2009, South Africa was ranked the fourth highest country in sub-Saharan Africa with people living with HIV (UNAIDS, 2010).

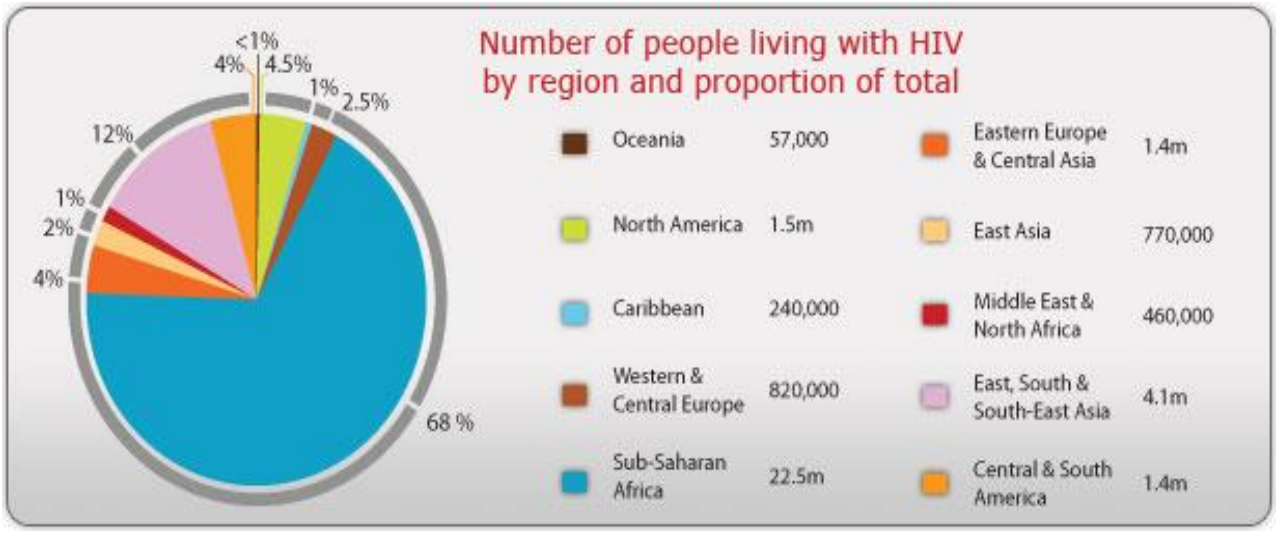


Figure 1.1 Number of people living with HIV worldwide in 2009 (UNAIDS Global Report 2010).

Antiretroviral therapy (ART) was introduced because it has shown to be able to reverse Acquired Immunodeficiency syndrome (AIDS) (Bangsberg *et al.*, 2001). ART reduces the HIV

replication, allowing the regeneration of the patient's immune system (KwaZulu-Natal Department of Health, 2005). ART is given to patients for the rest of their lives as part of HIV clinical care; hence making AIDS a manageable chronic illness (Bangsberg *et al.*, 2000).

However, the widespread use of effective ART has resulted in metabolic abnormalities and complications which range from manageable to life-threatening complications (Chow *et al*., 2003**).** This has had an impact on patient's non-compliance and mortality (Piliero, 2003)**.** The prevalence of metabolic complications in HIV-infected individuals is high (Chow *et al.,* 2003) but sometimes they are not assessed or monitored by the clinicians since they are not included in the National Antiretroviral Treatment Guidelines of South Africa (DoH SA, 2004). There are a limited number of tests that are performed as part of monitoring of patients who receive ART based on the National Antiretroviral Treatment Guidelines of South Africa (DoH SA, 2004). Life-threatening metabolic complications such as pancreatitis, dyslipidaemia, hepatotoxicity that could lead to death of many patients may be missed with these monitoring guidelines (David, 1999). It is believed that these complications, once identified, can be managed and this could prevent patients from non-compliance and subsequent death (Bangsberg *et al.,* 2001).

The aim of the study was to investigate whether patients on antiretroviral therapy (ART) in Durban KwaZulu-Natal, developed the common metabolic side effects such as pancreatitis, dyslipidaemia and hepatotoxicity that has been described in the literature, which were not routinely monitored for, in the National Antiretroviral Treatment Guidelines of South Africa (DoH SA, 2004). These metabolic side effects were determined by laboratory testing of blood levels of specific biomarkers; amylase, lipase, cholesterol, triglycerides, alanine aminotransferase (ALT) and aspartate aminotransferase (AST). These biomarkers were tested at stipulated intervals. Any significant change in the blood levels of these specific biomarkers was identified. The objectives of the study were to investigate the time of onset of these side effects in relation to the specific antiretroviral drugs taken, and also determine the incidence of the side effects in the study population

1.2 HISTORY OF HIV AND AIDS

The Acquired Immunodeficiency syndrome (AIDS) was first recognised in 1981 in the United States (Centre for Disease Control, 2001) in the gay community. At that time it was called gay related immune deficiency (GRID), since it was believed to be sexually transmitted between males (Cichocki, 2007). The human immunodeficiency virus (HIV) was later isolated by tissue culture in 1983 and it was believed to be related to the outbreak of AIDS (Cichocki, 2007).

In South Africa, the first recorded case of AIDS was diagnosed in 1982 (Pembrey, 2009) in a gay man. By 1985, cases involving sexual transmission between men and women were also identified (Pembrey, 2009). In 1990 the first national antenatal HIV survey in South Africa found that 0.8% of pregnant women were HIV positive (Swanevelder *et al.*, 1998). Ever since this first South African survey, the number of HIV positive pregnant women has been rising, with KwaZulu-Natal having the highest prevalence (Noble, 2009).

In 2009, the HIV prevalence had risen to 29.4% amongst pregnant women and KwaZulu-Natal still had the highest prevalence (DoH SA, 2009).

1.3 HISTORY OF ANTIRETROVIRAL THERAPY

Antiretroviral therapy was first introduced in 1996 in countries like America and Canada where a combination of three or more drugs was used (Palella *et al.*, 1998). The use of three or more drugs is known as Highly Active Antiretroviral Therapy (HAART) (Avert, 2009). The choice of drug combination is depended on the following factors: anticipated viral load reduction, differential side effects, drug interaction, patient tolerance, dosing schedules and other patient factors such as gender and pre-existing medical conditions (Rachlis *et al*., 1998).

Introduction of ART has improved the life expectancy of HIV infected people dramatically in affluent countries, like America, Europe and Canada (The Antiretroviral Therapy Cohort Collaboration, 2008). A study conducted by Concerted Action on Seroconversion to AIDS and

Death in Europe (CASCADE) collaboration in 2002 suggested that there was an increase in the survival rate for people with HIV-1 since the introduction of HAART (CASCADE collaboration, 2003).

1.4 SOUTH AFRICAN NATIONAL ANTIRETROVIRAL THERAPY PROGRAMME

In South Africa, the government was initially hesitant to provide ARV therapy to HIV infected people. In 2001, the government was supporting the views of the AIDS denialist community who propagated that HIV does not cause AIDS (Duesberg, 1988, Gellman, 2000), and was investigating alternate treatment for AIDS. The government made a decision to supply the ARV drugs in March 2004 to patients in Gauteng (Pembrey, 2009). In essence, this was eight years later than other countries, where ARVs were made available in 1996. In KwaZulu-Natal the ART programme was implemented in March 2004 (KwaZulu Natal Epidemiology Bulletin, 2005).

In 2004, the South African government estimated that there were already between 5.7 and 6.2 million people were living with HIV/AIDS. According to Pembrey (2009), the distribution of ARVs has been slow with only about 28% of HIV affected people, by the end of 2007, receiving ARV treatment (Pembrey, 2009).

AIDS deaths are common throughout the country and according to the Actuarial Research Centre and South African Medical Research Council (MRC) report on the Impact of HIV/AIDS in South Africa (2006), 71% of deaths among those aged between 15 and 49 years in South Africa are caused by AIDS. The health care inequity and infrastructural deficits within the public sector were major obstacles in the ART rollout task (Ojikutu *et al.,* 2007). The other challenge noted was that poorer populations who live in rural areas where ART rollout sites (district hospitals) are far from their homes, could not access treatment even though it is free (Ojikutu *et al.*, 2007).

1.5 ANTIRETROVIRAL THERAPY SIDE EFFECTS AND CHALLENGES

After a decade or more since the inception of ART, there has been evidence and reports of drug induced side effects (Chow *et al.*, 2003, George, 2006, Forna *et al.*, 2007). The side effects range from mild and manageable to severe and life-threatening. These side effects are sometimes unpleasant for patients which leads to patient's non-adherence to therapy (Piliero, 2003). Since ART is life-long, non-adherence has a negative effect on HIV control as the person may develop full-blown AIDS, which may eventually lead to their death and may lead to the development of resistancy against therapy (Bangsberg *et al*., 2007).

1.6 POTENTIAL BENEFITS OF THE STUDY

This study would assist in identifying whether life-threatening metabolic disorders occur in patients on ART. The detection of the development of these disorders would improve monitoring of patients on ART and hence enhance life expectancy of HIV infected patients. The outcomes may make a contribution in developing further patient management policies; this could play a role in ensuring the reduction of patient non-compliance and mortality due to ART. Further to this, vigilance amongst clinicians who are uninformed as to the ART side effects management may be improved.

This study will investigate the incidence of metabolic side effects, thus highlighting the importance of including these laboratory biomarkers in the National Antiretroviral Treatment Guidelines of South Africa and to be able to readdress the South African antiretroviral treatment plan.

This study will support, by evidence, the side effects of ART described in the literature.

1.7 SUMMARY

Some of the critical issues surrounding HIV and ARVs were highlighted and the aims and objectives of the study listed. The next chapter concentrates on the current issues surrounding HIV and ARVs nationally and internationally.

1.8 AIMS AND OBJECTIVES OF THE STUDY

Hypothesis statement

The antiretroviral therapy causes the development of metabolic side effects.

Aim of the study

It was to investigate whether patients on antiretroviral therapy (ART) in Durban KwaZulu Natal, developed metabolic side effects such as pancreatitis, dyslipidaemia and hepatotoxicity which were not routinely monitored for, in the National Antiretroviral Treatment Guidelines of South Africa (DoH SA, 2004).

Objectives of the study

1. To evaluate change in the laboratory values of the specified biomarkers from the blood specimens of the participants over time.

2. To determine whether patients on ARV in Durban KwaZulu Natal develop metabolic side effects when on the prescribed regimen as per published data.

3. To determine the extent and period of onset of these side effects in this study population.

4. To determine the prevalence of the side effects in the Durban KwaZulu Natal population.

CHAPTER TWO LITERATURE REVIEW

2.1 STUDY BACKGROUND

2.1.1 Introduction

Human immunodeficiency virus (HIV) is a virus that infects the immune system of humans. It is transmitted by transfer of body fluids from an infected person to an uninfected person. It attacks and damages the immune system, specifically the T helper lymphocytes, which are important in protecting the person by fighting infections. After progressive destruction of these cells, the person is unable to fight off any other diseases and may die from these diseases. There is no cure for viral infections, including HIV infection. HIV disease is a lifelong disease. HIV infected individuals become severely immunosuppressed and develop acquired immune deficiency syndrome (AIDS).

AIDS develops when the immune system of the HIV infected person is damaged. The symptoms of AIDS are primarily the result of conditions that do not normally develop in individuals with healthy immune systems. Most of these conditions are infections caused by bacteria, viruses, fungi and parasites that are normally destroyed or controlled by a functional immune system, which in this case, has been damaged by HIV. These infections affect almost every system of the body and are commonly called opportunistic infections.

Before the introduction of antiretroviral drugs (ARVs), progression to AIDS and death was known to be rapid. A study by Morgan *et al.* (2002) on HIV progression in rural areas of Africa found that the median time from seroconversion to death was 9.8 years. This finding was similar to industrialised countries, like Zaire, indicating that socio-economic status did not delay the progression of HIV to death. The AIDS epidemic has an impact in causing profound social and economic problems due to the stigma associated with the disease, and due to absenteeism from work, cost of medical care and reduced productivity because the individual cannot function. This has placed a heavy toll on existing health care systems, especially in Southern Africa where

health care resources are limited. HIV and AIDS (HIV/AIDS) have increased the demand for and cost of public health care services (Stewart *et al.*, 2004).

In 1996, the highly active antiretroviral therapy (HAART) was developed and made available in high-income countries such as America and Canada. Literature reports that AIDS related mortality declined markedly in the following two to three years but has since plateaued (Grinspoon *et al.*, 2008). According to the Brinkhof *et al.* (2009), the mortality rate among patients with HIV/AIDS is comparable to that among patients with diabetes and other chronic conditions, provided ART are initiated timeously. Antiretroviral therapy has changed HIV infection from a fatal disease to a chronic, manageable disease (Bangsberg *et al.*, 2000, Marins *et al*., 2003). Since the antiretroviral treatments are unable to eradicate HIV from infected individuals, therapy must be life-long and this exposes the individual to short- and long-term side effects (Palmisano *et al.*, 2011). Whilst the benefits of ART are evident in the literature, the metabolic side effects may be unpleasant and even life-threatening, thus making patients discontinue treatment. Hence, it is important to monitor and evaluate patients so that treatment regimens can be altered or adjusted timeously and clinicians are able to make well planned decisions on the correct regimes to be implemented.

Successful treatment and management of HIV rely on patient's adherence to treatment, and since patient's non-adherence or discontinuation of treatment has been linked to the development of serious side effects and drug resistance; monitoring, early identification and management of side effects is important. According to the First National Antiretroviral Treatment Guidelines of South Africa (DoH SA, 2004), the tests that were performed as part of the management of ART were very limited. The guidelines did not cater for life-threatening metabolic side effects which, if identified early, can be managed; thus preventing the pattern of non-compliance and subsequent death of patients.

2.1.2 HIV structure and subtypes

HIV is a Lentivirus, which means 'slow virus' because it takes a long time to produce any adverse effects in the body (Kanabus and Allen, 2009). Lentiviruses are part of the retrovirus family (Kanabus and Allen, 2009). Retroviruses are viruses that have genes composed of ribonucleic acid (RNA) and have a reverse transcriptase enzyme which converts RNA to deoxyribonucleic acid (DNA) (Coffin *et al.*, 1997)**.** This is a unique characteristic found in this family of viruses. The virus has nine genes which are important in its replication. Figure 2.1 below shows that the HIV has two identical strands of RNA, each containing a copy of the virus's nine genes (Hope, 2000). Three of the genes namely: 'gag', 'pol' and 'env' contain information required to construct structural proteins for new virus particles. The other six: namely 'tat', 'rev', 'nef', 'vif', ' vpr' and 'vpu' are required for the synthesis of proteins that control the ability of HIV to infect a cell, replicate or cause disease (Noble, 2009).

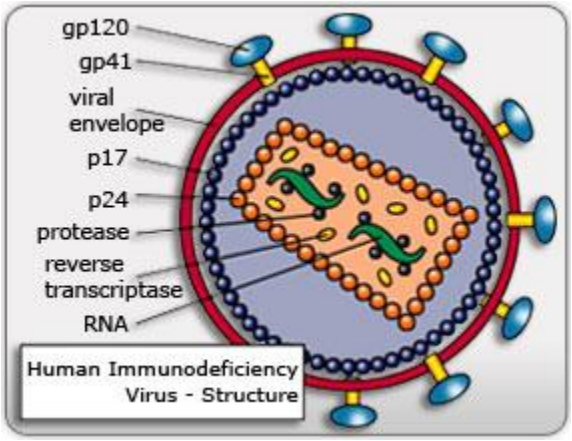


Figure 2.1 HIV structure from avert.org/virus.htm (2009), describing the various genes and proteins of the virus, also showing the reverse transcriptase enzyme and the two single stranded RNA.

There are two types of HIV: HIV-1 and HIV-2. The predominant type worldwide is HIV-1. It is the most pathogenic strain of the virus (Hunt, 2009) and is responsible for the AIDS pandemic (Centre for Communicable Diseases and Infection Control (CCDIC), 2010). HIV-2 is less common and found mainly in West Africa (CCDIC, 2010). HIV-1 is divided into groups: Group

M (major or main), Group N (non-M, non-O), Group O (outlier), based on genetic variability. More than 90% of HIV-1 infections belong to group M (Noble, 2009). According to Public Health Agency of Canada (PHAC), HIV Update Document (2010), HIV-1 infections are almost exclusively caused by Group M viruses. Group M is further classified into nine subtypes or clades. These are subtypes A, B, C, D, F, G, H, J and K. There are also over 40 circulating recombinant forms (CRFs), which are the combinations of these subtypes. The HIV-1 subtypes and CRFs are typically associated with certain geographical regions, with the most prevalent globally being subtypes A and C as indicated in Table 2.1 below.

Table 2.1: Worldwide Prevalence of the Most Represented HIV-1 Subtypes and Circulating Recombinant Forms (adapted from AIDS, 2004).

HIV-1 subtype / CRFs	**Global prevalence (%)**
C	50
A	12
B	10
F, G, H, J and K	7
CRF01_AE	5
CRF02_AG	5
D	3
Others	8

CRFs=Circulating recombinant forms.

Figure 2.2 illustrates the worldwide distribution of HIV-1 subtypes. In South Africa, the predominant subtype is C.

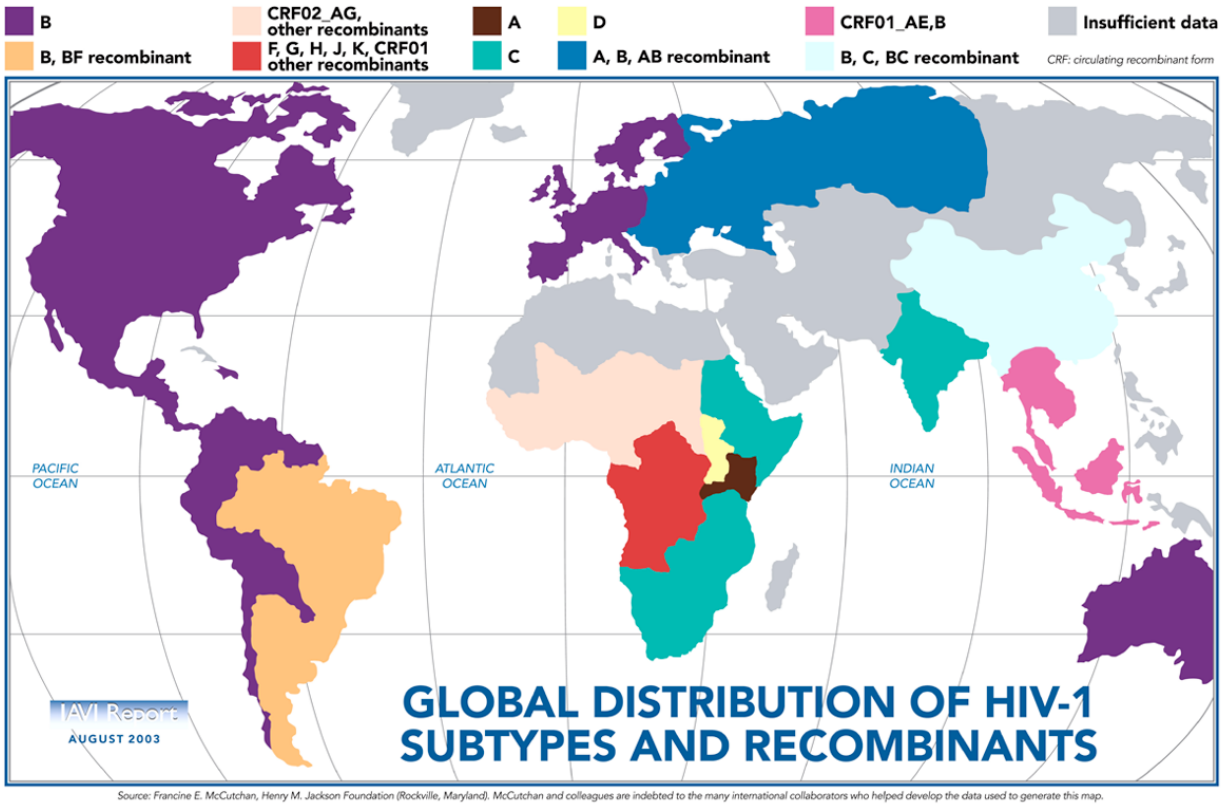


Figure 2.2 Global distribution of HIV-1 subtypes from International AIDS vaccine Initiative (IAVI report.org 2009), showing the distribution of subtypes throughout the world. In Central Africa, a large number of recombinants denoted (red and pink) and in South Africa Subtype C predominates (green).

In a study by John Hopkins University, it was suggested that HIV subtype predicts the likelihood of rapid death from AIDS (Kiwanuka *et al.*, 2008). It was found that HIV subtype D progresses more rapidly to AIDS than HIV subtype A. This study was conducted in Uganda and it was found that Ugandans infected with subtype D or recombinant strains incorporating subtype D developed AIDS sooner than those infected with subtype A, and also died sooner, if they did not receive ARVs timeously (Kiwanuka *et al.*, 2008). Another study by Baeten *et al.* (2007) found that Kenyan women infected with subtype D had more than twice the risk of death compared with those infected with subtype A. The study by Kiwanuka *et al.* (2008) had similar findings as the study by Baeten *et al.* (2007).

Two studies in Thailand showed that HIV subtype E was the most virulent than other subtypes of the virus (AIDS, 2007). An earlier study by Galai *et al. (*1997) showed that the progression rate of HIV subtype C and subtype B were similar. Kanki *et al.* (1999) also showed that individuals infected with subtypes C, D and G were eight times more likely to develop AIDS than individuals infected with subtype A.

From the literature reviewed, it can be concluded that HIV subtype C has a similar progression rate to AIDS, as with subtype B, if the patients do not receive ART timeously. Subtype A is the slowest progressor and subtype D is the fastest progressor (Kanki *et al.*, 1999, Kiwanuka *et al.*, 2008).

Before ART was initiated, progression to AIDS and death was very rapid. The patient's cluster of differentiation (CD4+) cell count and HIV RNA levels in the blood were used as indicators for the progression of HIV to AIDS. Table 2.2 is adapted from a Multicenter AIDS Cohort Study (MACS) (Mellors *et al.*, 1997) that was conducted by four American Universities and was used to predict HIV progression to AIDS and death within a 10 year period.

Table 2.2: Correlation of HIV – 1 RNA, CD4+ cell count, progression to AIDS and Death (adapted from Mellors *et al.*, 1997) showing that the higher the HIV-1 RNA copies the quicker the disease progression and death.

HIV-1 RNA level (copies / ml)	**Progression to AIDS within 6 years**	**Death from AIDS within 6 years**	**Change in CD4+ count per year (cells/mm^3)**
< 500	5.4%	0.9%	-36.3
501 – 3,000	16.6%	6.3%	-44.8
3,001 – 10,000	31.7%	18.1%	-55.2
10,001 – 30,000	55.2%	34.9%	-64.8
> 30,000	80.0%	69.5%	-76.5

Another collaborative study between 1997 – 2001 (CASCADE collaboration, 2003), found that, before the introduction of HAART, the median survival after HIV-1 seroconversion was estimated to be 12.5 years for people aged 15 – 24 years and 7.9 years for those aged 45 – 54 years; 99.7% of people aged 25 – 29 years were expected to survive 1 year after seroconversion, falling to 59.5% at 10 years.

2.1.2 HIV entry to the body

To understand how the ARVs function or the mode of action, it is important to first understand how HIV causes infection.

HIV cannot grow on artificial culture media, like bacteria or reproduce on its own, like parasites (Noble, 2009). It requires cells of living organisms in order to make new viral copies (Noble, 2009). HIV requires human cells with CD4+ receptors, like white blood cells, which act as the host.

Once HIV gets into the human body it gets attracted to CD4+ receptor cells and attaches. Its goal is to penetrate the cell, take control and make copies of itself, releasing copy after copy of HIV. The CD4+ receptor cell that has been invaded by HIV dies and the new copies of HIV will infect other CD4+ receptor cells.

Understanding how HIV replicates allows for the development of ways to block the process and in turn suppress HIV's attack on the immune system (Cichocki, 2007). This information also helps in the development of antiretroviral therapy.

HIV enters the body through exposure to infected body fluids, such as blood, and semen. Once in the body, the HIV attaches firmly to the host cell, CD4+ receptor cell via complimentary proteins. Once it is attached to the cell, HIV injects its own proteins into the cytoplasm of the CD4+ receptor cell and fusion occurs. The coat surrounding the HIV RNA is dissolved, exposing the RNA. This RNA is converted to the double stranded DNA by reverse transcriptase. This is then referred to as a copy DNA (cDNA). This enzyme uses bioelements from the CD4+ receptor cell. This HIV cDNA integrates into the CD4+ receptor cell DNA (nucleus) and the process of replication is initiated, new viral RNAs are formed and are used to make viral proteins. New

viral RNA and proteins move to the cell surface to assemble into a new immature HIV. The virus matures by protease and it is released into circulation, Figure 2.4 describes the replication cycle. The ART regimen targets the different areas of the replication cycle, thus inhibiting the replication of the virus.

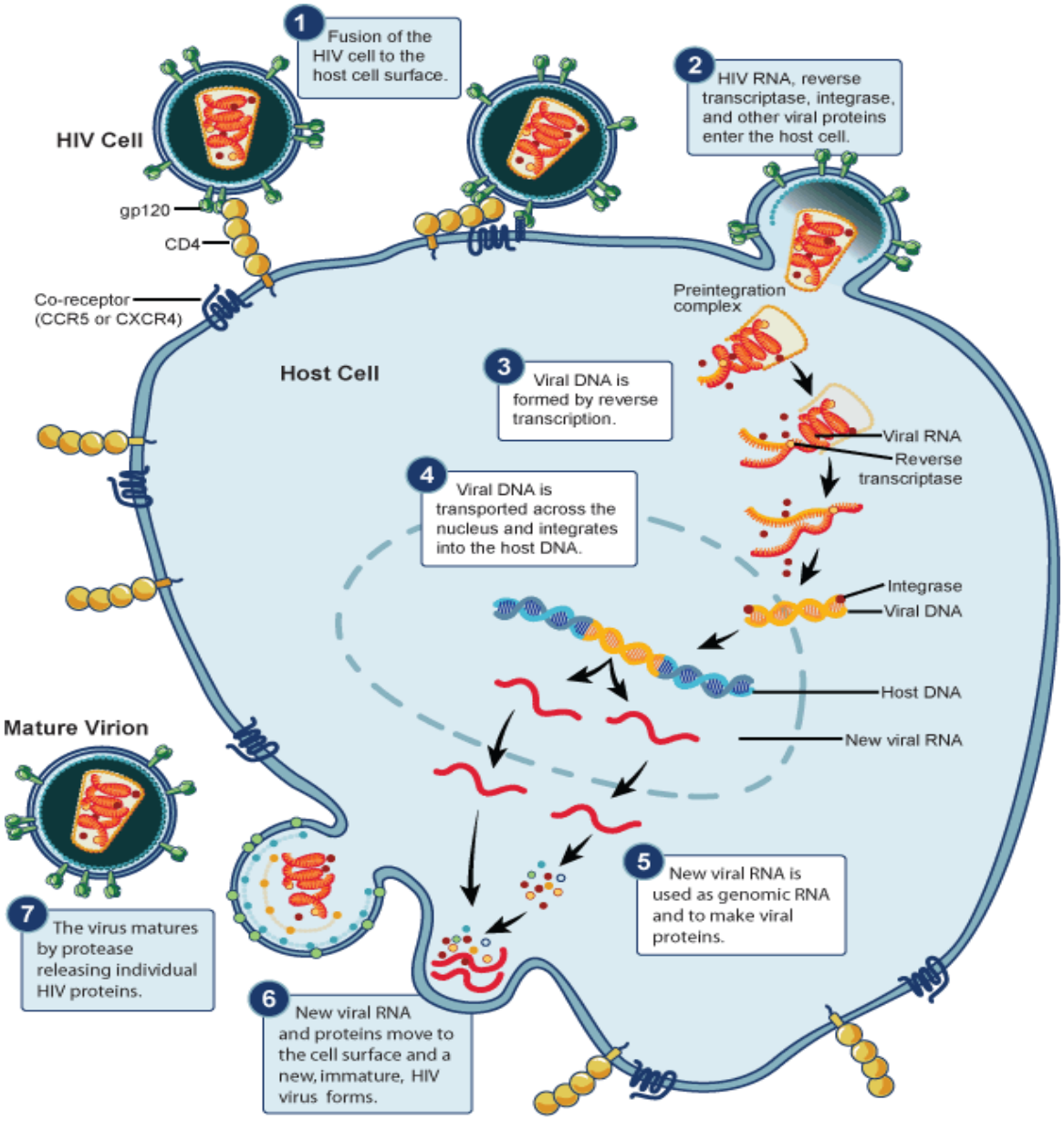


Figure 2.4 HIV replication cycle from www3.niaid.nih.gov/ (2009), describing the detailed process of HIV replication from the point of viral attachment to the CD4 receptor, integration, reverse transcription to produce cDNA, and replication to produce new viral copies. This is followed by assembly of new viral particles that are released into circulation.

HIV destroys the immune system, leaving the infected individual susceptible to other infections like tuberculosis, pneumonia and candidiasis (San Francisco AIDS Foundation, 2008). Before the introduction of ART, majority of HIV infected people died within ten years of infection (Mandell *et al.*, 2010) and they are called rapid progressors. According to the researchers from the Antiretroviral Cohort Collaboration, on average, a patient initiating an anti-HIV drug regimen, aged 35 could live to the age of 67 (The Antiretroviral Therapy Collaboration, 2008). This shows that ART has improved quality of life, increased life expectancy and hence decreased mortality. More details are described in the text at later stage.

2.1.3 CD4 receptor cells

Cluster of differentiation 4 (CD4) receptors are mainly found on T cell lymphocytes, other cells such as dendritic cells and Langerhans have also been found to have CD4 receptors (Lynch *et al.*, 2003). The T cell lymphocytes are the cells that send signals to activate the body's immune response when they detect foreign substances entering the body, like bacteria or viruses. They initiate the body's response to infections.

HIV requires cells containing DNA from a host to make more copies of itself. It attaches to CD4 receptor cells, allowing the virus to enter and infect the CD4 receptor cells, thus damaging them in the process. When a person is infected with HIV, the number of cells with CD4 receptors decrease, making the immune system weaker, therefore vulnerable to infections and illnesses.

The CD4 count test provides the information on the number of functioning CD4 receptor cells of the T cell lymphocytes in the blood and is used as an indicator of the strength or status of the immune system. CD4 counts are used together with the viral load to estimate disease progression in an infected person and according to the 2006 World Health Organisation (WHO) ART Guidelines, CD4 cell counts are also used as an indicator to initiate ART.

The CD4 count in untreated persons can be affected by the following factors; time of day, stress, infections, and vaccination.

Table 2.3: CD4 cell count and the status of the immune system, describing the immune system at the various levels of CD4 counts (adapted from AIDS Education and Training Center).

Count (cells/mm^3)	Interpretation
500 – 1200	Normal range. Healthy immune system
350 -499	The immune system is weakening
< 350	The immune system is suppressed. HIV treatment is recommended
< 200	Severe immune damage. The immune system is severely weakened and the HIV infected person is at a greater risk of opportunistic infections.

2.1.4 Antiretroviral therapy

Antiretroviral drugs (ARVs) are medications for the treatment of infection by retroviruses, primarily HIV. The main objective of HIV ARVs is to suppress viral replication and this is usually achieved by initiating combination therapy with two or more antiretroviral agents (Maenza *et al.,* 1998). Successful viral suppression is indicated by plasma viral levels that are below the lower limit of detection for routine HIV-RNA assays, usually less than 50 copies/ml. Viral suppression allows immune recovery as measured by an increase in circulating CD4 cells.

An effective combination should decrease a patient's viral load by at least 1 log (tenfold) after three to four weeks of treatment (Maenza *et al.*, 1998).

A study by Easterbrook *et al.* (2010) also compared the virological response of different HIV subtypes to ARVs. It was found that there were no significant differences between the subtypes in the time to achieve viral load suppression after initiation of ART. This also emphasizes the success of ART in suppressing HIV, irrespective of the subtype. This means that HIV subtype C,

which is the predominant strain in South Africa should respond well to ARVs even though the ARVs were mainly designed around the HIV subtype B and once the patient starts treatment, good viral load suppression will be achieved.

2.1.4.1 Classes of Antiretroviral drugs

There are five different classes of antiretroviral drugs. They are classified by the stage in the HIV life cycle that the drug inhibits (Medic8, 2009).

- **Reverse transcriptase inhibitors (RTIs)** target construction of viral DNA by inhibiting activity of reverse transcriptase. There are two subtypes; nucleoside- analogue (NRTI) and non-nucleoside- analogue (NNRTI) which distort the binding potential of the reverse transcriptase enzyme.

  NRTI blocks HIV's ability to copy a cell's DNA, which the virus needs in order to duplicate.

  NNRTI blocks the same protein as the NRTI, but are chemically different. Resistance to this class of medications develops quickly if not used in combination with an NRTI.
- **Protease inhibitors (PI)** target viral assembly by inhibiting the activity of protease, an enzyme used by HIV to cleave nascent proteins for final assembly of new virions.
  PI blocks protease, an enzyme that the HIV virus needs in order to duplicate.
- **Fusion inhibitor** blocks HIV from fusing with a cell's membrane to enter and infect it.
- **Integrase inhibitor** blocks the process of integration (when HIV's DNA is incorporated into the CD4 cell's DNA), hence blocking replication.
- **Entry inhibitor** blocks HIV-1 from the host cell by binding beta chemokine receptor Type 5 (CCR5), a molecule on the viral membrane termed a co-receptor that HIV-1 normally uses for entry into the cell. CCR5 inhibits viral infection before it enters the cell at the point of attachment.

Figure 2.5 highlights the different stages in the HIV replication cycle which each class of ARVs blocks.

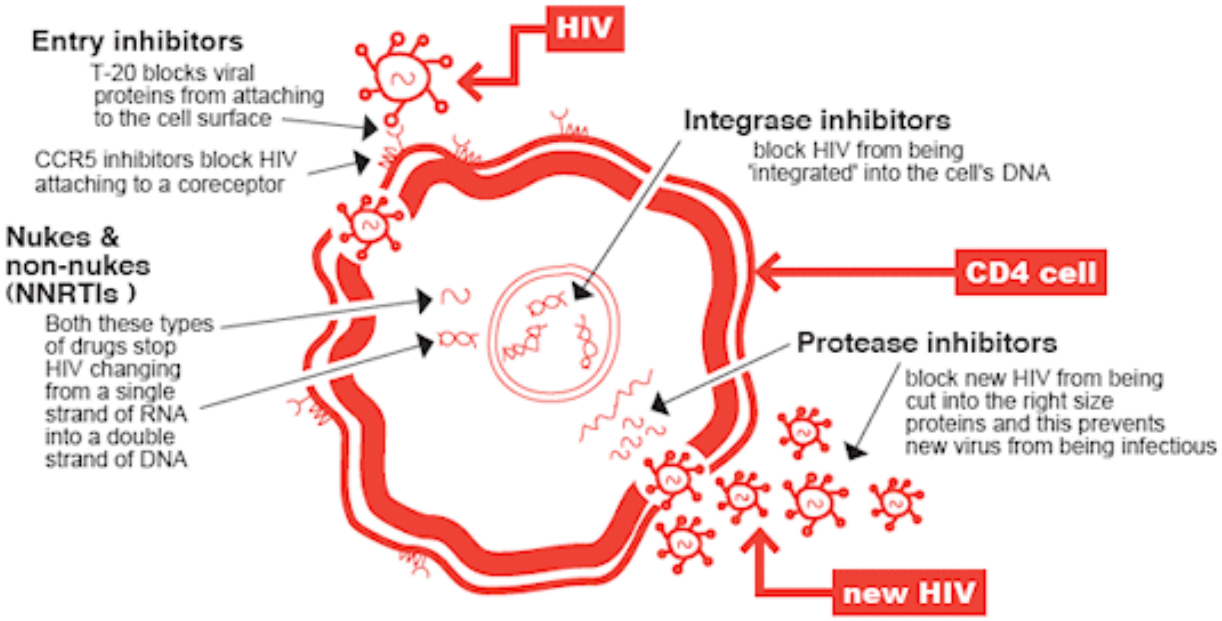


Figure 2.5 Life cycle and drug targets for HIV from http://i-base.info (July 2010), showing the different classes of ARVs and the target areas.

Table 2.4 indicates the names of ARV drugs and year in which each drug was approved by the United States Food and Drug Administration (FDA) (2011).

Table 2.4: United States Food and Drug Administration approved AIDS Drugs (United States Department of Health and Human Services, 2011).

ARV class	Drug generic name	Drug brand name	Abbreviation	Date of FDA approval
NRTIs	Lamivudine	Epivir	3TC	17 November 1995
	Abacavir	Ziagen	ABC	17December 1998
	Zidovudine	Retrovir	AZT or ZDV	19 March 1987

	Drug generic name	**Drug brand name**	**Abbreviation**	**Date of FDA approval**
NRTIs	Stavudine	Zerit	d4T	24 June 1994
	Zalcitabine	Hivid	ddC	19 June 1992
	Didanosine	Videx	ddI	09 October 1991
	emtricitabine	Emtriva	FTC	03 July 2003
	Tenofovir	Viread	TDF	26 October 2001
Combined NRTIs	Abacavir + Lamivudine	Kivexa or Epzicom	ABC + 3TC	02 August 2004
	Abacavir + Zidovudine + Lamivudine	Trizivir	ABC + AZT + 3TC	15 November 2000
	Zidovudine + Lamivudine	Combivir	AZT + 3TC	26 September 1997
	Tenofovir+ emtricitabine	Truvada	TDF + FTC	02 August 2004
NNRTIs	Delavirdine	Rescriptor	DLV	04 April 1997
	Efavirenz	Stocrin or Sustiva	EFV	17 September 1998
	Etravirine	Intelence	ETR	18 January 2008
	Nevirapine	Viramune	NVP	21 June 1996
	rilpivirine	Edurant		20 May 2011

ARV class	Drug generic name	Drug brand name	Abbreviation	Date of FDA approval
PIs	Amprenavir	Agenerase	APV	15 April 1999
	fosamprenavir	Telzir or Lexiva	FOS-APV	20 October 2003
	Atazanavir	Reyataz	ATV	20 June 2003
	Darunavir	Prezista	DRV	23 June 2006
	Indinavir	Crixivan	IDV	13 March 1996
	Lopinavir + ritonavir	Kaletra or Aluvia	LPV/RTV	15 September 2000
	Nelfinavir	Viracept	NFV	14 March 1997
	Ritonavir	Norvir	RTV	01 March 1996
	Saquinavir	Fortovase or Invirase	SQV	07 November 1997 06 December 1995
	Tipranavir	Aptivus	TPV	22 June 2005
Fusion I	Enfuvirtide	Fuzeon	T-20	13 March 2003
Entry I	Maraviroc	Celsentri or Selzentry	MVC	18 September 2007
Integrase I	Raltegravir	Isentress	RAL	12 October 2007

In the 2010 South African National Antiretroviral programme, the ARVs that are currently in use are the reverse transcriptase inhibitors and protease inhibitors. Table 2.5 provides the list of the regimen.

Table 2.5: Standardised National Antiretroviral Therapy Regimens for Adults and Adolescents from Clinical Guidelines for the management of HIV and AIDS in Adults and Adolescents, (adapted from National Department of Health, South Africa 2010).

ARV class	Drug generic name	Drug brand name	Abbreviation
NRTI	Tenofovir	Viread	TDF
	Lamivudine	Epivir	3TC
	Emtricitabine	Emtriva	FTC
	Stavudine	Zerit	d4T
	Zidovudine	Retrovir	AZT
NNRTI	Efavirenz	Stocrin or Sustiva	EFV
	Nevaripine	Viramune	NVP
PI	Lopinavir +Ritonavir	Kaletra or Aluvia	LPV/RTV

2.2 HIV EPIDEMIOLOGY AND THE ANTIRETROVIRAL THERAPY PROGRAMME

2.2.1 Impact of HIV/AIDS in sub-SAHARAN Africa

Figure 2.6 Map of sub- Saharan African countries (pbs.org 2010).

The impact of AIDS in sub-Saharan Africa (Figure 2.6) is severe with 22.5 million people living with HIV/AIDS during 2009 (HIV / AIDS statistics, 2010). An estimated 1.3 million people died from AIDS related illnesses in 2009, according to UNAIDS report on the global AIDS epidemic (UNAIDS, 2010). In 2009, South Africa had the highest number of people living with HIV and AIDS in sub-Saharan Africa, according to the Sub-Saharan HIV and AIDS statistics (UNAIDS, 2010). As indicated in Table 2.6, South Africa is faced with the largest and fastest growing HIV epidemic in sub-Saharan Africa.

Table 2.6: The Top 10 Sub-Saharan African Countries with highest number of people living with HIV and AIDS at the end of 2009 (adapted from UNAIDS Global Report 2010).

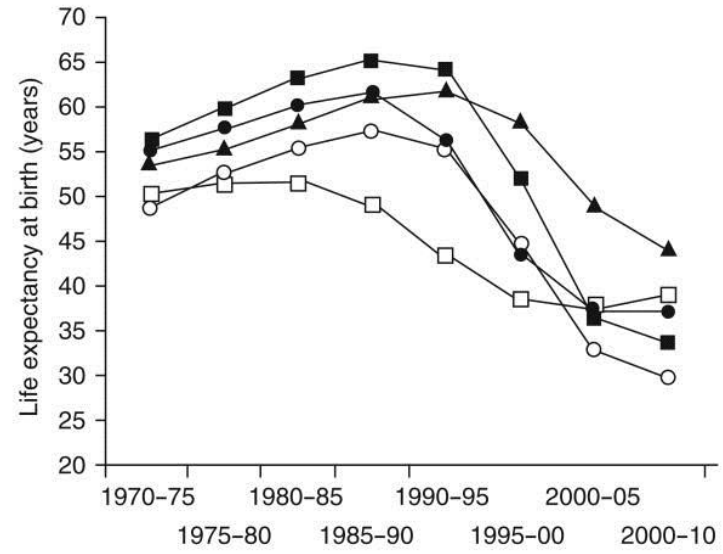
Country	People living with HIV/AIDS	Adult prevalence (%) (15 -49 years)	Women with HIV/AIDS	AIDS deaths	Orphans due to AIDS
South Africa	5,600,000	17.8	3,300,000	310,000	1,900,000
Nigeria	3,300,000	3.6	1,700,000	220,000	2,500,000
Kenya	1,500,000	6.3	760,000	80,000	1,200,000
Mozambique	1,400,000	11.5	760,000	74,000	670,000
United Republic of Tanzania	1,400,000	5.6	730,000	86,000	1,100,000
Zimbabwe	1,200,000	14.3	620,000	83,000	1,000,000
Uganda	1,200,000	6.5	610,000	64,000	1,200,000
Zambia	980,000	13.5	490,000	45,000	690,000
Malawi	920,000	11.0	470,000	51,000	650,000
Cameroon	610,000	5.3	320,000	37,000	330,000

When using the HIV adult prevalence to rank these countries, Swaziland is the highest ranking country, followed by Botswana, then Lesotho and South Africa, as indicated in Table 2.7 below.

Table 2.7: The Top 10 Sub-Saharan African Countries with highest HIV Adult prevalence at the end of 2009 (adapted from UNAIDS Global Report 2010).

Country	Adult prevalence (%) (15 -49 years)	People living with HIV/AIDS
Swaziland	25.9	180,000
Botswana	24.8	320,000
Lesotho	23.6	290,000
South Africa	17.8	5,600,000
Zimbabwe	14.3	1,200,000
Zambia	13.5	980,000
Namibia	13.1	180,000
Mozambique	11.5	1,400,000
Malawi	11.0	920,000
Uganda	6.5	1,200,000

Figure 2.7 shows the change in the life expectancy in a few Sub- Saharan countries, including the countries with the highest prevalence. It includes the pre- HIV era (before 1985) and post HIV era (after 1985). Figure 2.7 shows the change in the life expectancy in a few Sub- Saharan countries, including the ones with the highest prevalence. It includes the pre- HIV era and HIV era. The graph clearly shows the best life expectancy prior to 1985 (Pre HIV) and a marked decline in the 2000s (HIV era). It also shows the negative impact that HIV has on life expectancy in the sub-Saharan African countries, re-emphasizing the need for ART to improve life expectancy. As discussed, ARVs have improved life expectancy in all countries that have successfully initiated a treatment plan (The Antiretroviral Therapy Collaboration, 2008).



Botswana; South Africa; Swaziland; Zambia; Zimbabwe.

Figure 2.7 Impact of HIV on life expectancy in Africa (UNAIDS, 2009).

According to the South African Department of Health National Antenatal HIV and Syphilis prevalence survey conducted in 2009 (DoH SA, 2010), KwaZulu-Natal had the highest prevalence of HIV compared to other provinces in South Africa as shown in Table 2.8. Records from 2001 to 2009 show that KwaZulu-Natal has always had the highest prevalence (DoH SA, 2010). The records are based on women attending antenatal clinics across all nine provinces (DoH SA, 2010). The study findings had limitations because it did not reflect HIV infection in men and children.

Table 2.8: Estimated HIV prevalence (%) among antenatal attendees in South Africa by Province (adapted from Department of Health, South Africa 2009).

Province	2001	2004	2008	2009
KwaZulu-Natal	33.5	40.7	38.7	39.5
Mpumalanga	29.2	30.8	35.5	34.7
Free State	30.1	29.5	32.9	30.1
Gauteng	29.8	33.1	29.9	29.8
North West	25.2	26.7	31.0	30.0
Eastern Cape	21.7	28.0	27.6	28.1
Limpopo	14.5	19.3	20.7	21.4
Northern Cape	15.9	17.6	16.2	17.2
Western Cape	8.6	15.4	16.1	16.9

A National HIV survey, which is a household survey, was conducted in 2008. In this study participants had on site HIV tests performed in their homes. Based on this survey, it was estimated that 10.9% of all South Africans over 2 years old were living with HIV (Human Sciences Research Council, 2008)). Among females, HIV prevalence is highest between 25 and 29 year olds; among males, the prevalence is highest between 30 and34 year olds. This study was used to address the gaps from the antenatal survey where children and men were excluded.

2.2.1.1 AIDS orphans

In 2007 it was estimated that 1.4 million children in South Africa were orphaned as a direct consequence of HIV/AIDS (Avert, 2008). The UNAIDS (2010) report estimated that there are 1.9 million AIDS orphans (Table 2.6). This has put pressure on older relatives to become

primary carers and siblings may be forced to live apart, which may cause harm with their development (Ojikutu *et al.*, 2007).

New prevention of mother-to-child transmission (PMTCT) guidelines recommends that HIV positive pregnant women be advised to start ARV treatment when their CD4 count is below 350cells/mm^3 (DoH SA, 2010). The guidelines, shown in Table 2.9, also advise that all pregnant women who test HIV positive should begin receiving treatment at 14 weeks rather than in the last term of pregnancy.

Table 2.9: Standardised National ART and ARV regimens for women who are HIV positive and pregnant from Clinical Guidelines (Prevention of mother–to-child transmission) National Department of Health, South Africa, National AIDS Council, 2010.

Maternal regimens	
Women	**Regimen**
Eligible for lifelong ART (i.e. CD4 $\leq$ 350 or WHO clinical stage 3 or 4)	Tenofovir + Lamivudine/Emtricitabine+ Nevirapine
Currently on lifelong ART	Continue ART
Contraindication to Tenofovir (renal disease)	Zidovudine+ Lamivudine/Emtricitabine+ Nevirapine
Not eligible for ART (i.e. CD4 > 350 and WHO stage 1 or 2)	Zidovudine from 14 weeks Single dose nevirapine + zidovudine 3hourly in labour Tenofovir + emtricitabine single dose post- delivery
Unbooked and presents in labour	Single dose nevirapine + zidovudine 3hourly in labour Tenofovir + emtricitabine single dose post- delivery

The new guidelines are believed to decrease the mortality of mothers, thus decreasing the number of AIDS orphans.

2.2.1.2 Economic impact

According to a report by Professor Aardt of the Bureau of Market Research (2004) on the projected economic impact of HIV/AIDS in South Africa, the economy would be negatively affected in a number of ways: loss of personal income resulting in negative outcomes for the gross domestic product (GDP) of the country, which is defined as the total value of goods produced and services provided in a country during one year (Aardt, 2004). It was projected that by 2015, GDP decline would be between 1.3% and 6%. This supports an earlier report by Booysen *et al.* (2003) on the impact of HIV on the South African economy, that the growth in the GDP would be 0.6 % lower than if there were no HIV/AIDS. Professor Aardt (2004) further projected that by 2012 households would be spending a lot more on transport, insurance services, health services and funeral services than would have been in a non-HIV/AIDS scenario. It is important to note that in 2004, South Africa was in the early stages of ART implementation when these projections were made. Currently, there is no study published to support the 2004 projections.

According to the Avert report (2010), AIDS has affected the economy by reducing the labour supply through increased mortality and illness. It has also limited the country's ability to attract industries that depend on low-cost labour and has made investments in African businesses less desirable.

A South African company established a workplace and community HIV/AIDS project in 2001 to provide treatment services to its employees and dependants, so as to decrease absenteeism and mortality amongst HIV infected workers and dependants (World Economic Forum, 2002). It estimated that expenses related to HIV/ AIDS were equivalent to 4% of all their salaries (World Economic Forum, 2002). All these reports mentioned above were re-emphasizing the need for

antiretroviral therapy in South Africa to prevent the negative effects on the South African economy.

2.2.2 Introduction of antiretroviral therapy (worldwide)

Antiretroviral therapy was first introduced in 1996 in countries like America and Canada where a combination of three or more drugs was used (Palella *et al*., 1998). The drug combination is referred to HAART (Avert, 2009). The choice of the combination depended on some factors such as anticipated viral load reduction, differential side effects, drug interaction, patient tolerance, dosing schedules and other patient factors such as pre-existing medical conditions and is a female who is pregnant or is planning to be pregnant (Rachlis *et al.,* 1998). The treatment of choice is a combination of two NRTIs and one PI (Rachlis *et al.,* 1998).

2.2.3 Benefits of antiretroviral therapy

Introduction of ART has improved the life expectancy of HIV infected people dramatically in affluent countries, like United States of America, France and Canada (The Antiretroviral Cohort Collaboration, 2008). Life expectancy at a certain age is a demographic indicator that measures the additional years that will be lived by a person after that age. It has also decreased mortality rates (Palella and Delaney, 1998). There is a plethora of literature supporting that the use of ART has resulted in tremendous reduction in morbidity and mortality in HIV-infected individuals, thus improving life expectancy (CASCADE collaboration, 2002, Fang *et al.*, 2006, The Antiretroviral Therapy Cohort Collaboration, 2008, Mills *et al.*, 2011).

In an earlier study compiled by Palella and Delaney (1998) in eight United States cities, the mortality rate decreased from 29.4% to 8.8% in two years of the ART programme and the incidence of opportunistic infections declined from 21.9% to 3.7% in the three years the ART programme was studied.

A study conducted by CASCADE collaboration (Concerted Action on Seroconversion to AIDS and Death in Europe) in 2002, suggested that there was an increase in the survival rate for people with HIV-1 since the introduction of HAART in 1996.

A study by Fang *et al.* (2006) that was conducted in Taiwan found that there was a mean survival time of 10.6 years for patients that had developed AIDS at diagnosis and a mean survival time of 21.5 years for patients who had not developed AIDS at diagnosis, provided they are receiving ART.

Another life expectancy study, The Antiretroviral Therapy Cohort Collaboration, a multinational collaboration of HIV cohort studies in Europe and North America, found that mortality rates decreased from 16.3 deaths per 1 000 person – years in 1996 – 99 to 10.0 deaths per 1 000 person-years in 2003 -05, and life expectancy increased as indicated in Table 2.10.

Table 2.10: Life expectancy in years over time after introduction of Antiretroviral treatment (adapted from The Antiretroviral Therapy Cohort Collaboration, 2008.)

Life expectancy	**1996 – 99**	**2000 – 02**	**2003 – 05**
At exact age 20 years	36.1 years	41.2 years	49.4 years
At exact age 35 years	25.0 years	30.1 years	37.3 years
Percentage of people surviving from 20 to 44 years	75.5%	79.5%	85.7%

Table 2.10 shows that between 1996 – 99 and 2003 -05, there was a gain in life expectancy for people at age 20 years of about 13 years. Life expectancy at age 20 years increased from 36.1 years to 49.4 years. Percentage of people surviving from 20 to 44 years increased from 76% (1996) to 86% (2005). This was probably due to improved ART availability, improved patient understanding and tolerance of ART.

Another study by Mills *et al.* (2011) that was conducted in Uganda, which is classified as a low-income country, found that there was an addition of 26.7 years for a 20 year old patient receiving ART and an addition of 27.9 years for a 35 year old patient receiving ART.

In South Africa, Smart (2006) indicated that with the National rollout of ART in South Africa, a 50% -80% reduction in the incidence of Tuberculosis (TB) has been observed in ART-treated adults with HIV. According to a World Health Organisation (WHO) study done in Khayelitsha, South Africa (2003), - a study funded by a President's Emergency Plan for AIDS Relief (PEPFAR) grant - the survival rate of infected individuals after 18 months on treatment was 84%.

This stresses the importance of ART availability to individuals infected with HIV in South Africa, so that life expectancy of these individuals can be improved.

2.2.4 South African National Antiretroviral Treatment Programme

According to estimates from UNAIDS Global Report 2010, around 30.8 million adults were living with HIV at the end of 2009. HIV/AIDS in South Africa is still a prominent health concern because South Africa is believed to have more people with HIV/AIDS than any other country (Table 2.3).

It has been mentioned that the South African government was hesitant about providing antiretroviral therapy to HIV positive people, prior to 2003. In 2001 the government was openly supporting the views of AIDS denialist community that HIV does not cause AIDS (Duesberg,1988, Gellman 2000), and was investigating alternate treatment for AIDS. The government only started supplying ARVs in March 2004 in Gauteng (Pembrey, 2009) . In KwaZulu-Natal the ART programme was also implemented in March 2004 (KZN Epidemiology Bulletin, 2005).

In 2004, the South African government estimated that between 5.7 and 6.2 million people were living with HIV/AIDS.and by the end of 2007 only 28% of people in need were receiving treatment (Pembrey, 2009) resulting regularly in many people dying of AIDS. According to Actuarial Research Centre and South African Medical Research Council (MRC) report (2006),

71% of deaths among those aged between 15 and 49 are caused by AIDS. According to the statistics South Africa Report on population estimates (2010), there were 920 000 adults (15 and over) receiving ART in 2009. This report also estimated that there were 1 555 000 adults in need of ART in 2010.

According to the 2003 South African National ART Guidelines, all HIV infected individuals are only legible for ART if the CD4 count is less than 200 cells/mm^3, and since 2010, the guidelines have been updated (DoH SA, 2010). According to the 2010 South African National ART guidelines, HIV infected individuals co-infected with TB or pregnant are legible for ART if the CD4 count is less than 350 cells/mm^3. Between March 2004 and May 2005 77,352 HIV positive adults and children were tested for CD4 and 37,564 were found to have CD4 less than 200 cells/mm^3.

Table 2.11 shows the 2003 South African Antiretroviral regimen guidelines. The participants in this study followed these guidelines.

Table 2.11: Antiretroviral regimens used in South Africa (adapted from Operational Plan for Comprehensive HIV and AIDS care, Management and Treatment in South Africa, 2003)

Regimen	**Drugs**	**Class combination**
1a: First-line	Stavudine + Lamivudine + Efavirenz	Two nucleoside reverse trancriptase inhibitor (NRTI) + one non-nucleoside reverse transcriptase inhibitor (NNRTI)
1b : alternate first- line	Stavudine + Lamivudine + Nevaripine	Two NRTI + one NNRTI
2 : second-line	Zidovudine + Didanosine + Lopinavir / Ritonavir	Two NRTI + one protease inhibitor (PI)

Due to improved knowledge on ARV side effects, the guidelines have been revised and updated as follows:

Table 2.12: The South African Antiretroviral Treatment Guidelines (adapted from Department of Health, 2010), showing the most updated treatment guidelines used.

Regimen	**Drugs**	**Combination**
First line : for new patients	Tenofovir + Lamivudine / Emtricitabine+ Efavirenz	Two NRTI + one NNRTI
First line : For patients currently on stavudine based regimen with no side effects	Stavudine + Lamivudine + Efavirenz / Nevaripine	Two NRTI + one NNRTI
First line : For child bearing women, not on reliable contraception	Tenofovir + Lamivudine / Emtricitabine+ Nevirapine	Two NRTI + one NNRTI
First line : For patients who have adverse effects to Tenofovir	Zidovudine + Lamivudine + Efavirenz / Nevirapine	Two NRTI + one NNRTI
Second line : For patients failing on a stavudine or zidovudine based first line	Tenofovir + Lamivudine / Emtricitabine+ Lopinavir + ritonavir	Two NRTI + one protease inhibitor (PI)
Second line : For patients failing on a tenofovir based first line	Zidovudine + Lamivudine + Lopinavir + ritonavir	Two NRTI + one protease inhibitor (PI)

Even though ARVs reduce the mortality rate, the fact that patients may take these drugs long term (even up to 50 years), does not prevent them from developing side effects (David, 1999). Complications also arise due to drug resistance and adherence patterns (Bangsberg *et al.*, 2007).

2.2.5 Antiretroviral therapy side effects and challenges

Antiretroviral regimens (Table 2.11 and 2.12) are complicated and difficult for patients to follow and they can have serious side effects including metabolic side effects as explained in Table 2.13 (DoH SA, 2004, DHHS, 2006). As mentioned before, the development of side effects directly affects the adherence to treatment and may result in the discontinuation of treatment by patients.

Table 2.13: Side effects caused by Antiretroviral drugs (adapted from National Antiretroviral Treatment Guidelines; National Department of Health, South Africa 2004 and the Department of Health and Human Services, 2006).

Antiretroviral drug	**Adverse effect**
Stavudine (d4T)	Peripheral neuropathy, hepatic steatosis, lactic acidosis, pancreatitis, lipoatrophy (loss of peripheral subcutaneous fat in the extremities, buttocks and face)
Lamivudine (3TC)	Diarrhoea, pancreatitis, lactic acidosis
Efavirenz (EFV)	CNS disturbance, GIT symptoms
Nevaripine (NVP)	Skin rash, nausea, vomiting, hepatitis (can be fatal)
Zidovudine (AZT)	Bone marrow suppression, GIT symptoms, lactic acidosis, myopathy, cardiomyopathy in children
Didanosine (ddL)	Pancreatitis, peripheral neuropathy, GIT effects (bloating, flatulence, nausea, diarrhoea), lactic acidosis
Lopinavir/Ritonavir	GIT symptoms, lipid abnormalities, lipodystrophic changes

The severity of adverse effects or events (AE) related to HAART may differ from country to country due to a number of factors like diseases affecting each country, host genetics, nutritional status, socio-economic status of the country (Subbaraman *et al.,* 2007). The adverse effects may also relate to the antiretroviral drug administered. The discussion will focus on the antiretroviral drugs that are used in the 2004 National ART plan and the drug regimen for this study population, which are stavudine, lamivudine and efavirenz.

2.2.5.1 Adverse effects associated with stavudine

Studies (Moyle *et al.,* 1998, Scarcella *et al*., 2002, van Oosterhout *et al.,* 2005) indicated that 10% to 56% patients exposed to stavudine therapy developed peripheral neuropathy which is defined as degenerative changes of the peripheral nerves. This abnormality results in the distortion and interruption of messages between the brain and the rest of the body which affects the functioning of the body.

Stavudine is also associated with lipoatrophy of face, arms and buttocks (Pujari *et al*.,2005) and this was reported in a number of studies (Paton *et al.,* 2002, Puttawong *et al*., 2004, Pujari *et al.,* 2005, Tin *et al*., 2005, van Griensven *et al*., 2006). Even though lipoatrophy may not be considered a life-threatening effect but it affected the social relations and increased stigma of the patient (Paton *et al.,* 2002, Pujari *et al.,* 2005).

Lactic acidosis is another adverse effect associated with stavudine, although infrequent (Hosseinipour *et al*., 2006). Lactic acidosis is a life- threatening condition that is defined as persistently and remarkably elevated serum lactate levels (> 5 mmol/L) with low pH value and decreased bicarbonate concentration. Lactic acidosis may be divided into two types. Type A is lactic acidosis with clinical evidence of tissue hypoxia. Type B is lactic acidosis without tissue hypoxia. Lactic acidosis observed on stavudine patients is considered Type B, and the prevalence ranges from 15% to 35% (Calza *et al.,* 2004). It is usually accompanied by tachypnoea, dyspnoea, hyperventilation, tachycardia, cardiac arrhythmia, arterial hypotension, seizures and abnormal mentation (Calza *et al.,* 2004) and this may lead to multiorgan dysfunction and haemodynamic instability, which in most cases, is fatal. The mortality rate among patients with

lactic acidosis ranges from 30% to 60% (Calza *et al*., 2004; Brivet *et al*., 2000) and Chow (2003) indicated it was associated with lactate level above 10 mmol/L.

Lactate production is a normal physiological function, with circulating lactate held within narrow limits which are 1.6 to 2.1 mmol/L. However about 5 – 25% of HIV patients on stavudine have raised lactate levels (Datta *et al*., 2003). According to Osler's South African study (2007), there was a 1.5% annual risk of symptomatic hypelactataemia with stavudine.

2.2.5.2 Adverse effects associated with lamivudine

Lamivudine is well tolerated even though in the 2004 South African National Antiretroviral Therapy Guidelines some adverse events were documented. However, in the 2010 South African National Antiretroviral Therapy Guidelines, the adverse events have been removed and substituted with well tolerated. Minor effects such as headache and dry mouth have been reported. Discontinuation of this drug on patients co-infected with hepatitis B may cause a flare up of this disease. (Honkoop *et al*., 2000)

2.2.5.3 Adverse effects associated with efavirenz

Efavirenz may be associated with central nervous system adverse effects like sleep disorders, cognitive disorders and mood disorders (Hawkins *et al.*,2005) during the first three months of treatment and the prevalence of these disorders decreases thereafter (Lochet *et al.*,2003).

In an efavirenz developmental toxicity study, malformations were observed in three of 20 foetus of cynomolgus monkeys (Bristol-Myers Squibb, 2005), indicating that this drug may not be safe to use by pregnant women. A Botswana study (Bussman *et al.,* 2007) found that 42.1% pregnancies were affected by first trimester efavirenz exposure, resulting in miscarriages. A France study of 12 pregnant women (Jeantils *et al.,* 2006) found that two women had

miscarriages and seven had live births. Out of the seven, three had abnormalities. Another pregnancy registry study of women who were exposed to efavirenz in the first trimester reported 14 out of 477 live births had congenital defects (Antiretroviral Pregnancy Steering Committee Registry, 2009).

As part of the antiretroviral therapy programme, the drugs mentioned above are administered in combination and other potentially life-threatening adverse effects that may develop as a result of that. The discussion will focus on the metabolic side effects that were evaluated in this study.

2.2.5.4 Hepatotoxicity

Hepatotoxicity is the term used to define liver damage caused by medications and other chemicals (Zimmerman, 1999).The liver is one of the largest and most important organs in the human body. It carries out a number of vital functions that the body requires to remain healthy and stable (Zelman *et al.*, 2011). For HIV positive people who are prone to opportunistic infection, liver plays a vital role since it is responsible for making new proteins needed by the immune system which helps the body to resist infection (Stoppler, 2011). The liver also detoxifies drugs, including those used to treat HIV and AIDS related infections (Stoppler, 2011). During this process, the liver can become overworked which can lead to liver damage (Aidsinfo, 2005). When the liver is damaged, it can no longer perform all of its necessary tasks (Zelman *et al*, 2011).

There are conditions that fall within the hepatotoxicity category. These conditions are hepatitis, hepatic necrosis or cirrhosis and hepatic steatosis (Aidsinfo, 2005). When the liver is damaged, its enzymes are released into the blood stream and the levels can be measured by blood tests (Aidsinfo, 2005). The hepatic cell damage marker enzymes are alanine aminotransfearse (ALT) and aspartate aminotransferase (AST) (Stoppler, 2011). These enzymes are involved in the synthesis of proteins in the liver, within the hepatocytes (Nabili, 2011).

The signs and symptoms of hepatotoxicity are non-specific and may vary depending on the extent of liver damage. These may include nausea, vomiting, abdominal pain, loss of appetite

and diarrhoea (Aidsinfo, 2005). The more specific signs are jaundice (yellowing of the skin and eyes) and liver enlargement (hepatomegaly) (Stoppler, 2011).

Hepatotoxicity is seen in patients receiving stavudine, lamivudine or nevirapine (Kontorinis and Dieterich, 2003). Severe hepatotoxicity is characterised by a five-fold increase in serum levels of liver enzymes, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) (Division of AIDS, 2004). A 12-month study performed at two HIV clinics in Nigeria teaching hospitals (Ikekpeazu *et al.*, 2009) revealed that HAART caused elevation of ALT and AST which resolved in most cases. The elevation was seen in the first six months in the group on NNRTI and at only three months in the NRTI and PI group. PI was not included in the 2004 South African regimen because of the cost of antiretroviral drugs. Since the cost of these drugs have been revised, the 2010 South African ART guidelines have included the PI group (DoH SA, 2010).

2.2.5.5 Dyslipidaemia

Dyslipidaemia, is the elevation of plasma cholesterol, triglycerides or both or a decreased high density lipoprotein (HDL). The relationship between length of exposure to ART and increased incidence of cardiovascular events have also increased concerns that ART-associated dyslipidaemia will result in increased rates of cardiovascular disease among ART treated patients (Mallon, 2006).

Cholesterol is a fat that the body needs to work properly (Lab Tests Online, 2008). It is an important component of cell membranes and is important in the establishment of proper membrane permeability and fluidity (Lab Tests Online, 2008). It is also an important component of hormone production, bile acids synthesis and vitamin D synthesis (Lab Tests Online, 2008) Despite its importance, high levels of cholesterol in the blood may cause atherosclerosis which is an important predisposing factor to coronary heart disease (Zelman *et al.*, 2011).

Triglycerides are the chemical forms in which fat exists in the body. Triglycerides in blood originate either from fats in food or are made in the body from carbohydrates (Lab Tests Online, 2009). Glucose which is not metabolised immediately by the tissues, is converted into

triglycerides and stored as adipose tissue. Higher concentrations of triglycerides in blood, usually correlates with the consumption of starchy and other foods that are high in glucose. They play an important role as energy sources. Increased levels of triglycerides in blood, together with increased cholesterol levels are associated with increased risk of coronary heart disease (Lab Tests Online, 2009).

Dyslipidaemia is characterised by cholesterol levels above 6.19 mmol/L and triglycerides levels above 5.65 mmol/L. Patients on long-term ART, particularly retroviral PI drugs (Yarasheski *et al.,* 2003) are usually prone to this condition. *In vitro* evidence indicates that some PI drugs reduce lipoprotein lipase (LPL) and hepatic lipase (HL) expression and activities (Yarasheski *et al.*, 2003). Lipoprotein lipase is the enzyme primarily responsible for triglyceride clearance from the circulation (Yarasheski *et al.*, 2003). Hepatic lipase is an enzyme that can also hydrolyse triglycerides. Efavirenz, a retroviral NNRTI drug has been associated with hypercholesterolaemia (DoH AIDS Institute, New York, 2004). Elevated triglycerides are also associated with pancreatitis (DoH AIDS Institute, New York, 2004). According to a report by Coffey, in the Guide for HIV/AIDS clinical care (2012), dyslipidaemia is caused by a combination of factors related to HIV disease, ART regimen and individual patient characteristics. Coffey (2012) also reported that not all ART-treated patients experience lipid abnormalities to the same degree, because of its association with coronary heart disease, identification and management of this condition is important.

2.2.5.6 Pancreatitis

Pancreatitis (inflammation of the pancreas) can be an acute complication of ART, is potentially fatal (Piliero, 2003) and has been linked to the use of lamivudine and stavudine, the retroviral NRTI drugs (Piliero, 2003). Both these drugs are the cornerstone of the South African ART regimen and are used in combination. Pancreatitis is characterised clinically by abdominal pains, nausea, vomiting and biochemically by elevations of lipase and /or amylase, specifically by a three-fold and above increase in serum amylase and lipase levels. Amylase (pancreatic) is the

enzyme that is found within the pancreas. It is released when it is required for digestion of carbohydrates. The increase in blood levels signifies pancreatic damage. The extent of the damage is directly proportional to the blood levels. Lipase is another enzyme that is also found within the pancreatic cells. It is released when digestion of lipids is required. The increase in blood levels also signifies pancreatic damage. The extent of the damage is directly proportional to the blood levels.

A study involving 3 000 participants from five large muliticentres in the United States of America (1996 - 2001), who were on antiretrovirals (any combination) found that the incidence of Grade 4 clinical-and/or laboratory defined pancreatitis was 0.85 per person-years. The participants involved in this study received different combinations of ARVs; group 1- PI or 2 PI + 2 NRTIs, group 2 -NNRTI + 2 NRTIs, group 3 -1 or 2 PIs + NNRTI + 1 or 2NRTIs. The South African ART combination is NNRTI + 2NRTI.

Another study in Uganda from 2003 to 2004 that assessed the clinical toxicity of ARVs found that of 1 029 participants, $\leq$ 0.5% developed pancreatitis (Forna *et al.*,2007). The study group was on stavudine, lamivudine with nevirapine or efavirenz. A EuroSIDA study that was investigating the incidence of pancreatitis in patients on stavudine and / or didanosine concluded that there was no evidence to associate pancreatitis with cumulative exposure to stavudine and / or didanosine (AIDS, 2008). The authors observed that higher baseline CD4 cell counts were associated with a decreased risk of pancreatitis.

From the discussion it is clear that stavudine, lamivudine and efavirenz, which are the South African first line regimen show evidence of metabolic side effects or adverse effects. The severity of the metabolic side effects range from mild to potentially life-threatening (severe) and the biomarker blood levels help to determine the severity. The Division of AIDS (DAIDS) Table for grading the severity of adverse events (2004) is used. The grading criterion for the biomarkers in this study is indicated in Table 2.14.

Table 2.14: Toxicity grading using the biomarker blood levels (adapted from Division of AIDS Table for grading the Severity of Adults and Paediatric Adverse events, 2004).

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE THREATENING
Hepatotoxicity biomarkers				
ALT	1.25 – 2.5 X ULN	2.6 – 5.0 X ULN	5.1 – 10.0 X ULN	> 10.0 X ULN
AST	1.25 – 2.5 X ULN	2.6 – 5.0 X ULN	5.1 – 10.0 X ULN	> 10.0 X ULN
Dyslipidaemia (hyperlipidaemia) biomarkers				
Cholesterol (fasting)	5.18 - 6.19 mmol/L	6.20 - 7.77 mmol/L	> 7.77 mmol/L	NA
Triglycerides (fasting)	NA	5.65 - 8.48 mmol/L	8.49 - 13.56 mmol/L	> 13.56 mmol/L
Pancreatitis biomarkers				
Amylase (pancreatic)	1.1 – 1.5 X ULN	1.6 – 2.0 X ULN	2.1 – 5.0 X ULN	> 5.0 X ULN
Lipase	1.1 – 1.5 X ULN	1.6 – 3.0 X ULN	3.1 – 5.0 X ULN	> 5.0 X ULN

ULN – upper level of the Normal Range

2.2.6 South African antiretroviral therapy rollout challenges

2.2.6.1 Infrastructure

When the public sector ART rollout was initiated, most programmes were started at large government hospitals where the infrastructure is better than clinics. For rural residents, the public health care system proved to be overcrowded and difficult to access (Ojikutu *et al.*, 2007). Without access to therapy at the community level in local clinics, patients were left on long hospital waiting lists and had to wait many months before receiving treatment.

There is also a significant human resource deficit in South Africa according to Nattrass (2006). There were 100 nurses/100 000 uninsured persons, many of whom rely on public health services, making the national ART plan which promotes nurse-centred approach to rollout difficult to implement.

2.2.6.2 Alternative medicines

According to the WHO, 80% of Africa's population uses traditional medicine for primary health care (WHO, 2006). In South Africa, 75% of HIV-infected people use remedies dispensed by the traditional healers (WHO, 2006). The low cost, proximity to the community and respect for traditional leadership are the reasons that drive the demand for services provided by the healers. Several studies have shown the toxicity of these therapies (Steenkamp *et al.*, 2000, Popat *et al.*, 2001, Luyckx *et al.*, 2004, Kales *et al.*, 2007). A study by Luyckx *et al*. (2004) reported high rates of dehydration, vomiting, diarrhoea and renal failure among patients on traditional remedies. When these remedies are used together with ART, severe toxicities may occur and the efficacy of ARVs may be compromised.

The promotion of alternative treatments for HIV infection has led to an enormous amount of confusion among people living with HIV and AIDS (Ojikutu *et al.*, 2007). Also in 2006, the then Health minister, Dr. Manto Tshabalala-Msimang advocated a diet of garlic, olive oil,

beetroot and lemon to cure the disease, showing that the government did not fully support the ART programme. Concoctions called 'immune boosters' are sold throughout South Africa and there is evidence to suggest that South Africans are choosing these and other alternative therapies over the ART (IRIN News report, 2006).

2.2.6.3 Co-infection with Tuberculosis

Tuberculosis (TB) is the leading cause of death in HIV infected individuals in South Africa (Coetzee *et al.,* 2004). People living with HIV are at a higher risk of developing active TB as a weakened immune system will facilitate the development of the disease (WHO, 2009). South Africa has one of the highest co-infection rates with an HIV prevalence of 75% among people with incident TB, according to the WHO report on Global tuberculosis control report of 2009. The increased number of TB co-infection had delayed the initiation of ARVs, since according to the National Treatment plan (2003), people that are diagnosed with TB prior to ARV initiation should be first treated with anti-TB drugs for two months before being put on ARVs. This guideline has since changed to address the problem of increased mortality in co-infected individuals. The 2010 South African National ART Guidelines recommend that patients with TB and HIV are eligible to start ARV treatment when the CD4 count is at 350 cells/mm^3 or less and patients co-infected with multi drug resistant TB (MDR-TB) or extensively drug resistant TB (XDR-TB) are eligible for ART, irrespective of the CD4 count.

The development of drug-resistant strains of TB has complicated the management of TB and HIV. The MDR-TB strain is resistant to both isoniazid and rifampicin drugs which are the cornerstone of TB treatment. The XDR-TB strain which is resistant to both first-line and many second-line anti-tuberculosis drugs also emerged. This strain was almost exclusively in HIV-infected patients and was associated with an accelerated fatality rate (Gandhi *et al.,* 2006). All these developments have had severe impact on mortality in Africa and have made management of HIV challenging (Sterling *et al.,* 2010).

There has been a call for an integration of care for the two diseases (Karim *et al.*, 2009). This integration system has made it easier for people with one disease to be tested and treated for the other (South African National AIDS Council (SANAC), 2007). In 2007, over 33% of HIV positive TB patients were provided with antiretroviral therapy and 66% received co-trimoxazole prophylaxis (WHO report on Global Tuberculosis Control Report of 2009). In 2010 patients that were co-infected began ARV treatment at CD4 count of 350cells/mm^3 or less (DoH SA, 2010)

The South African HIV infected population is faced with a lot of challenges when it comes to the antiretroviral therapy programme ranging from being in a country with the highest number of infected people with all of them in need or will be in need of treatment in a health care system with limited resources. This is further compounded by the tuberculosis and the emergence of highly drug resistance strains. The enhanced management of ART will decrease the burden of challenges faced by this population, reduce patient non-compliance and mortality due to ART and hence enhance life expectancy.

CHAPTER THREE METHODOLOGY

3.1 INTRODUCTION

The aim of the study was to investigate whether patients in KwaZulu-Natal, South Africa who were on antiretroviral drugs (ARVs) developed metabolic side effects. Laboratory biomarker tests were used to assess side effects. The biomarkers tested for were, alanine aminotransferase (ALT), aspartate aminotransferase (AST), cholesterol, triglycerides, amylase, and lipase. Blood samples drawn from the patients were used to detect the levels of these biomarkers. These biomarkers were tested at different intervals: before initiation of ARVs (baseline), six months and twelve months. Any significant change was noted in accordance with the Division of AIDS 2004 Guidelines. A significant change for amylase and lipase was a two-fold and above increase from the upper limit of normal in the laboratory value. For ALT and AST, a three-fold and above increase was used. For cholesterol, a value greater than 6.19 mmol/L and triglycerides, a value greater than 5.65 mmol/L (Division of AIDS, 2004).

3.2 STUDY DESIGN

The study design was a descriptive, longitudinal survey. The study included 92 patients who were already selected for the ART programme, according to the 2004 South African National Antiretroviral Treatment Guidelines (DoH SA, 2004). The patients were recruited from uMlazi Medical Centre. The participants were candidates for treatment regimen first-line (a), which is stavudine (d_4T), lamivudine (3TC) and efavirenz (EFV) drugs (DoH, South Africa, 2004). All laboratory testing was conducted at Global Clinical and Viral Laboratory, Durban. This laboratory service was routinely utilised by the uMlazi Medical Centre. The data collection resulted in a sample size of 276. The number of patients recruited for this study was verified by the biostatistician to be sufficient to show statistical significance.

3.3 PARTICIPANT SELECTION

Patients enrolled for the study were required to meet the inclusion criteria.

3.3.1 Inclusion criteria

- 18 years or older
- HIV infected with laboratory evidence of their status, medical records and consent forms were filed at the clinic
- Candidates for ART regimen first-line (a); stavudine, lamivudine and efavirenz drugs.
- Enrolled patients with sufficient pre-treatment data that is required for this study (CD4 count, full blood count (FBC), viral load) from the medical records
- Any ethnic group
- Both male and female candidates will be enrolled
- Communicate in English or isiZulu
- Be willing to answer questions on the questionnaire for the study

3.3.2 Exclusion criteria

- Patients who have active TB clinically or on anti TB treatment
- Patients who are on alternate HIV/AIDS treatment
- Pregnant women
- Not willing to comply with protocol

3.4 ETHICAL CONSIDERATION

Before the commencement of the investigation, ethical approval was obtained from the Durban University of Technology Ethical Committee. Permissions were obtained from the uMlazi Medical Centre (appendix 2) and the Global Clinical and Viral Laboratory (appendix 3) where all the specimen testing took place. The research plan was discussed with ART programme coordinator of the uMlazi Medical Centre and laboratory staff who received specimens for processing. Similarly, the research plan was also discussed with the staff members who printed and sent the results to the medical centre.

Patients who met the inclusion criteria were recruited at uMlazi Medical Centre. A consent form drawn up by the principal investigator in both English and IsiZulu (appendix 5A and 5B) was presented to all patients who were willing to participate in the study. Participants were informed about the objective, requirements and benefits of the study. This information was also in the subject information leaflet which was given to the participants (appendix 4A and 4B). Participants were also informed that their right to participate in the study was voluntary and that they were free to withdraw at any point from the study without affecting their treatment plan and follow up visits to the medical centre. They were also assured that all the information used in the study would remain confidential and that any data reported in the thesis and scientific journals or published data would not include information identifying them as participants in the study.

3.5 METHODOLOGY OUTLINE

Once the patients were selected for the antiretroviral treatment programme, they underwent ART counseling and clinical assessment, which was two weeks prior to initiation of therapy. During the counseling week, baseline blood samples were collected from patients. The following laboratory tests: CD4 count, Full blood count, and alanine aminotransferase (ALT) were performed as per the 2004 South African National Antiretroviral Treatment Guidelines. An additional request form specific to this study was sent to the laboratory together with these blood samples (Appendix 7). The additional tests that were performed were amylase, lipase, cholesterol, triglycerides and aspartate aminotransferase (AST). All laboratory tests for the study

were performed using the same samples that were already drawn from the patient as part of their clinical assessment. For the triglyceride test, participants were required to fast. They were asked not to eat anything, except water, from 9 pm of the previous night.

A blood tube without an anticoagulant (plain or red top or yellow top tube) was used for the analysis of these biomarkers: lipase level, amylase level, alanine aminotransferase (ALT), aspartate aminotransferase (AST), cholesterol level and triglyceride level.

Table 3.1: Biomarkers commonly used for the specific metabolic side effects.

Biomarker	**Metabolic side effect**
amylase lipase	Pancreatitis
cholesterol triglyceride	Dyslipidaemia
ALT AST	Hepatotoxicity

A questionnaire was also administered by the principal investigator. Other participants preferred that the questionnaire be administered by the ART coordinator. The questionnaire provided information on the demographic details, other treatment/s that the participant is on, nutritional status of participant on ART, participant's knowledge on side effects and management of ART. Once the laboratory results were received and evaluated by the clinician, the patients were given their initial ART package. All the laboratory results for this study were recorded and filed in a separate folder, which was only accessed by the principal investigator.

The six month follow up date and 12 month follow up date were diarized by the principal investigator and the participant.

The six month follow up and 12 month follow up blood samples were processed following the same procedure as the baseline samples.

3.6 LABORATORY TESTING

All laboratory testing was conducted at Global Clinical and Viral Laboratory, Durban. Fully automated laboratory systems were used for testing, in compliance with International Organization for Standardization (ISO) standards. Spectrophotometric methods used in the automated instrument, Unisel, DXC 600, Beckman were based on the principle that when a patient's serum sample or control is mixed with or more appropriate chemical reagents, a substance which is a chromophore, is produced that has the ability to absorb light at a specific wavelength. The assay method for each analyte or biomarker incorporates a coupled enzymatic reaction using an enzyme as the indicator reaction and monitors the change in absorbance at 340nm. The amount of light absorbed at the end of the reaction is proportional to the concentration of the analyte being measured. The step by step laboratory methodology for each biomarker is attached (Appendix 9A – 9F).

The instruments used were calibrated as per the Laboratory protocol and manufacturer's instruction. Quality controls were included with each batch to ensure validity of results. On completion of laboratory testing, blood specimens were suitably preserved, by refrigeration at 4^0C, in case retesting was required for the study. Random samples were run twice to assure reproducibility. External quality control programmes were evaluated to ensure quality of data.

3.7 DATA COLLECTION

Pre-treatment data, including clinical status and immune status as determined by CD4+ cell count and viral load, were obtained from participants by means of a questionnaire administered

in English or isiZulu by the principal investigator, as well as information obtained from the participants' medical records.

The first part of the questionnaire provided information on the demographics of participants. This information was used in the assessment of incidence of side effects in this population.

The second part of the questionnaire provided the information on participant's adherence to treatment and participant's knowledge of the possible side effects of the regimen.

The third part provided information on the participant's other illnesses and medication which may have been the predisposing factors or confounders to the side effects.

Laboratory results generated from the Global Clinical and Viral Laboratory, were recorded and any significant change was noted. A significant change for amylase and lipase was a two-fold and above increase from the upper limit of normal in the laboratory value. For ALT and AST, a significant change was a three-fold and above increase. For cholesterol, a significant change was a value greater than 6.19 mmol/L and for triglyceride a value greater than 5.65 mmol/L was a significant change (Division of AIDS, 2004).

3.8 STATISTICAL METHODOLOGY

1. The laboratory data; at baseline, six months and 12 months were compared. The repeated measures analysis of variance (ANOVA) to compare changes in biomarkers over time method was used.
2. The side effects of antiretroviral drugs encountered by the participants were compared with the commonly encountered side effects published (Table 2.13) by looking at the frequency and the time of onset of each side effect.
3. The severity of each side effect was assessed by grading each biomarker laboratory result using the DAIDS toxicity grading table (Table 2.14).
4. The biomarker laboratory results (in participants with blood levels that were suggestive of metabolic disorders) was correlated with the clinical symptoms or information provided by the clinician after assessing the patient (Appendix 8). Regression analysis was used.

5. Presumptive nutritional status of the patient was assessed by using the household income information on the questionnaire which indicates food affordability means. This was also correlated with their responses on the dietary question on the questionnaire.
   Regression analysis was used.

CHAPTER FOUR RESULTS

4.1 DEMOGRAPHICS AND BASELINE CHARACTERISTICS

Ninety two participants were enrolled into the study and all 92 completed follow up assessments. The participants were all from the black ethnic group. The study consisted of 59 (64%) females and 33 (36%) males as shown in Figure 4.1.

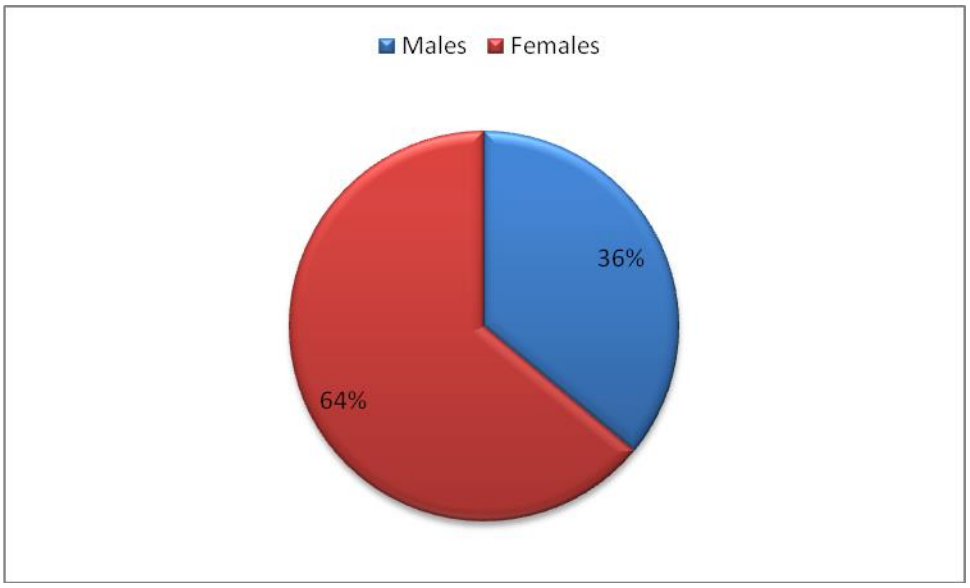


Figure 4.1: Pie chart on gender distribution of participants.

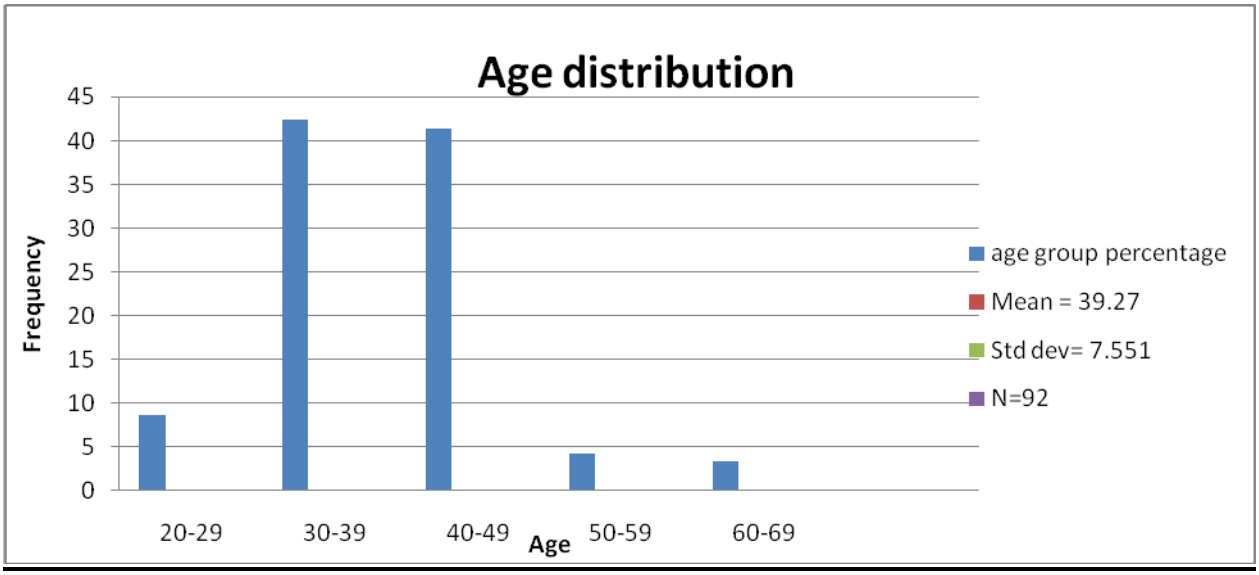


Figure 4.2: Age distribution of participants.

The mean age of participants was 39.27 years and the age range was 25 to 64 years as shown in Figure 4.2. The highest number of participants was between the age groups of 30 – 39 years (42.4%) and 40 - 49 years (41.3%), respectively. This age group represents the economically active group in the country. The lowest age percentage of participants (3%) is from the age group of 60 – 69 years.

Since the study has 59 females and 33 males, Figure 4.3 indicates age distribution in each gender.

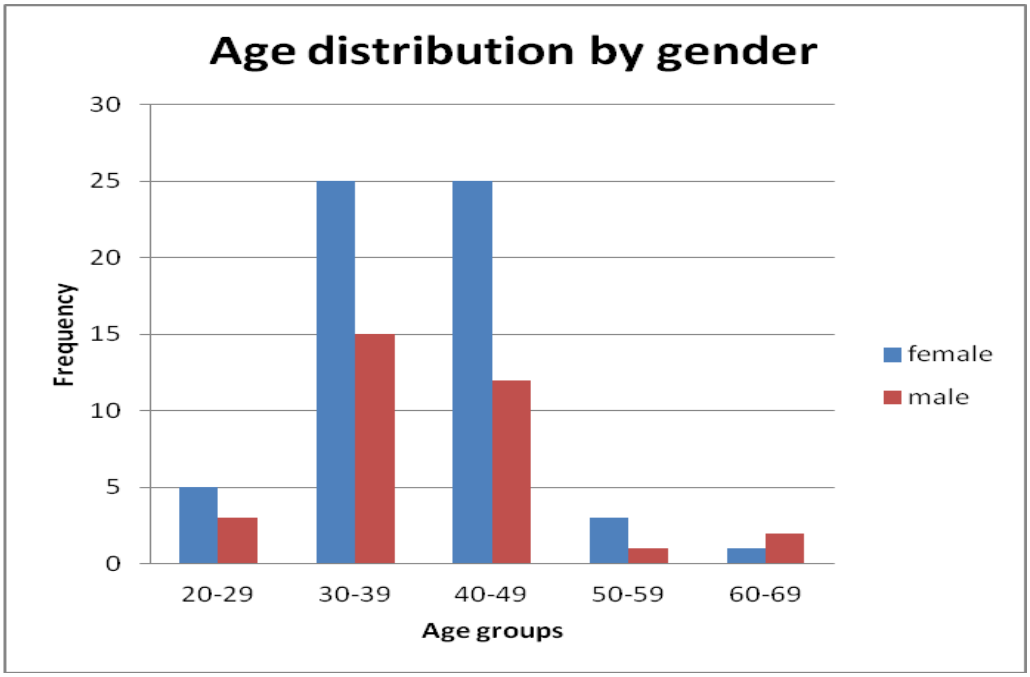


Figure 4.3: Age distribution of participants by gender.

Baseline parameters were taken for all patients as depicted in Table 4.1. Also depicted are the highest and lowest reading recorded for each biomarker. Alanine aminotransferase (ALT), aspartate aminotransferase (AST) are enzymes released by the liver. The reference range for ALT is 10 - 45 U/L and AST is 5 - 40 U/L. Cholesterol and triglycerides are part of the lipid profile. The reference range for cholesterol is 3.5 - 5.2 mmol/L and triglycerides is 0.28 -2.3 mmol/L. Amylase and lipase are the enzymes that are released by the pancreas. The reference range for amylase is 28 - 110 U/L and lipase is 7 - 60 U/L. The baseline results for all the participants were within the reference ranges for all the stipulated parameters. All reference ranges used in this study were based on those that have been calibrated for the laboratory where the testing was performed.

Table 4.1: Baseline Results for all Biomarkers.

Biomarker	[1]Reference Range	Mean Results at baseline N=92	Highest value*	Lowest value**
ALT	10-45 U/L	19.52	33.0	6.0
AST	5-40 U/L	24.67	41.0	12.0
Cholesterol	3.5-5.2 mmol/L	3.72	4.84	2.51
Triglyceride	0.28- 2.3 mmol/L	1.04	2.63	0.26
Amylase	28-110 U/L	48.61	84.0	29.0
Lipase	7-60 U/L	36.8	55.0	22.0

[1]Reference ranges used in accordance with the Laboratory where testing was conducted

*of the collective data of 92 participants **of the collective data of 92 participants

4.2 ASSESSMENT / COMPARISON OF BIOMARKER VALUES OVER TIME

The first objective of the study was to determine whether ART patients develop any metabolic side effects when on the prescribed regimen. This was determined by assessing and identifying any increase in specified biomarker levels in blood samples over time. For each biomarker, blood

levels at six month interval and twelve month interval results were each compared to baseline results. The repeated measure analysis of variance (ANOVA) was used to compare the equality of the means and it subsequently plots trends or any significant difference in levels at the different time points. The mean of each set of data were compared and any change was noted in the follow up data. Statistically significant changes were noted. Comparisons using ANOVA is described in the text.

4.2.1 Comparison of ALT data; at baseline and six month interval and baseline and twelve month interval

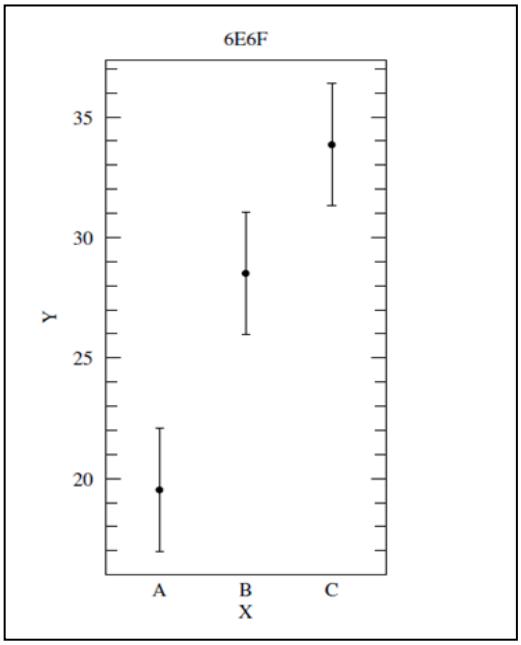
Alanine aminotransferase (ALT) is one of the enzymes that are within the hepatocytes. The increase in blood levels signifies hepatocyte damage. The extent of the damage is directly proportional to the blood levels. The upper limit of normal (ULN) of this enzyme is 45 U/L. This is based on the reference range that was used by the laboratory where the study samples were analysed.

Statistical analysis of the six month data revealed a mean of 28.51 which is below 45 U/L and the 12 month mean is 33.85 which is also below the upper limit of normal value as seen in Table 4.2. The results indicate that there is no ALT abnormality after 12 months of treatment *(p*<0.0001).

Table 4.2: Summary statistics for ALT at six month and 12 month intervals.

Biomarker	Reference Range	Highest value*	Lowest value**	Mean Results at baseline N=92	Mean Results at 6 months N=92	Mean Results at 12 months N=92
ALT	10-45U/L	33.0	6.0	19.52	28.51	33.85

*of the collective data of 92 participants **of the collective data of 92 participants



Y axis: ALT values

X axis:

A: Baseline

B: 6 months

C: 12 months

Figure 4.4: Comparisons of ALT values at baseline, six months and 12 months showing changes in levels that do not have a diagnostic impact.

What has been noted is the gradual increase (18.7%) in the ALT value over time (from six to 12 months) as seen in Figure 4.4, indicating that ALT levels are not significantly altered within 12 months of ARV treatment. It can be speculated that ALT abnormalities may be detected after a longer period of treatment.

4.2.2 Comparison of AST data; at baseline and six month interval and baseline and twelve month interval

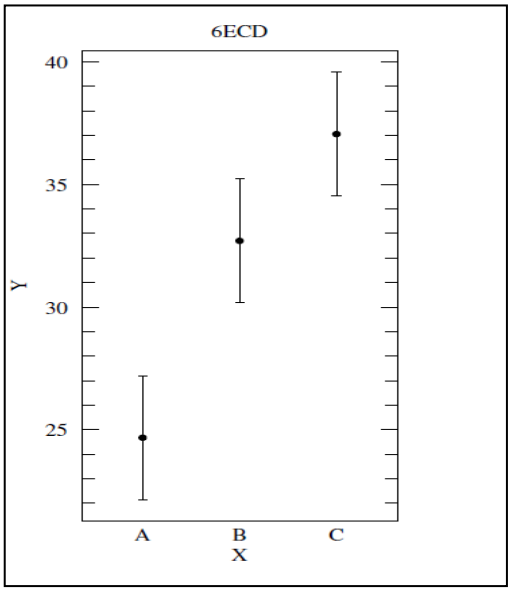
Aspartate aminotransferase (AST) is another enzyme that is also found within the hepatocytes. The increase in blood levels also signifies hepatocyte damage. The extent of the damage is directly proportional to the blood levels. The ULN of this enzyme is 40 U/L. This is based on the reference range that was used by the laboratory when the study samples were analysed.

Table 4.3 shows that at six months, the mean AST level was 32.70 and a 12 month, the mean was 37.05. Both the results are below the upper limit value, of 40 U/L. This indicates that after 12 months of treatment, there are no AST abnormalities noted ($p<0.0001$).

Table 4.3: Summary statistics for AST at baseline, six month and 12 month intervals.

Biomarker	**Reference Range**	**Highest value***	**Lowest value****	**Mean Results at baseline N=92**	**Mean Results at 6 months N=92**	**Mean Results at 12 months N=92**
AST	5-40 U/L	41.0	12.0	24.67	32.70	37.05

*of the collective data of 92 participants **of the collective data of 92 participants



Y axis: AST levels

X axis:

A: Baseline

B: 6 months

C: 12 months

Figure 4.5: Comparisons of AST mean values at baseline, six months and 12 months showing changes in levels that do not have a diagnostic impact.

There is a gradual increase (13.4%) over time as reflected in Figure 4.5. Similarly to ALT, it can be speculated that AST abnormalities may be detected after prolonged periods of treatment.

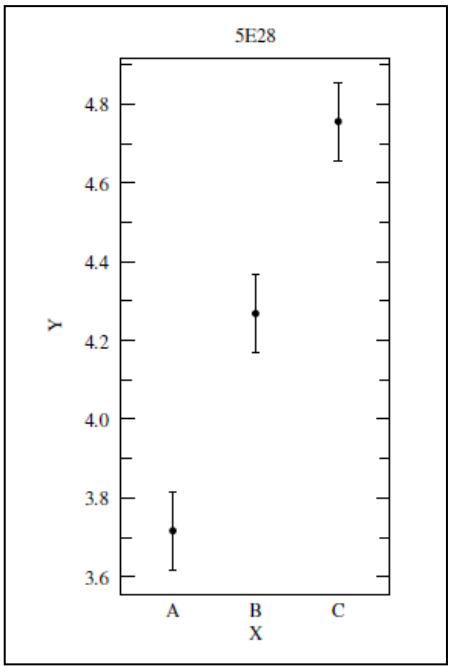
4.2.3 Comparison of cholesterol data; at baseline and six month interval and baseline and twelve month interval

The reference range of cholesterol blood levels (fasting) is 3.5 – 5.2 mmol/L. This is based on the reference range that was used by the laboratory when the study samples were analysed. Table 4.5 below shows that at six months the mean cholesterol level was 4.268 and a 12 month the mean was 4.756. Both the results are below the upper limit value, of 5.2mmol/L. This indicates that after 12 months of treatment, cholesterol levels in blood are not abnormally elevated (p<0.0001).

Table 4.4: Summary statistics for cholesterol at baseline, six month and 12 month intervals.

Biomarker	Reference Range	Highest value*	Lowest value**	Mean Results at baseline N=92	Mean Results at 6 months N=92	Mean Results at 12 months N=92
Cholesterol	3.5-5.2 mmol/L	4.84	2.51	3.72	4.27	4.76

*of the collective data of 92 participants **of the collective data of 92 participants



Y axis: Cholesterol levels

X axis

A: Baseline

B: 6 months

C: 12 months

Figure 4.6: Comparisons of cholesterol mean values for baseline, six month and 12 month showing no clinical significance when compared to normal ranges.

It is noted that there is a gradual increase (11.4%) over 6 and 12 months as reflected in Figure 4.6. It can be speculated that abnormalities may be detected after prolonged periods of treatment.

4.2.4 Comparison of triglyceride data; at baseline and six month interval and baseline and twelve month interval

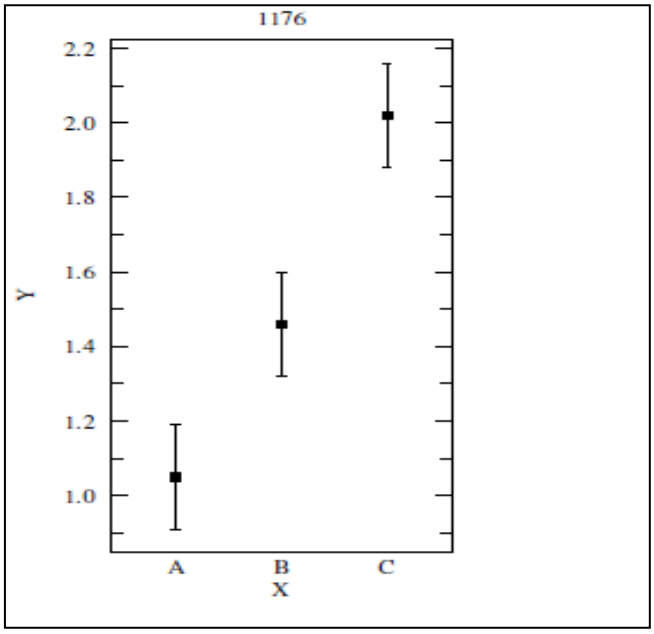
The reference range of triglycerides blood levels (fasting) is 0.28 – 2.3 mmol/L. This is based on the reference range that was used by the laboratory when the study samples were analysed.

The Table 4.5 below shows that at six months, the mean triglyceride level was 1.46 and a 12 month, the mean was 2.02. Both the results are below the upper limit value, of 2.3 mmol/L. This indicates that after 12 months of treatment, triglyceride levels in blood are not abnormally elevated ($p<0.0001$).

Table 4.5: Summary statistics for triglycerides at six month and 12 month intervals.

Biomarker	**Reference Range**	**Highest value***	**Lowest value****	**Mean Results at baseline N=92**	**Mean Results at 6 months N=92**	**Mean Results at 12 months N=92**
Triglyceride	0.28- 2.3 mmol/L	2.63	0.26	1.04	1.46	2.02

*of the collective data of 92 participants **of the collective data of 92 participants



Y axis: Triglycerides values

X axis:
A: Baseline

B: 6 months

C : 12 months

Figure 4.7: Comparisons of triglycerides mean values at baseline, six month and 12 month showing no significant changes.

There is a gradual increase (38.4%) noted, over 6 and 12 months as reflected in Figure 4.7. Factors, including change in the participants' diet, should be assessed as contributors to the gradual increase.

4.2.5 Comparison of amylase data; at baseline and six month interval and baseline and twelve month interval

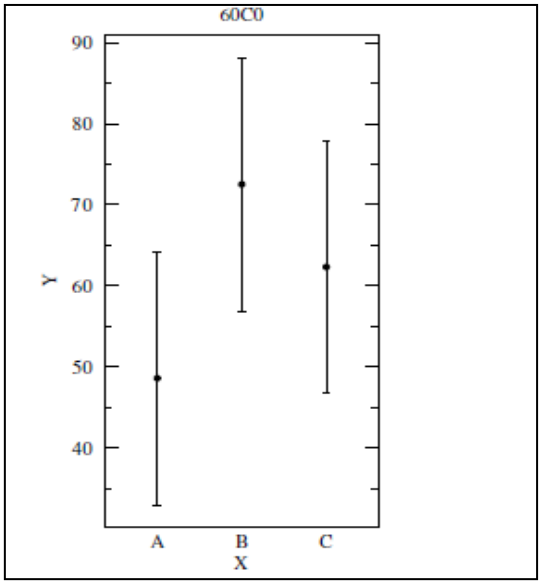
The upper limit of normal (ULN) of this enzyme is 110 U/L. This is based on the reference range that was used by the laboratory when the study samples were analysed.

The mean amylase level was 72.51 at the six month interval and 62.33 at the 12 month interval as shown in Table 4.6. Both the results are below the upper limit value of 110 U/L. This indicates that after 12 months of treatment, amylase levels in blood are not elevated (p=0.1).

Table 4.6 Summary statistics for amylase at 6 month and 12 month intervals.

Biomarker	Reference Range	Highest value*	Lowest value**	Mean Results at baseline N=92	Mean Results at 6 months N=92	Mean Results at 12 months N=92
Amylase	28-110 U/L	84.0	29.0	48.61	72.51	62.33

*of the collective data of 92 participants **of the collective data of 92 participants



Y axis: amylase levels

X axis:

A: Baseline

B: 6 months

C: 12 months

Figure 4.8: Comparisons of amylase mean at baseline, 6 month and 12 month time points showing no significant change.

At the six month interval the mean amylase level increased but there was a decrease at the 12 month interval, as shown in Figure 4.8. There is no pancreatic abnormality indicated after 12 months of treatment.

4.2.6 Comparison of lipase data; at baseline and six month interval and baseline and twelve month interval

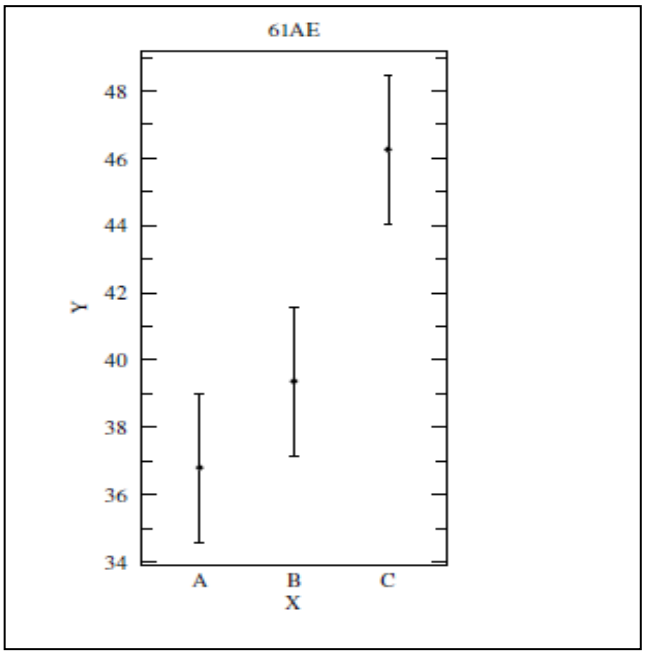
The upper limit of normal (ULN) of this enzyme is 60 U/L. This is based on the reference range that was used by the laboratory when the study samples were analysed.

The mean lipase level was 39.37 at the six month interval and 46.26 at the 12 month interval as shown in Table 4.7. Both the results are below the upper limit value of 60 U/L. This indicates that after 12 months of treatment, lipase levels in the blood are not elevated (p<0.0001).

Table 4.7: Summary statistics for lipase at six month and 12 month intervals.

Biomarker	**Reference Range**	**Highest value***	**Lowest value****	**Mean Results at baseline N=92**	**Mean Results at 6 months N=92**	**Mean Results at 12 months N=92**
Lipase	7-60 U/L	55.0	22.0	36.8	39.37	46.26

*of the collective data of 92 participants **of the collective data of 92 participants



Y axis: lipase levels

X axis:

A: Baseline

B: 6 months

C: 12 months

Figure 4.9: Comparisons of lipase mean at baseline, six month and 12 month showing no significance with timelines.

The mean lipase level shows a gradual increase from baseline to six month and 12 month intervals, as shown in Figure 4.9. But, there is still no pancreatic abnormality indicated after 12 months of treatment.

4.3 TOXICITY GRADING OF THE BIOMARKER LABORATORY RESULTS

Another objective was to assess the laboratory results that were higher than the stipulated reference range or upper level of normal (ULN) and determine whether they were clinically significant by using the established toxicity grading system (DAIDS, 2004). The biomarkers with results higher than the ULN were graded according to the Division of AIDS Table for Grading the severity of Adult and Paediatric Adverse Events (DAIDS AE Grading Table) (DAIDS, 2004). It grades the laboratory results from Grade 1, which is mild adverse events (AE) to Grade 4, which is potentially life-threatening.

4.3.1 ALT results grading

According to the DAIDS Table, the adverse event (AE) or toxicity grading of this enzyme is as follows: Grade 1 (mild AE) is 1.25 -2.5 X ULN, Grade 2 (moderate AE) is 2.6 – 5.0 X ULN, Grade 3 (severe AE) is 5.1 -10.0 X ULN and Grade 4 (potentially life-threatening AE) is >10.0 X ULN. This, in essence translates to Grade 1 as 56.25 – 112.5 U/L, Grade 2 is 117 -225 U/L, Grade 3 is 229.5 – 450 U/L and Grade 4 is > 450 U/L.

At six month interval, blood was collected from each participant for ALT analysis. Out of the 92 participants, ALT results of eight participants were higher than the ULN, which is 45 U/L; two were females and six were males as indicated in Table 4.8.

Table 4. 8: Table indicating the number of participants of different age groups with lower and higher than the upper limit of normal values of ALT at six month interval.

Gender			ALT at 6 months (U/L)		
			10 - 45	**> 45**	**Total**
Females	**Age**	20 - 29	5	0	5
		30 - 39	23	2	25
		40 - 49	25	0	25
		50 - 59	3	0	3
		60 - 69	1	0	1
		Total	**57**	**2**	**59**
Males	**Age**	20 - 29	2	1	3
		30 - 39	12	3	15
		40 - 49	10	2	12
		50 - 59	1	0	1
		60 - 69	2	0	2
		Total	**27**	**6**	**33**

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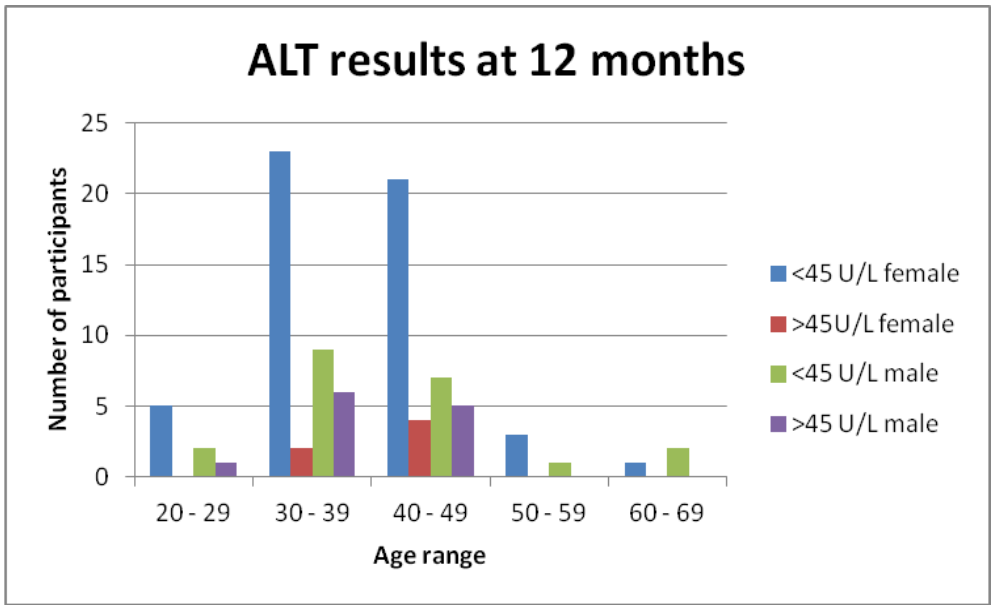

Figure 4. 10: The bar graph indicating the number of participants of different age groups with lower and higher than the upper limit of normal values of ALT at 12 month interval

At the 12 month interval, ALT was assessed again. Out of the 92 participants, 18 participants had ALT results that were higher than ULN as indicated in Figure 4.10. Six were females and twelve were males.

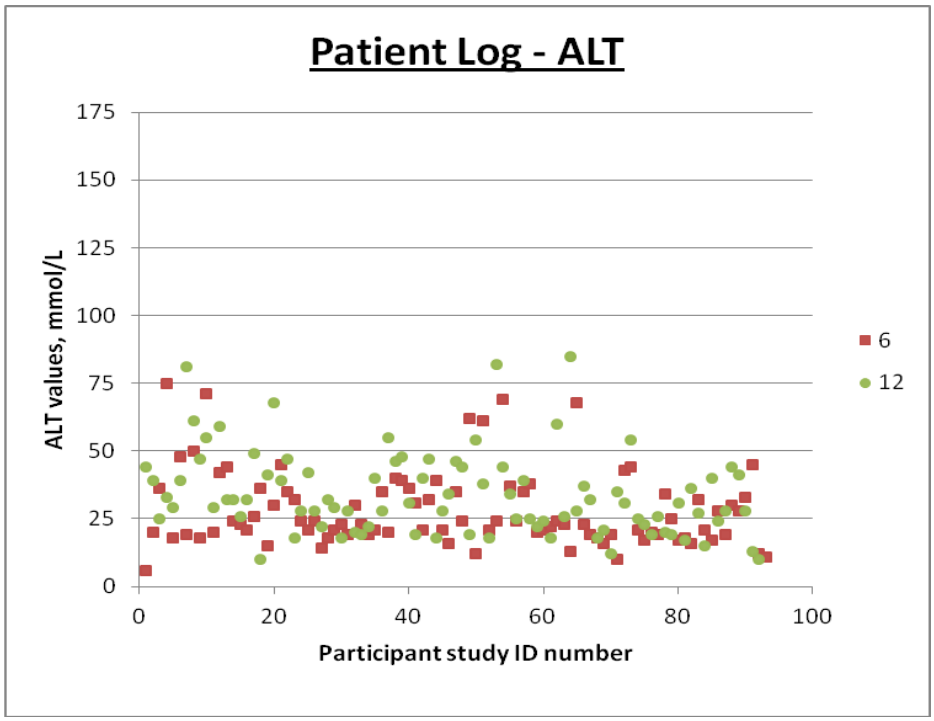

Figure 4.11: This scatter plot shows all 92 patients' ALT levels (absolute values) showing the different grades of toxicity at 6 months and 12 months of treatment

Out of the eight participants with higher than ULN results at the six months interval (Table 4.8), six participants, 6.5% had grade 1 toxicity which is mild, according to the DAIDS AE toxicity grading system. This grading is not classified as clinically significant change and is not regarded as life-threatening. The other two participants' results were below grade 1. The distribution of the results of the eight participants with grade 1 toxicity is indicated in Figure 4.11.

Out of the 18 participants with ALT results that are higher than ULN at 12 month interval, seven (7.6%) had grade 1 toxicity, which is mild. This was not clinically significant and not life-threatening. The other 11 participants had lower than grade 1 toxicity. The distribution of the results, identifying the grade 1 toxicity is shown in Figure 4.11. All results plotted between 56 and 112 (Y axis, range) are indicators of grade 1 toxicity.

4.3.2 AST results grading

The adverse event (AE) or toxicity grading of this enzyme is as follows: Grade 1 (mild AE) is 1.25 -2.5 X ULN, Grade 2 (moderate AE) is 2.6 – 5.0 X ULN, Grade 3 (severe AE) is 5.1 -10.0 X ULN and Grade 4 (potentially life-threatening AE) is >10.0 X ULN. This, in essence translates to Grade 1 as 50 – 100 IU/L, Grade 2 is 104 -200 IU/L, Grade 3 is 204 – 400 IU/L and Grade 4 is > 400 IU/L.

At six month interval, AST was assessed. Out of the 92 participants, 18 participants (19.6%) had AST results that were higher than ULN, which was 40 IU/L as indicated in the Table 4.9. Seven were females and eleven were males.

Table 4. 9: Table indicating the number of participants of different age groups with lower and higher than the upper limit of normal values of AST at the six month interval.

Gender			AST at 6 months (U/L)		
			5 - 40	**> 40**	**Total**
Females	**Age**	20 - 29	5	0	5
		30 - 39	21	4	25
		40 - 49	22	3	25
		50 - 59	3	0	3
		60 - 69	1	0	1
		Total	**52**	**7**	**59**
Males	**Age**	20 - 29	2	1	3
		30 - 39	10	5	15
		40 - 49	7	5	12
		50 - 59	1	0	1
		60 - 69	2	0	2
		Total	**22**	**11**	**33**

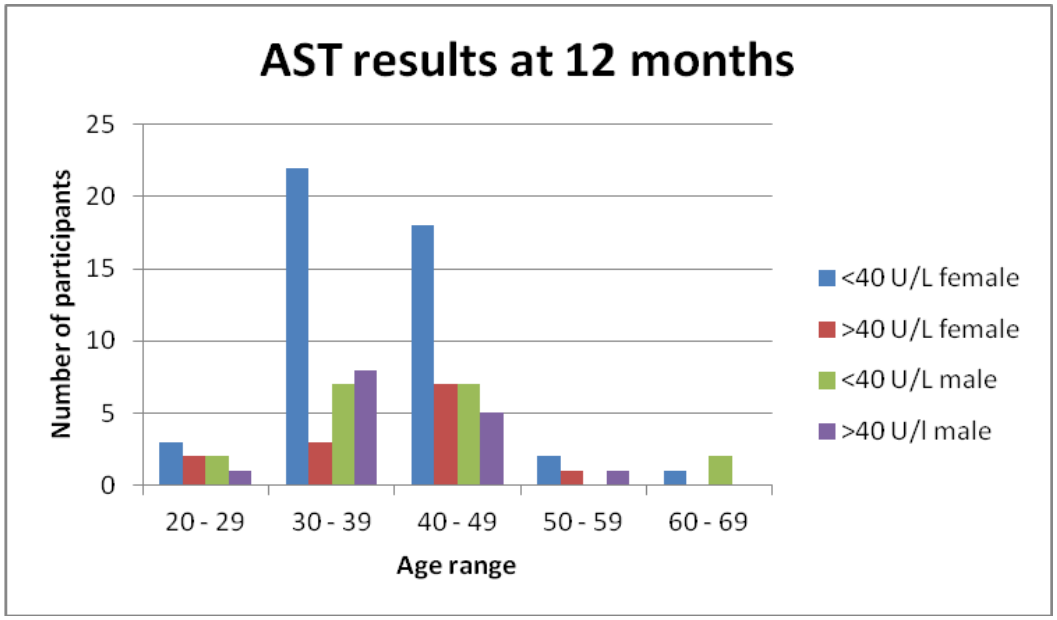

Figure 4.12: The bar graph indicating the number of participants of different age groups with lower and higher than the upper limit of normal values of AST at the 12 month interval.

At the 12 month interval, AST was assessed. Out of the 92 participants, 28 participants (30.4%) had AST results that were higher than ULN as indicated in Figure 4.12. Thirteen were females and 15 were males.

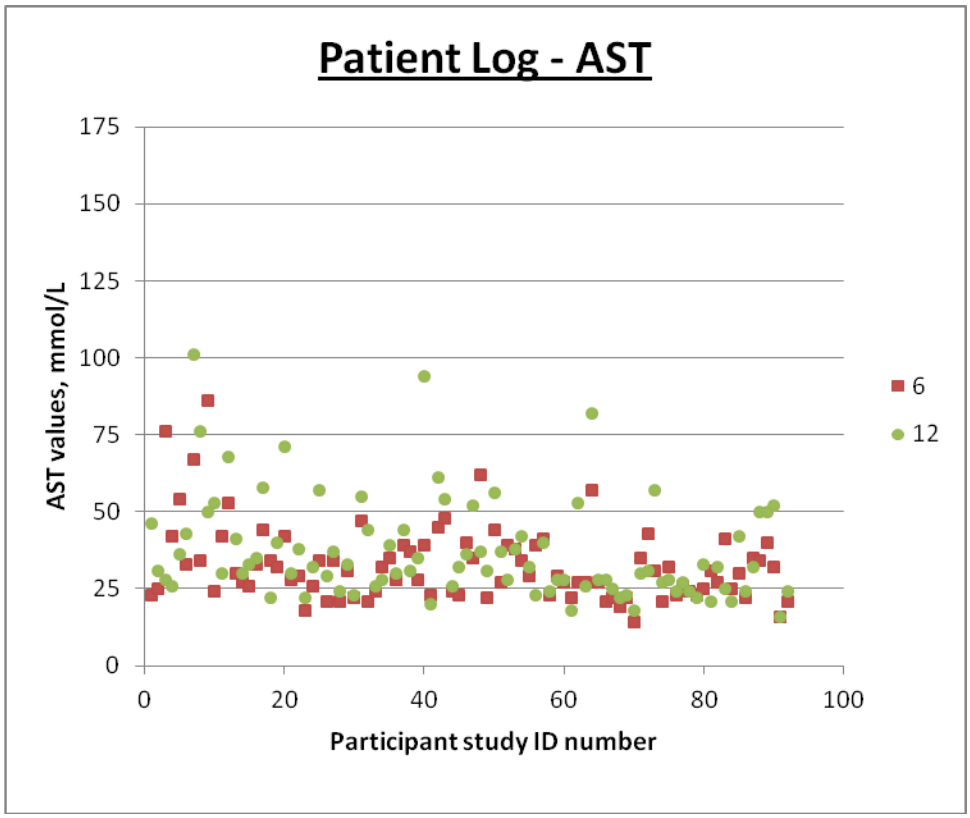

Figure 4.13: This scatter plot shows all 92 patients' AST levels (absolute values) showing the different grades of toxicity

Out of the 18 participants with AST results that are higher than ULN at the six month interval, seven, (7.6%) had grade 1 toxicity, which is mild. This was not clinically significant and not life-threatening. The other 11 participants had lower than grade 1 toxicity. The distribution of the participants indicating grade 1 toxicity is shown in Figure 4.13.

Out of the 28 participants with AST results that are higher than ULN at 12 month interval, 19 participants (20.7%) had grade 1 toxicity, which is mild. This was not clinically significant and not life-threatening. One participant, (1.1%) had grade 2 toxicity, which is moderate. This was also not clinically significant and not life-threatening. The other eight participants had lower than grade 1 toxicity. The distribution of the participants indicating toxicity is shown in Figure 4.13. The plots that indicate toxicity are those from 50 U/L and above.

4.3.3 Cholesterol results grading

The adverse event (AE) or toxicity grading of this enzyme is as follows: Grade 1 (mild AE) is 5.18 -6.19 mmol/L, Grade 2 (moderate AE) is 6.20 – 7.77 mmol/L and Grade 3 (severe AE) is > 7.77 mmol/L.

At the six month interval, three out of 92 participants, had cholesterol levels that were higher than ULN, which was 5.2 mmol/L as indicated in Table 4.10. There were two females and one male.

Table 4.10: Table indicating the number of participants of different age groups with lower and higher than the upper limit of normal values of cholesterol at the six month interval.

Gender			Cholesterol at the 6 month interval (mmol/L)		
			< 5.2	**> 5.2**	**Total**
Females	**Age**	20 - 29	5	0	5
		30 - 39	23	2	25
		40 - 49	25	0	25
		50 - 59	3	0	3
		60 - 69	1	0	1
		Total	**57**	**2**	**59**
Males	**Age**	20 - 29	3	0	3
		30 - 39	14	0	14
		40 - 49	13	0	13
		50 - 59	0	1	1
		60 - 69	2	0	2
		Total	**32**	**1**	**33**

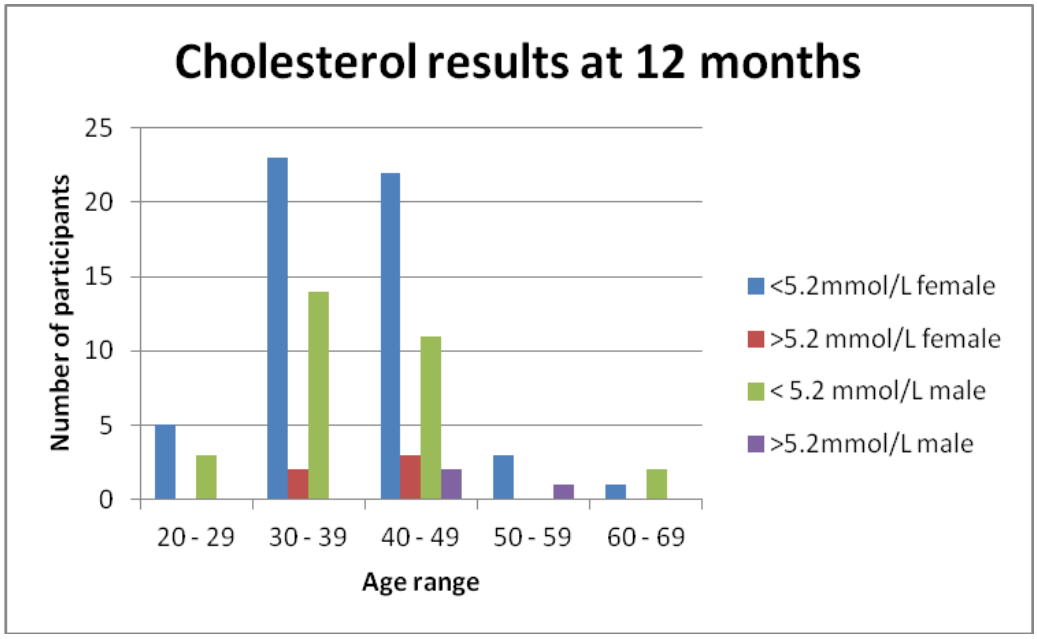

Figure 4.14: The bar graph indicating the number of participants of different age groups with lower and higher than the upper limit of normal values of cholesterol at 12 month interval.

At the 12 month interval, eight out of 92 participants, had cholesterol levels that were higher than ULN as indicated in Figure 4.14. Five were females and three were males.

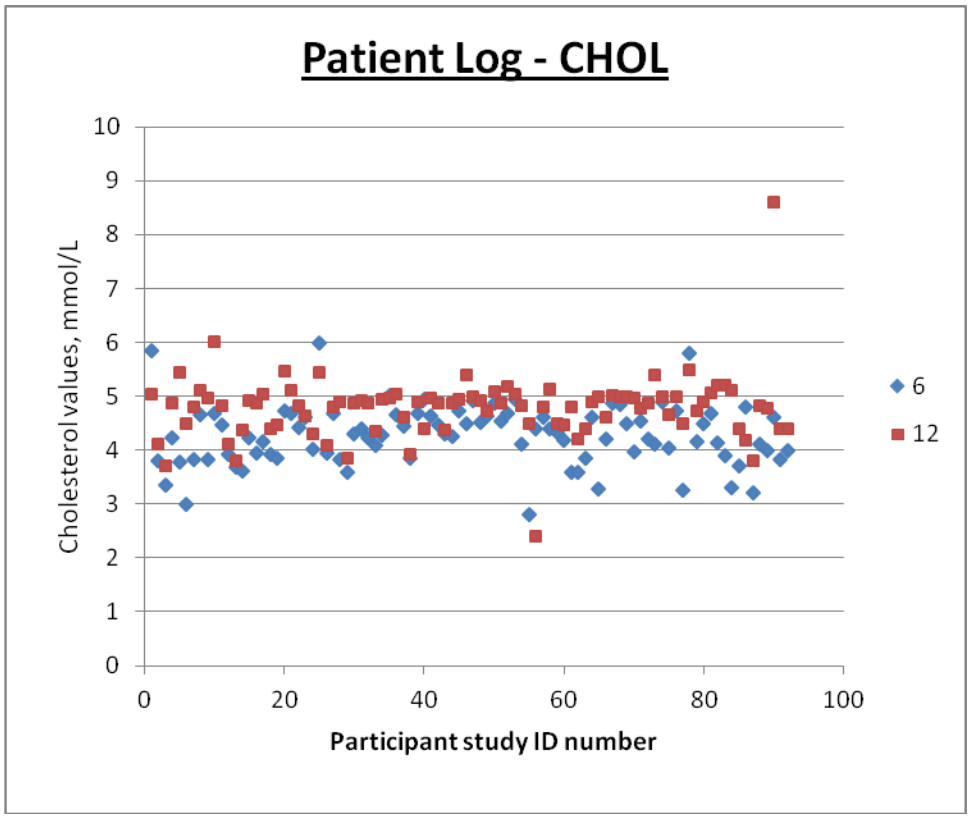

Figure 4.15: This scatter plot shows all 92 patients cholesterol levels (absolute values) showing the different grades of toxicity.

The three participants with higher than ULN results, had grade 1 toxicity, which is mild. This was not clinically significant and not life-threatening. The distribution of results is indicated in Figure 4.15.

At the 12 month interval, all eight participants with higher than ULN results, had grade 1 toxicity, which is mild but not life-threatening.The distribution of results is indicated in Figure 4.15.

4.3.4 Triglyceride results grading

The adverse event (AE) or toxicity grading of this enzyme is as follows: Grade 2 (moderate AE) is 5.65 – 8.48 mmol/L, Grade 3 (severe AE) is 8.49 – 13.56 mmol/L and Grade 4 is > 13.56 mmol/L.

At the six month interval, six out of 92 participants (one female and five males), had triglyceride levels that were higher than ULN, which was 2.3 mmol/L as indicated in Table 4.11.

Table 4.11: Table indicating the number of participants of different age groups with lower and higher than the upper limit of normal values of triglyceride at six month interval

Gender			Triglyceride at six month (mmol/L)		
			<60	**>60**	**Total**
Females	**Age**	20 - 29	4	1	5
		30 - 39	25	0	25
		40 - 49	25	0	25
		50 - 59	3	0	3
		60 - 69	1	0	1
		Total	**58**	**1**	**59**
Males	**Age**	20 - 29	3	0	3
		30 - 39	12	2	14
		40 - 49	11	2	13
		50 - 59	0	1	1
		60 - 69	2	0	2
		Total	**28**	**5**	**33**

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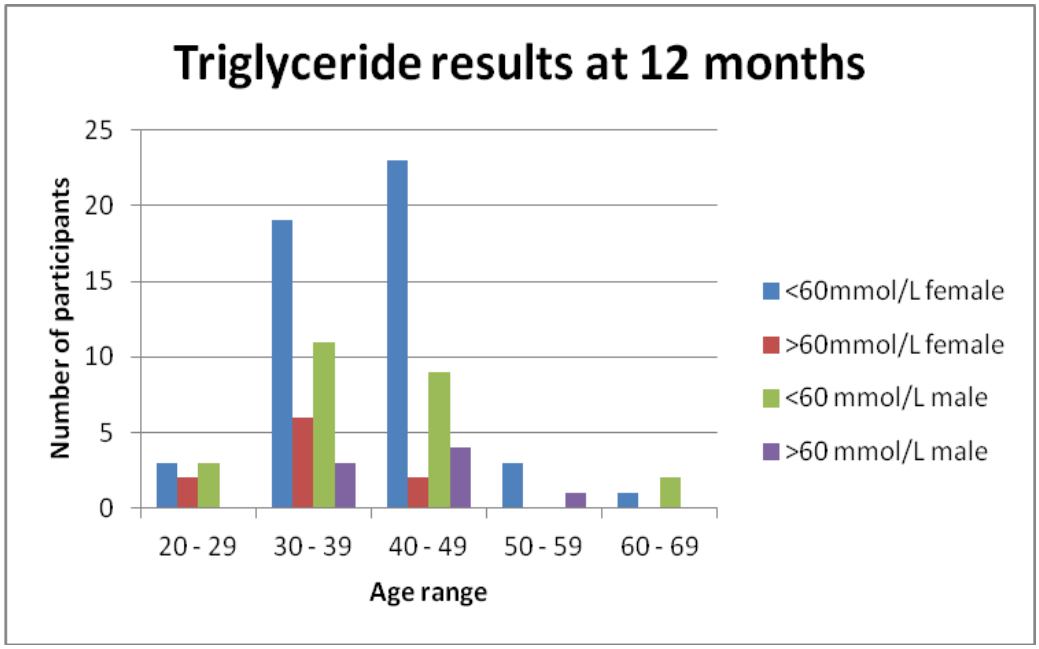


Figure 4.16: The bar graph indicating the number of participants of different age groups with lower and higher than the upper limit of normal values of triglyceride at 12 month interval.

At the 12 month interval, 18 out of 92 participants, had triglyceride levels that were higher than ULN, which was 2.3 mmol/L as indicated in Figure 4.16. Ten were females and eight were males.

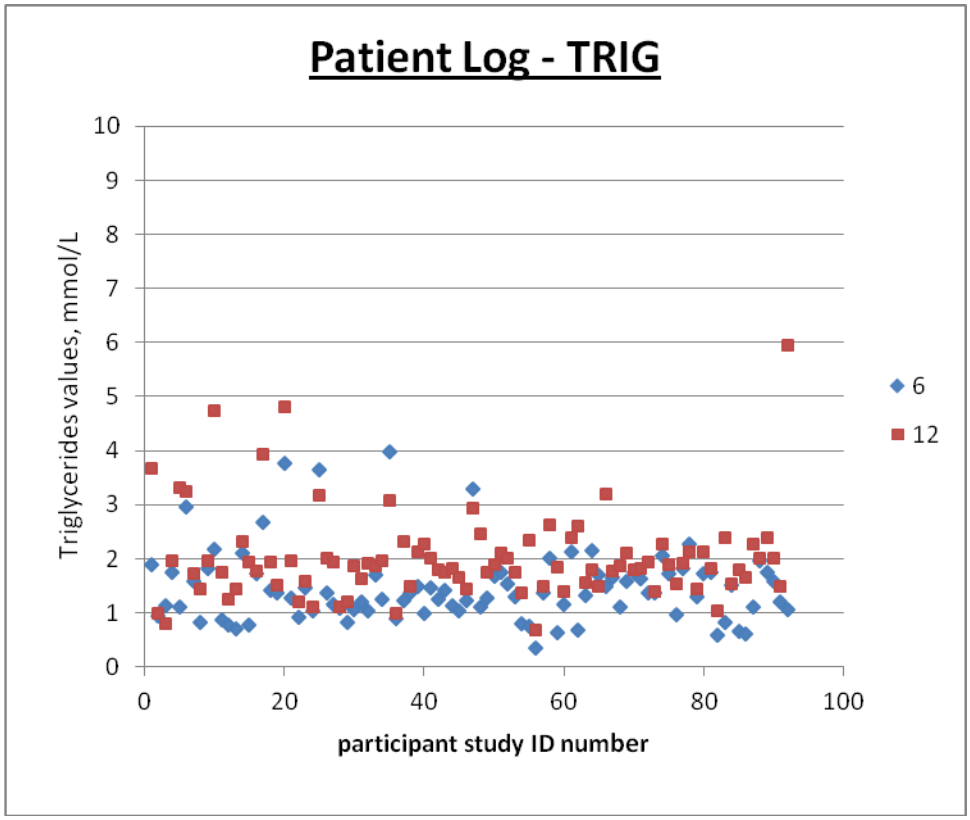


Figure 4.17: This scatter plot shows all 92 patients triglyceride levels (absolute values) showing the different grades of toxicity.

Two participants with greater than ULN of triglycerides at six months, had less than grade 2 toxicity, which is mild. The distribution of results can be seen in Figure 4.17. This was not clinically significant and not life-threatening. There is no grade 1 toxicity level for the triglyceride estimation.

One participant with greater than ULN of triglycerides at 12 months, had grade 2 toxicity, which is moderate AE. This is clinically significant but not life-threatening. The other 17 participants had less than grade 2 toxicity, which is not clinically significant. The grade 2 toxicity results are plotted from 5.65 mmol/L and above.

4.3.5 Amylase result grading

The adverse event (AE) or toxicity grading of this enzyme is as follows: Grade 1 (mild AE) is 1.1 -1.5 X ULN, Grade 2 (moderate AE) is 1.6 – 2.0 X ULN, Grade 3 (severe AE) is 2.1 -5.0 X ULN and Grade 4 (potentially life-threatening AE) is >5.0 X ULN. This translates to Grade 1 as 121 – 165 U/L, Grade 2 is 176 - 220 U/L, Grade 3 is 231 – 550 U/L and Grade 4 is > 550 U/L.

At six month interval, three out of 92 participants (two females and one male), had amylase levels that were higher than ULN, which was 110 U/L as indicated in Table 4.12.

Table 4.12: Table indicating the number of participants of different age groups with lower and higher than the upper limit of normal values of amylase at six month interval.

Gender			Amylase at six month interval (U/L)		
			28 - **110**	**>110**	**Total**
Females	**Age**	20 - 29	5	0	5
		30 - 39	24	1	25
		40 - 49	24	1	25
		50 - 59	3	0	3
		60 - 69	1	0	1
		Total	**57**	**2**	**59**
Males	**Age**	20 - 29	3	0	3
		30 - 39	13	1	14
		40 - 49	13	0	13
		50 - 59	1	0	1
		60 - 69	2	0	2
		Total	**32**	**1**	**33**

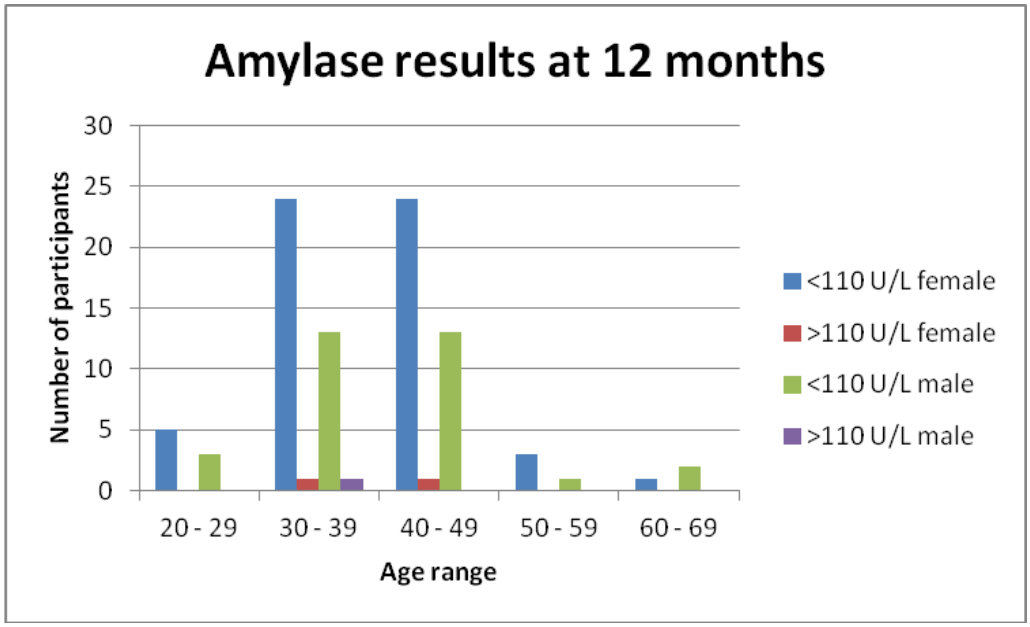


Figure 4.18: The bar graph indicating the number of participants of different age groups with lower and higher than the upper limit of normal values of amylase at 12 month interval.

At the 12 month interval, three out of 92 participants (two females and one male), had amylase levels that were higher than ULN, as indicated in Figure 4.18.

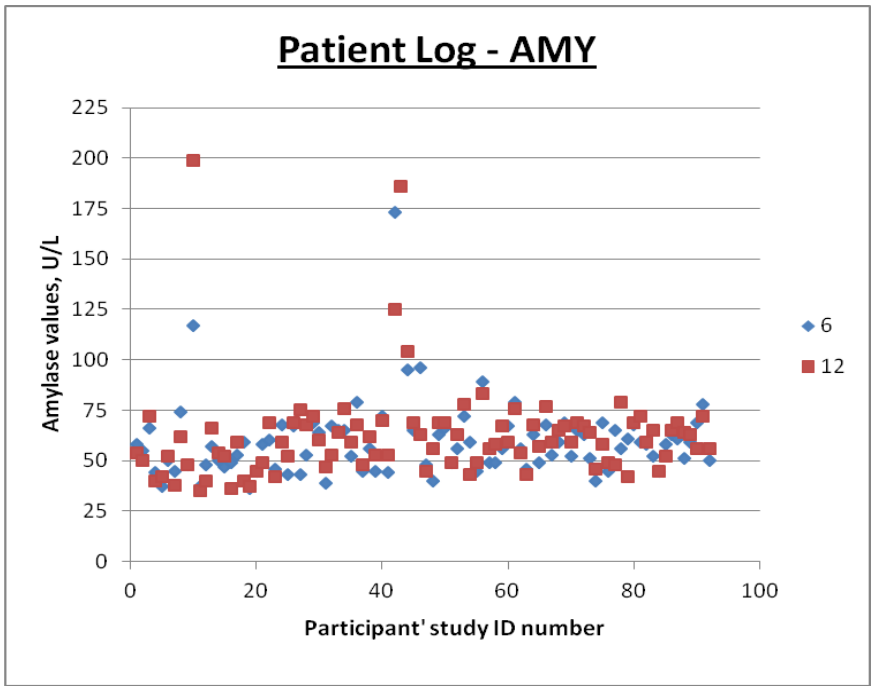


Figure 4.19: This scatter plot shows all 92 patients amylase levels (absolute values) showing the different grades of toxicity.

Out of the three participants with higher than ULN of amylase at six month interval, one had grade 2 toxicity, which is moderate AE and another one had grade 4 toxicity which is potentially life-threatening AE. The other one had less than grade 1 toxicity which is not clinically significant. This is shown in Figure 4.19.

Out of the three participants higher than ULN of amylase at 12 month interval, two had grade 2 toxicity, which is moderate AE and clinically significant. One had grade 1 toxicity, which is mild AE. This is shown in Figure 4.19.

4.3.6 Lipase result grading

The adverse event (AE) or toxicity grading of this enzyme is as follows: Grade 1 (mild AE) is 1.1 -1.5 X ULN, Grade 2 (moderate AE) is 1.6 – 3.0 X ULN, Grade 3 (severe AE) is 3.1 –

5.0 X ULN and Grade 4 (potentially life-threatening AE) is >5.0 X ULN. This translates to Grade 1 as 66 – 90 U/L, Grade 2 is 96 -180 U/L, Grade 3 is 186 – 300 U/L and Grade 4 is > 300 U/L.

At the six month interval, two out of 92 participants (one female and one male), had lipase levels that were higher than ULN, which was 60 U/L as indicated in Table 4.13.

Table 4.13: Table indicating the number of participants of different age groups with lower and higher than the upper limit of normal values of lipase at six month interval.

			Lipase at six month interval (U/L)		
Gender			**7-60**	**>60**	**Total**
Females	**Age**	20 - 29	5	0	5
		30 - 39	25	0	25
		40 - 49	24	1	25
		50 - 59	3	0	3
		60 - 69	1	0	1
		Total	**58**	**1**	**59**
Males	**Age**	20 - 29	3	0	3
		30 - 39	13	1	14
		40 - 49	13	0	13
		50 - 59	1	0	1
		60 - 69	2	0	2
		Total	**32**	**1**	**33**

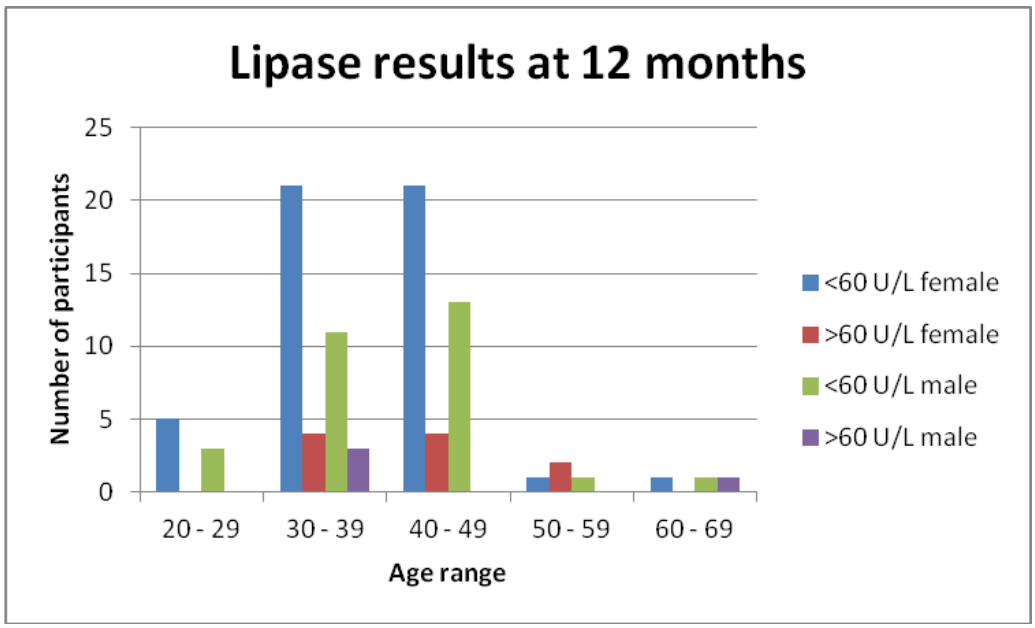


Figure 4.20: The bar graph indicating the number of participants of different age groups with lower and higher than the upper limit of normal values of lipase at 12 month interval.

At 12 month interval, 14 out of 92 participants (ten females and four males), had lipase levels that were higher than ULN, which was 60 U/L as indicated in Figure 4.20.

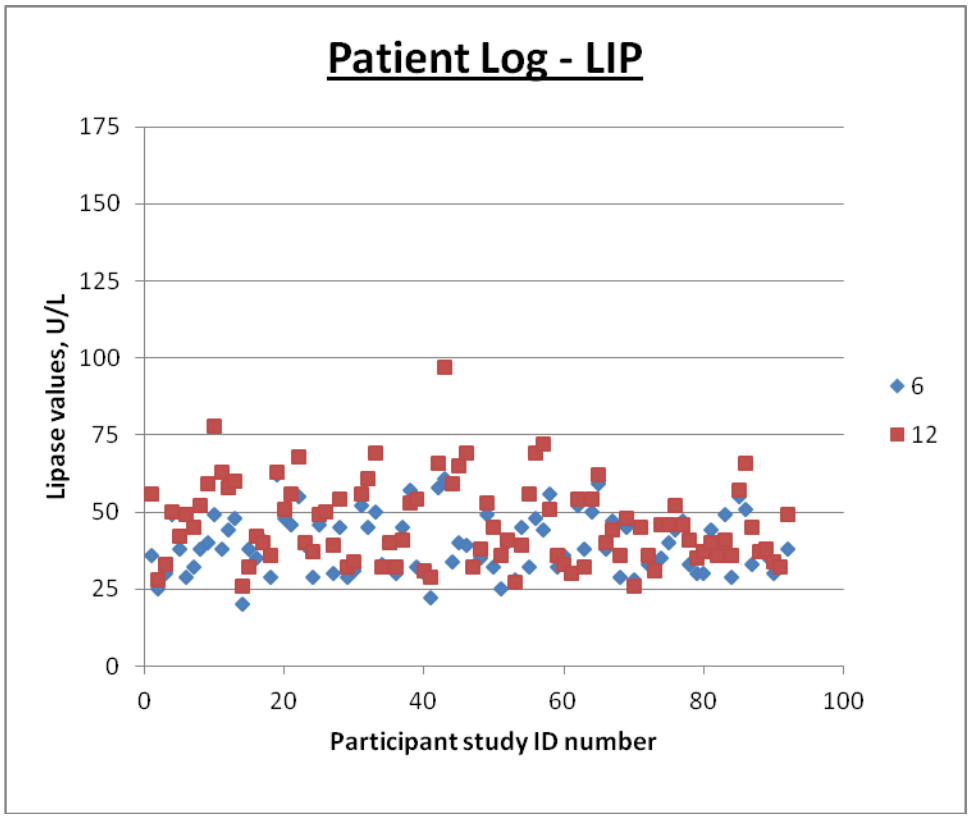


Figure 4.21 This scatter plot shows all 92 patients lipase levels (absolute values) showing the different grades of toxicity.

The two participants with higher than ULN results at six months, had less than grade 1 toxicity, which is not clinically significant and not life-threatening. The distribution of results is shown in Figure 4.21.

Out of the 14 participants with higher than ULN results at 12 months, one has grade 2 toxicity, which is moderate AE and clinically significant. The rest had grade 1 toxicity which is not clinically significant. The distribution of results is shown in Figure 4.21.

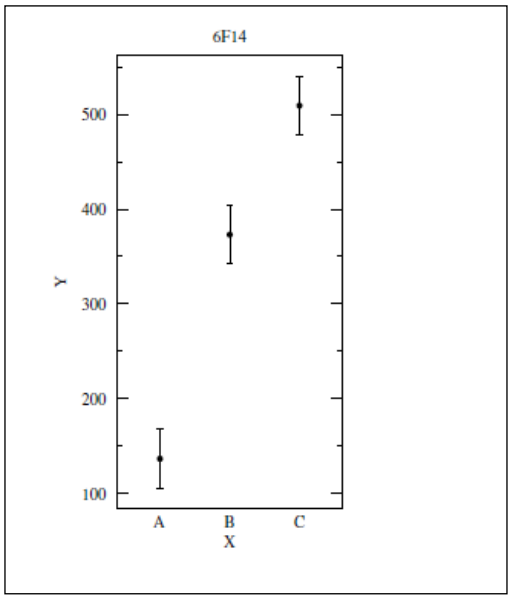
4.4 THE EFFECTS OF ART ON THE RECONSTITUTION OF THE IMMUNE SYSTEM

4.4.1 Viral load

The viral load for the 92 participants range from 4230 copies/ ml to 955 $X10^3$ copies/ml of blood at baseline. After six months and 12 months of treatment the viral load was below 40 copies/ ml of blood.

4.4.2 CD4 count

According to the 2004 South African National Antiretroviral Guidelines, patients are eligible for ART if the CD4 count is below 200 cells/mm^3. All 92 participants had a CD4 count below 200 at baseline. The mean at baseline was 136.4. at six months of treatment it increased to a mean of 373 and at 12 month evaluation, it was a mean of 509.5. The comparison of the mean values is indicated in Figure 4.22.



Y axis: CD4 count

X axis

A: baseline

B: 6 month

C: 12 month

Figure 4.22: Comparisons of CD4 count mean at baseline, six month and 12 month showing a significant increase.

CHAPTER FIVE DISCUSSION

The aim of the study was to investigate whether patients on antiretroviral therapy in Durban, KwaZulu- Natal developed metabolic side effects by performing laboratory biomarker tests. These side effects were not routinely monitored for, in the National Antiretroviral Treatment Guidelines of South Africa, 2003 and also in the 2010 Guidelines. The time of onset and the incidence of the side effects were investigated in the study population. The study included 92 participants who were already selected for the antiretroviral therapy programme according to the South African protocol. The participants were candidates for treatment regimen first-line (a) which is stavudine, lamivudine and efavirenz. All participants that enrolled for the study met the inclusion criteria. The blood samples were tested at different intervals; before initiation of antiretroviral drugs, six months and twelve months. Any significant change in the blood levels of the biomarkers was identified.

The alanine aminotransferase (ALT) is the biomarker for hepatic damage. An increase in the blood levels signifies hepatocyte damage, resulting in the release of this enzyme into the bloodstream. A clinically significant change for ALT is a three-fold and above increase; using 45 U/L as the upper level of normal (ULN) limit. According to the toxicity grading table, a three-fold increase is interpreted as Grade 2 toxicity of moderate adverse event. After 12 months of treatment, 7.6% (seven) participants had Grade 1 toxicity and no participants showed grade 2 and above toxicity. These results support the findings of a study carried out in Uganda by Kalyesubula, *et al.* (2011), where Grade 2 and above hepatotoxicity incidence on ART patients was low (4.2%). Another study in Botswana by Bussman *et al.* (2008) found a low incidence (1.1%) of hepatotoxicity after 6 months of treatment.

The aspartate amonitransferase (AST) is another biomarker for hepatic damage. A clinically significant change is a three-fold and above increase; using 40 U/L as the upper level of normal limit. According to the toxicity grading table, a three-fold increase is interpreted as Grade 2 toxicity of moderate adverse event. After 12 months of treatment, 1.1% of participants had

grade 2 toxicity and 20.7% of participants had grade 1 toxicity. In a study conducted in Uganda by Ocama *et al.* (2010), a low incidence (1.5%) of Grade 3 AST elevation after 36 months of treatment was found. This confirms that after 12 months of treatment, there is low or no Grade 2 toxicity when assessing the AST.

The risk of hepatic damage in patients who are on antiretroviral drugs is believed to be increased in patients with underlying chronic viral hepatitis (Reisler *et al.;* 2003). Alcohol is another factor that was identified in studies investigated by Nunez *et al.* (2001), Martin-Carbonero *et al.* (2003) and Ugiagbe *et al.* (2011) that puts ART patients at risk of developing hepatotoxicity. In the absence of the two major predisposing factors, hepatotoxicity incidences would be low.

Cholesterol is an important fat in the body, but blood levels are also regarded as a biomarker for dyslipidaemia which predisposes a person to atherosclerotic vascular disease and subsequently cardiovascular disease. The ULN of fasting cholesterol levels in blood is 5.2 mmol/L and the clinically significant change for cholesterol is a blood level that is greater than 6.19 mmol/L. After 12 months of treatment, 8.7% (eight) of participants had fasting cholesterol blood levels that were higher than ULN but lower than 6.19 mmol/L. No participants had blood levels that were greater than 6.19 mmol/L.

Hypercholesterolaemia is associated with protease inhibitor ARVs (Fontas *et al.*, 2004). In a report by George (2006), the prevalence of hypercholesterolaemia in patients receiving protease inhibitor ARV ranges from 30% to 80%, but other factors like age and lipodystrophy also contribute to this metabolic side effect. Hypercholesterolaemia was seen after 200 weeks of therapy in a study conducted by Nuesch *et al.* (2006) in Thailand. This increase in blood cholesterol levels was associated with ageing of the patients and the protease inhibitor drug, indinavir.

It is important to remember that cholesterol levels may be increased by excessive consumption of foods that are high in cholesterol, glucose, trans fats and saturated fats. But the development of

hypercholesterolaemia toxicity may take years to develop. My study population did not use any PI based regimen and were followed for a maximum of 12 months (52 weeks). So this may explain the absence of hypercholesterolaemia toxicity.

Triglycerides are the chemical forms of fats and are also regarded as biomarkers for dyslipidaemia. High levels of triglyceride in the blood predispose a person to coronary heart diseases. The ULN of fasting triglyceride blood levels is 2.3 mmol/L and a clinically significant change is a blood level that is greater than 5.65 mmol/L. According to the toxicity grading table, this is interpreted as a grade 2 and above toxicity. After 12 months of treatment, 1.1% of participants had grade 2 toxicity. Triglycerides levels change dramatically in response to meals. They may increase as much as five to ten times after eating. Triglycerides are associated with starchy and other high carbohydrate foods. When assessing blood levels for determining toxicity, it is important that patients fast; minimum of nine hours without food. All participants in the study were advised about the fasting but it may be difficult to validate that all triglycerides assessments were done on fasting blood.

Dyslipidaemia is defined as an abnormal amount of lipids in the blood, particularly elevation of levels (hyperlipidaemia). The lipid profile includes cholesterol and triglyceride measurements. Hyperlipidaemia is usually associated with cardiovascular disease, as the lipids may accumulate in the blood vessels, causing the narrowing and eventually blockage of blood supply to a number of organs. There are other factors that may increase the risk of cardiovascular diseases, but the study only focused on the levels of the two lipids. According to previous studies (Ramezani *et al.*, 2007, Nuesch *et al.*, 2006 and Murphy *et al.*, 2007) hyperlipidaemia toxicity is predominantly found in patients who are on PI based ART. In my study population, there was elevation of cholesterol levels and triglycerides, but did not reach the toxicity levels, except one patient with grade 2 triglyceride toxicity.

Amylase is the enzyme that is found within the pancreas and an increase in blood levels signifies pancreatic damage. A clinically significant change for amylase is a two-fold and above increase

from the ULN, which is 110 U/L. According to the grading toxicity table (Table 2.14), this translates to Grade 3 and above toxicity. After six months of treatment, 1.1% of participants had grade 4 toxicity. After 12 months of treatment, 2,2% of participants had Grade 2 toxicity and none had grade 3 or above toxicity.

There was history of alcohol abuse in the patient who had grade 4 toxicity at six months of treatment. It is believed that it may be a contributing factor.

Lipase is another enzyme that is found in the pancreas and its increase in the blood also signifies pancreatic damage. A clinically significant change for lipase is a two-fold and above increase from the ULN which is 60 U/L. According to the grading toxicity table, this translates to Grade 2 and above toxicity. After 12 months of treatment, 1.1% of participants had grade 2 toxicity.

Pancreatitis is described as inflammation of the pancreatic cells. Because of the inflammation, digestive enzymes that are kept within these cells, leak out through the highly permeable pancreatic cells to the bloodstream. Since the enzymes are catabolic, they may digest body cellular structures, resulting in life-threatening complications. In my study population, the incidence of pancreatic toxicity was low. This supports the findings of other studies. An earlier study by Reisler *et al.* (2005) involved 8 451 participants. Out of 6 287 participants, 217 showed increased levels of amylase and/or lipase. These participants were on various ARV combinations. The pancreatic incidences were higher in stavudine and didanosine based regimen. But, the overall pancreatic incidences were reported as low. Another study by Smith *et al.* (2008) also revealed low pancreatic incidences in patients on ARV. There were 43 pancreatic events in 9 648 participants. Another study conducted in Uganda (Forna *et al.,* 2007) on patients who were on the same regimen as my study found that there were < 0.5% pancreatitis incidences in the study population of 1 029. This population was monitored for 18 months.

The nutritional status of my study population was assumed to be higher than satisfactory, this was based on the assessment of the questionnaire that was done by the participants. All participants had a combined gross household income of more than R10 000 per month. Based on

this finding, it was fair to assume that the participants were able to afford food and maintain an acceptable nutritional status. Balanced diet is an important part of ART management and patients that enroll for ART are usually advised on diet. The nutritional assessment was meant to be supported by pre-albumin blood levels analysis. Unfortunately this was unsuccessful due to discontinuation of this test in the lab where all the tests for this study were performed.

Antiretroviral therapy has undoubtedly improved the lives of HIV infected individuals by decreasing the mortality rate due to HIV associated infections and has improved the life expectancy of HIV infected people as indicated by The Antiretroviral Therapy Cohort Collaboration study (2008). The treatment has successfully decreased the viral load to undetectable levels; less than 40 viral copies/ml of blood in all my study participants within six months of treatment. The treatment has also allowed reconstitution of the immune system, which is seen by an increase in the CD4+ cell count. For my study group, there was an increase in the CD4 mean cell count; at baseline it was 136.4, at six month interval it was 373 and at 12 month interval it was 509.5. By maintaining these standards, the participants can prolong their life span. Since HIV treatment is life-long, patients may experience side effects to these drugs, ranging from minor to life-threatening complications. The life-threatening complications become an obstacle in the successful management of ART. This was the finding of a study by Castelnuovo *et al.* (2011), where drug toxicity was the major cause for treatment changes and interruptions. Therefore, it becomes very important that patients are monitored closely for any indications of the development of life-threatening conditions. This is done by laboratory assessment of the biomarkers for these life-threatening conditions.

LIMITATIONS OF THE STUDY

The study was conducted in one HIV clinic and the participants were mainly from the middle socio-economic group, and can afford to support the treatment with necessary diet. This could have been a contributing factor towards their tolerance of the drugs, as compared to low socio-economic group. Hence the side effects noted did not exceed Grade 2 DAIDS grading, indicating

that there were no serious harmful drug toxicities noted in our patient group. Another limitation is that the participants were only from the black ethnic group and the study focused at one particular clinic. This limitation of the study does not allow the findings to be generalised to all clinics that do not have the same demographic profile.

The participants were followed for 12 months only on the advice of the biostatistician, because loss to follow-up rates are high on long term studies . There is evidence in literature that patients may develop significant toxicity after a minimum of 18 months on uninterrupted treatment (Nuesch *et al*., 2006).

CHAPTER SIX CONCLUSION

In this study, it was found that the incidences of hepatotoxicity, hyperlipidaemia and pancreatitis after 12 months of treatment were low. There was a gradual increase in the levels of hepatotoxicity biomarkers, which indicates that toxicity may develop after a long duration on treatment.

Regular laboratory testing may prove to be expensive, especially in South Africa as the resources may be limited. Since this study has found that the incidences of the toxicities after 12 months of treatment are low, regular testing of patients may not be necessary up to this time point. It is recommended that guidelines for patients that have been on the ART for longer durations should be revised to include scheduled annual toxicity screening assessments, irrespective of the type of antiretroviral drug that the patient is taking. Currently, the 2010 South African National Guidelines prescribe that laboratory testing for toxicity is performed when a patient presents with signs and symptoms of a particular side effect and it also depends on the type of antiretroviral drug that the patient is taking.

It is also recommended that early clinical indications should be closely monitored by the patient and all the health workers involved. This requires vigilance and proper training for the health workers. If there are indications in the early stages of treatment, appropriate laboratory assessments should be conducted and appropriate action taken, so as to save the lives of patients, make the patients comfortable with this lifelong treatment and avoid unnecessary discontinuation of treatment.

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APPENDIX 1

Biomedical Technology Department, M L Sultan Campus

To: Doctor in charge
Umlazi Medical Centre, V section

Dear Sir / Madam

RE: Application for permission to conduct research at your hospital/clinic.

I, Thandie Sylph Ndlovu, am a registered for a Master's degree in the category of Biomedical Technology at the Durban University of Technology. The title of my study is *An investigation of the metabolic side effects of antiretroviral therapy in HIV positive patients in KwaZulu Natal* .

I intend to conduct research on patients who are on antiretroviral drugs (ARV). The focus of my research is to investigate the development of any side effects of antiretroviral drugs (ARV)on the patients who are currently taking antiretroviral treatment in your clinic/ unit/ establishment. I intend to detect the side effects by performing certain laboratory tests on the blood specimens that are collected from patients during the routine ARV monitoring. All tests will be performed at an accredited laboratory and the patients will not pay for these tests. 125 patients are needed for my study and the tests will be performed at baseline, 3 months and 6 months interval as per the routine ARV monitoring. Medical records and related previous tests of the patients may also be reviewed.

I am aware of and will abide with the ethical obligations related to my research. Patients will not be subjected to any additional procedure or testing or any unnecessary trauma. All results will be communicated to the respective clinics. The results will be confidential and the details of all patients will not be disclosed to any other person than the principal investigator. All patients will be given confidential reference number or code so that they are not identified by their names. I have attached the proposal for my study.

Awaiting your favourable response.

Yours sincerely

T. S. Ndlovu (Miss)
Principal investigator
031 3735298

Dr Lorna Madurai
Supervisor
031 4626691

Mrs P Pillay
Supervisor
031 3735423

APPENDIX 2

UMLAZI MEDICAL CENTRE
V SECTION

DATE: 28 August 2008

To: Ms Thandie S Ndlovu

We hereby grant permission to Ms Thandie Ndlovu to use our clinic for purposes for your M Tech Degree.
Names of patients will not be disclosed to you to maintain patient confidentiality

Yours sincerely

[redacted]

Dr Aboobaker

APPENDIX 3

GLOBAL CLINICAL VIRAL LABORATORY

11 Dan Pienaar Drive
Amanzimtoti
4126
P.O Box 13026,
Jacobs 4026

PR NO: 0071609 CK NO: 2002/009697/23
INNOVATION THROUGH COMMITMENT

Tel: (+27 31) 9040500
Results:(+27 31) 9040520
Fax:(+27 31) 9040526
Logistics:(+27 31) 9040524/7/9
Insurance:(+27 31) 9040551
Emergency No's:
082 333 8230/ 083 412 0932

DATE: 28 August 2008

To: Ms Thandie S Ndlovu

We hereby grant permission to Ms Thandie Ndlovu to use our laboratory for purposes for your M Tech Degree.

Names of patients will not be disclosed to you to maintain patient confidentiality

Yours sincerely

[redacted]

D.NOWRUNGSAH

APPENDIX 4(A)

DEPARTMENT OF BIOMEDICAL TECHNOLOGY
(Global Clinical and Viral Laboratory)

PARTICIPANT'S INFORMATION LETTER

Dear Participant,

My name is Thandie Ndlovu and I wish to inform you of a study that I am embarking on towards my Master's degree in Technology. I am registered as a student at the Durban University of Technology (DUT).

The title of the study is **"An investigation of the metabolic side effects of antiretroviral therapy in HIV positive patients in KwaZulu-Natal"**

The focus of this study is to improve the monitoring of patients like yourself that are taking antiretroviral drugs. This study is aimed at identifying side effects such as example liver disease and others of the antiretroviral drugs. Monitoring of the side effects will be achieved by performing laboratory tests on patients' blood samples over a period of 6 months. The laboratory testing will be done on the specimens that are drawn from you routinely when you come for your scheduled follow up visits. The testing will be done at three and six month intervals. All the laboratory results from this study will be communicated back to your doctor. No additional blood samples will be drawn. There will be no charges for these tests. Before the study commences a questionnaire will be administered. Your medical records relevant to this study may also be reviewed.

The results of all the laboratory tests and your details will not be disclosed to any other person other than the principal investigator. You will be given a confidential reference number or code so that you cannot be identified by your name.

Participating in this study could benefit you directly since there may be more vigilant monitoring of your health with regard to the side effects. Also there is potential benefit to other patients' antiretroviral drugs.

You are welcome to contact me, the principal investigator, at anytime to discuss any issues that may be of concern to you. I thank you for your time and consideration.
If you require further information or details about this study you can contact any of the following people :

Miss Thandie Ndlovu	Dr Lorna Madurai	Mrs Pavitra Pillay
Principal investigator	Supervisor	Supervisor
Tel : 031 3735298	031 4626691	0313735423

Thank you for your participation

APPENDIX 4(B)

DEAPRTMENT OF BIOMEDICAL TECHNOLOGY
(Global Clinical and Viral Laboratory)

ULWAZI NGOCWANINGO

Siguli esithandekayo

Ngifisa ukukwazisa ngocwaningo oluzokwenziwa umfundi wase Durban University of Technology (DUT) ukuze azuze iziqu eziphakeme zemfundo.

Isihloko socwaningo ukuhlolwa imiphumela engathandeki yemishanguzo kubantu abanegciwane lengculazi abadla imishanguzo ekwaZulu Natal.

Lolucwaningo luzobheka iziguli ezidla imishanguzo kuze kube izinyanga eziyisithupha
Ukuthi aziqalwa yini imiphumela engathandeki yemishanguzo ngokwenza amatest elaboratory. Kuzosetshenziwa wona lawamagazi athathwa udokotela uma uze kuyena. Awekho amanye azokhishwa kuwena. Ukwenziwa kwalamatest engeziwe kuzokhokhelwa umcwaningi omkhulu. Imininingwane yakho yeziguli namanye neminye imiphumela yokuhlolwa akho amadala angase asetshenziswe.

Imiphumela yakho yamagazi nemininingwane yakho angeke idalulwe kumuntu ngaphandle komxwaningi omkhulu. Uzonikwa inombolo noma ikhodi eyimfihlo oyokwaziwa ngayo, hhayi igama lakho.

Ungangithinta mina, umcwaningi omkhulu, noma yinini uma ufuna ukucaciselwa nganoma yiluphi udaba oukuhluphayo.
Uma udinga olunye ulwazi noma incazelo ngalolucwaningo, ungathintana nomunye walaba abalandelayo :

Miss Thandie Ndlovu	Dr Lorna Madurai	Mrs Pavitra Pillay
Umcwaningi omkhulu	Umlawuli	Umlawuli
Ucingo : 031 3735298	031 4626691	031 3735423

Siyabonga ngokuzimbandakanya kwakho

APPENDIX 5 (A)

DEPARTMENT OF BIOMEDICAL TECHNOLOGY
(Global Clinical and Viral Laboratory)

INFORMED CONSENT

STUDY TITLE: AN INVESTIGATION OF THE METABOLIC SIDE EFFECTS OF ANTIRETROVIRAL THERAPY IN HIV INFECTED INDIVIDUALS IN KWAZULU NATAL

I ---------- (full name) have understood the details provided by the Doctor/ Nurse/Investigator about my participation in this study. I agree that the blood drawn for my routine laboratory testing be used for research purposes. I am aware that my details and laboratory results will be kept STRICTLY CONFIDENTIAL.

I agree that my previous results be used. Yes ☐ No ☐

I am willing to allow access to

all my medical records Yes ☐ No ☐

Under these conditions, I am willing to participate in this study.

---------- ----------
Signature of Patient Witness

Date: ---------- Date: ----------

Signature of Clinician/Nurse

Date: ----------

Please note that you are free to withdraw from the study at any time should you wish to.
If you require further information or details about this study, you can contact the following person:

Miss Thandie Ndlovu	Dr Lorna Madurai	Mrs Pavitra Pillay
Principal Investigator	Supervisor	Supervisor
Tel: 031 3735298	031 462 6691	031 373 5423

Thank you for your participation

APPENDIX 5(B)

DEPARTMENT OF BIOMEDICAL TECHNOLOGY
(Global Clinical and Viral Laboratory)

IMVUME ENOKUQONDA

ISIHLOKO SOCWANINGO : UKUHLOLWA IMPHUMELA ENGATHANDEKI YEMISHANGUZO KUZIGULI EZINENGCULAZI KWAZULU NATAL

Mina ---------- (igama eliphelele) ngiyayiqonda imininingwane engiyinikwe uDokotela/ uNesi/uMcwaningi ngokuzimbandakanya nalolucwaningo. Ngiyavuma ukuthi igazi elithathelwe ukuhlolwa okujwayelekile lisetshenziswe kulolucwaningo. Ngiyazi ukuthi imininingwane yami nemiphumela yamagazi izogcinwa IYIMFIHLO.

Ngiyavuma ukuthi imiphumela yami emidala

isetshenziswe. Yebo ☐ Cha ☐

Ngizimisele ukuvumela ukusetshenziswa

kwemininingwane yeziguli yakho Yebo ☐ Cha ☐

Ngaphansi kwalemigomo, ngizimisele ukumbandakanywa kulolucwaningo.

---------- ----------
Ukusayina kwesiguli Ufakazi

Usuku: ---------- Usuku: ----------

Ukusayina kukaDokotela/ uNesi

Usuku: ----------

Uma udinga olunye ulwazi noma incazelo ngalolucwaningo, ungathintana nalona olandelayo:

Miss Thandie Ndlovu	Dr Lorna Madurai	Mrs Pavitra Pillay
Umcwaningi omkhulu	Umphathi	Umphathi
Tel: 031 3735298	031 462 6691	031 3735423

Siyabonga ngokuzimbandakanya kwakho

APPENDIX 6

DATA COLLECTION QUESTIONNAIRE

To be filled by the investigator

Patient reference number ----------
Date ----------
ARV regimen ----------

For statistical purposes only
To be filled by the patient

PERSONAL DETAILS

Where do you live ? ----------

Race Black ☐ White ☐ Indian ☐ Coloured ☐

Date of birth (day/month/year) ☐ ☐ ☐

Gender Female ☐ Male ☐

If female, how many children do you have? ----------

Do you smoke ? Yes ☐ No ☐

Do you drink liquor ? Yes ☐ No ☐

Are you employed ? Yes ☐ No ☐

If yes, what is your income? ----------

How many members are employed in your family? ----------

ANTIRETROVIRAL THERAPY INFORMATION

Are you HIV infected? Yes ☐ No ☐

Which year were you diagnosed with HIV? ----------

Have you had a CD4 test done previously? Yes ☐ No ☐

If yes, what was the value? ----------

Have you had a viral load test done previously? Yes ☐ No ☐

If yes, what was the value? ----------

Are you taking any antiretroviral treatment? Yes ☐ No ☐

When did you start taking antiretroviral treatment? ----------

Are you taking any other treatment for HIV? Yes ☐ No ☐

If yes, specify. ----------

Did the nurse/ doctor/ anyone explain

about the treatment and its side effects? Yes ☐ No ☐

If yes, what were you told? ----------

Have you had any side effects since on ARV Yes ☐ No ☐

Are you taking your medication everyday? Yes ☐ No ☐

If No, why not? ----------

Are you on adequate dietary supplements? Yes ☐ No ☐

OTHER ILLNESSES

Do you have TB? Yes ☐ No ☐

Have you been tested for TB? Yes ☐ No ☐

If yes, when were you tested? ----------

What were the results? ----------

Do you suffer from any other illnesses? Yes ☐ No ☐

If yes, specify. ----------

Are you on treatment for your illness/es? ----------

Are you on any other treatment? Yes ☐ No ☐

If yes, specify. ----------

Are you willing to allow the study team to use

your blood for research purposes? Yes ☐ No ☐

Do you want to ask any questions? Yes ☐ No ☐

Questionnaire completed by:

Full name ----------

Designation ----------

Signature ----------

Your details will be kept strictly confidential. Your name does not appear on this form.

Thank you for your participation

APPENDIX 7

DEPARTMENT OF BIOMEDICAL TECHNOLOGY
(Global Clinical and Viral Laboratory)

STUDY TITLE: AN INVESTIGATION OF METABOLIC SIDE EFFECTS OF ANTIRETROVIRAL THERAPY USING LABORATORY BIOMARKERS IN HIV INFECTED INDIVIDUALS

Investigator : T S Ndlovu

LABORATORY REQUEST FORM

ARV SITE			
PATIENT'S REFERENCE NUMBER			
DATE OF SPECIMEN COLLECTION			
TESTING INTERVAL	Baseline	6-month	12-month

BIOCHEMISTRY TESTS to be performed (plain tube)

Name of test	
ALT	**X**
AST	**X**
LIPASE	**X**
CHOLESTEROL	**X**
TRIGLYCERIDES	**X**
PRE-ALBUMIN	**X**

BIOCHEMISTRY TEST to be performed on sodium fluoride tube

Name of test	
LACTATE	**X**

HAEMATOLOGY TEST to be performed on EDTA tube

Name of test	
FULL BLOOD COUNT	**X**

APPENDIX 8

DEPARTMENT OF BIOMEDICAL TECHNOLOGY

STUDY TITLE: AN INVESTIGATION OF METABOLIC SIDE EFFECTS OF ANTIRETROVIRAL THERAPY USING LABORATORY BIOMARKERS IN HIV INFECTED INDIVIDUALS

Investigator : T S Ndlovu

CLINICAL ASSESSMENT FORM

To be filled in by the clinician

ARV SITE		
PATIENT'S REFERENCE NUMBER		
DATE OF CLINICAL ASSESSMENT		
LABORATORY DATA INTERVAL	6-month	12-month

Name of test	**Laboratory result**	**Comment**
ALT		
AST		
LACTATE		
LIPASE		
CHOLESTEROL		
TRIGLYCERIDES		

CLINICAL EVALUATION

PANCREATIC DISORDER

	YES	**NO**
Nausea		
Vomiting		
Fever		
Sweating		
Abdominal tenderness		
sharp pain in the upper abdomen		

HEPATIC DISORDER

	YES	**NO**
Nausea		
Vomiting		
Abdominal pain		
Jaundice		

Hepatomegaly		
Diarrhoea		

Comments

Name of clinician:----------

Signature: ----------

Date: ----------

APPENDIX 9A

BECKMAN COULTER CE

SYNCHRON® System(s)
Chemistry Information Sheet

ALT
Alanine Aminotransferase

Kit Reorder # 442620 (200 tests/cartridge)
Kit Reorder # 476826 (400 tests/cartridge)

For *In Vitro* Diagnostic Use

ANNUAL REVIEW

Reviewed by:	Date	Reviewed by:	Date

PRINCIPLE

INTENDED USE

ALT reagent, when used in conjunction with SYNCHRON LX® System(s), UniCel® DxC 600/800 System(s), is intended for the quantitative determination of Alanine Aminotransferase activity in human serum or plasma.

CLINICAL SIGNIFICANCE

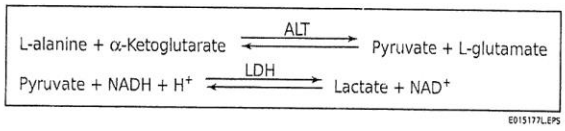
Alanine aminotransferase measurements are used in the diagnosis and treatment of certain liver diseases (e.g., viral hepatitis and cirrhosis) and heart diseases.

METHODOLOGY

ALT reagent is used to measure analyte activity by a kinetic rate method.[1,2] In the reaction, alanine aminotransferase catalyzes the reversible transamination of L-alanine and alpha-ketoglutarate to pyruvate and L-glutamate. The pyruvate is then reduced to lactate in the presence of lactate dehydrogenase (LDH) with the concurrent oxidation of reduced β-nicotinamide adenine dinucleotide (NADH) to β-nicotinamide adenine dinucleotide (NAD).

The SYNCHRON® System(s) automatically proportions the appropriate sample and reagent volumes into the cuvette. The ratio used is one part sample to 11 parts reagent. The system monitors the change in absorbance at 340 nanometers. This change in absorbance is directly proportional to the activity of ALT in the sample and is used by the System to calculate and express the ALT activity.

CHEMICAL REACTION SCHEME



APPENDIX 9B

BECKMAN COULTER CE

SYNCHRON® System(s)
Chemistry Information Sheet

AST
Aspartate Aminotransferase

Kit Reorder # 442665 (200 tests/cartridge)
Kit Reorder # 476831 (400 tests/cartridge)

For *In Vitro* Diagnostic Use

ANNUAL REVIEW

Reviewed by:	Date	Reviewed by:	Date

PRINCIPLE

INTENDED USE

AST reagent, when used in conjunction with SYNCHRON LX® System(s), UniCel® DxC 600/800 System(s), is intended for the quantitative determination of Aspartate Aminotransferase activity in human serum or plasma.

CLINICAL SIGNIFICANCE

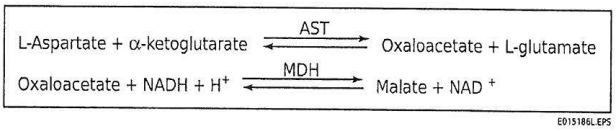
Aspartate aminotransferase measurements are used in the diagnosis and treatment of certain types of liver and heart disease.

METHODOLOGY

The AST reagent is used to measure aspartate aminotransferase activity by an enzymatic rate method.[1,2] In the assay reaction, the AST catalyzes the reversible transamination of L-aspartate and α-ketoglutarate to oxaloacetate and L-glutamate. The oxaloacetate is then reduced to malate in the presence of malate dehydrogenase (MDH) with the concurrent oxidation of β-Nicotinamide Adenine Dinucleotide (reduced form) (NADH) to β-Nicotinamide Adenine Dinucleotide (NAD).

The SYNCHRON® System(s) automatically proportions the appropriate sample and reagent volumes into a cuvette. The ratio used is one part sample to 11 parts reagent. The system monitors the rate of change in absorbance at 340 nanometers over a fixed-time interval. This rate of change in absorbance is directly proportional to the activity of AST in the sample and is used by the SYNCHRON® System(s) to calculate and express the AST activity.

CHEMICAL REACTION SCHEME



APPENDIX 9C

BECKMAN COULTER CE

SYNCHRON® System(s)
Chemistry Information Sheet

CHOL
Cholesterol
Kit Reorder # 467825

For *In Vitro* Diagnostic Use

ANNUAL REVIEW

Reviewed by:	Date	Reviewed by:	Date

PRINCIPLE

INTENDED USE

CHOL reagent, when used in conjunction with SYNCHRON LX® System(s), UniCel® DxC 600/800 System(s) and SYNCHRON® Systems Multi Calibrator, is intended for quantitative determination of Cholesterol concentration in human serum or plasma.

CLINICAL SIGNIFICANCE

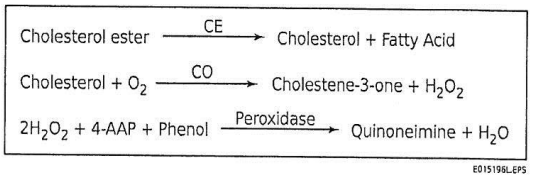
Cholesterol measurements are used in the diagnosis and treatment of atherosclerotic coronary artery disease. Cholesterol measurements are also used in the diagnosis of metabolic disorders involving lipids and lipoproteins. Total serum cholesterol concentrations depend on many factors including age, gender, diet, physical activity, liver disease, and other metabolic disorders.

METHODOLOGY

CHOL reagent is used to measure cholesterol concentration by a timed-endpoint method.[1,2,3] In the reaction, cholesterol esterase (CE) hydrolyzes cholesterol esters to free cholesterol and fatty acids. Free cholesterol is oxidized to cholestene-3-one and hydrogen peroxide by cholesterol oxidase (CO). Peroxidase catalyzes the reaction of hydrogen peroxide with 4-aminoantipyrine (4-AAP) and phenol to produce a colored quinoneimine product.

The SYNCHRON® System(s) automatically proportions the appropriate sample and reagent volumes into the cuvette. The ratio used is one part sample to 100 parts reagent. The system monitors the change in absorbance at 520 nanometers. This change in absorbance is directly proportional to the concentration of CHOL in the sample and is used by the System to calculate and express CHOL concentration.

CHEMICAL REACTION SCHEME



APPENDIX 9D

BECKMAN COULTER CE

SYNCHRON® System(s)
Chemistry Information Sheet

TG
Triglycerides GPO
Kit Reorder # 445850

For *In Vitro* Diagnostic Use

ANNUAL REVIEW

Reviewed by:	Date	Reviewed by:	Date

PRINCIPLE

INTENDED USE

TG reagent, when used in conjunction with SYNCHRON LX® System(s), UniCel® DxC 600/800 System(s) and SYNCHRON® Systems Multi Calibrator, is intended for quantitative determination of total Triglycerides concentration in human serum or plasma.

CLINICAL SIGNIFICANCE

Triglyceride measurements are used in the diagnosis and treatment of patients with diabetes mellitus, nephrosis, liver obstruction, other diseases involving lipid metabolism, or various endocrine disorders.

METHODOLOGY

Triglycerides GPO reagent is used to measure the triglycerides concentration by a timed endpoint method.[1,2] Triglycerides in the sample are hydrolyzed to glycerol and free fatty acids by the action of lipase. A sequence of three coupled enzymatic steps using glycerol kinase (GK), glycerophosphate oxidase (GPO), and horseradish peroxidase (HPO) causes the oxidative coupling of 3,5-dichloro-2-hydroxybenzenesulfonic acid (DHBS) with 4-aminoantipyrine to form a red quinoneimine dye.

The SYNCHRON® System(s) automatically proportions the appropriate sample and reagent volumes into the cuvette. The ratio used is one part sample to 100 parts reagent. The system monitors the change in absorbance at 520 nanometers. This change in absorbance is directly proportional to the concentration of TG in the sample and is used by the System to calculate and express the TG concentration.

APPENDIX 9E

BECKMAN COULTER CE

SYNCHRON® System(s)
Chemistry Information Sheet

AMY
Amylase
Kit Reorder # 442775

For *In Vitro* Diagnostic Use

ANNUAL REVIEW

Reviewed by:	Date	Reviewed by:	Date

PRINCIPLE

INTENDED USE

AMY reagent, in conjunction with SYNCHRON LX® System(s), UniCel® DxC 600/800 System(s) is intended for the quantitative determination of total Amylase activity in human serum, plasma or urine. Use of this product, in conjunction with the SYNCHRON® Systems Enzyme Validator Set, will result in assay values which are compatible with the methods recommended by the International Federation of Clinical Chemistry (IFCC).[1]

CLINICAL SIGNIFICANCE

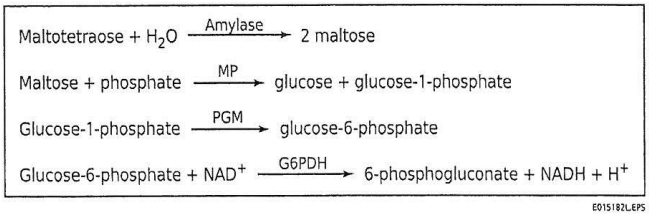
Amylase measurements are used primarily in the diagnosis and treatment of pancreatitis.

METHODOLOGY

AMY reagent is used to measure amylase activity by an enzymatic rate method.[2] In the reaction, amylase catalyzes the hydrolysis of the defined substrate, maltotetraose, to maltose. The rate of formation of maltose is measured through the use of three coupled reactions catalyzed by maltose phosphorylase (MP), β-phosphoglucomutase (PGM), and glucose-6-phosphate dehydrogenase (G6PDH) which results in the production of reduced β-nicotinamide adenine dinucleotide (NADH) from β-nicotinamide adenine dinucleotide (NAD).

The SYNCHRON® System(s) automatically proportions the appropriate sample and reagent volumes into the cuvette. The ratio used is one part sample to 21 parts reagent. The system monitors the change in absorbance at 340 nanometers. This change in absorbance is directly proportional to the activity of AMY in the sample and is used by the System to calculate and express the total AMY activity.

CHEMICAL REACTION SCHEME



E015182LEPS

APPENDIX 9F

BECKMAN COULTER CE

SYNCHRON® System(s)
Chemistry Information Sheet

LIP
Lipase

Kit Reorder # 465126 (30 tests/cartridge)
Kit Reorder # 476851 (60 tests/cartridge)

For *In Vitro* Diagnostic Use

ANNUAL REVIEW

Reviewed by:	Date	Reviewed by:	Date

PRINCIPLE

INTENDED USE

LIP reagent, when used in conjunction with SYNCHRON LX® System(s), UniCel® DxC 600/800 System(s) and SYNCHRON® Systems Enzyme Validator Set, is intended for the quantitative determination of Lipase activity in human serum or plasma in random access mode.

CLINICAL SIGNIFICANCE

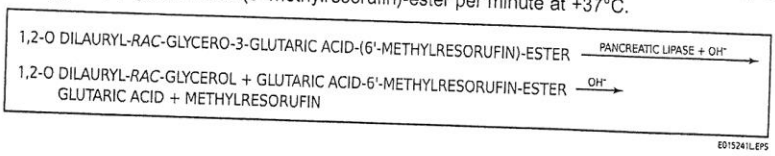
Lipase measurements are used primarily in the diagnosis and treatment of pancreatic disorders.

METHODOLOGY

The Random Access Lipase reagent utilizes the methodology of Panteghini to determine pancreatic lipase activity in serum and plasma.[1] The SYNCHRON® System(s) monitors the rate of formation of methylresorufin which forms spontaneously from two coupled reactions which utilize a 1,2-O-dilauryl-rac-glycero-3-glutaric acid-(6'-methylresorufin)-ester as a substrate. The measured rate of color formation at 560 nm is directly proportional to the pancreatic lipase activity.

The SYNCHRON® System(s) automatically proportions the appropriate sample and reagent volumes into the cuvette. The ratio used is one part sample to 54 parts reagent. The system monitors the change in absorbance at 560 nanometers. The rate of formation of the methylresorufin is directly proportional to the activity of LIP in the sample and is used by the System to calculate and express the LIP activity.

One unit (U) is defined as the amount of enzyme activity which liberates 1 µmol of methylresorufin from 1,2-O-dilauryl-rac-glycero-3-glutaric acid-(6'-methylresorufin)-ester per minute at +37°C.



APPENDIX 10

FULL BLOOD COUNT RAW DATA

			RCC	RCC	RCC	Hb	Hb	Hb	Plt	Plt	Plt
Participant code	**Gender**		**B**	**6**	**12**	**B**	**6**	**12**	**B**	**6**	**12**
DUTUML001	F		3.25	3.85	3.68	11.8	12.1	11.8	470	526	372
DUTUML002	F		3.8	3.27	3.4	14.5	12.6	13.1	273	207	344
DUTUML003	F		4.3	4.2	3.79	12.5	12.3	12.1	533	498	415
DUTUML004	M		3.6	4.7	4.63	10.8	14.6	14.6	176	195	155
DUTUML005	M		3.76	3.6	3.4	14.5	14.1	13.6	211	207	323
DUTUML006	M		3.7	3.68	3.4	11.5	13.5	13.2	312	230	287
DUTUML007	M		3.67	4.05	3.29	12.1	15	10.4	350	269	369
DUTUML008	M		3.29	3.43	3.18	12	11.2	11.8	348	485	256
DUTUML009	M		4.1	3.7	4.53	15.1	13.6	14.9	251	233	151
DUTUML010	F		3.6	3.41	3.42	11.6	11.1	11.4	557	531	534
DUTUML011	F		3.21	3.89	4.14	8.7	11.1	11.7	259	237	232
DUTUML012	F		1.21	3.13	4.2	8.8	9	11.2	69	205	178
DUTUML013	F		3.81	3.49	3.74	11.3	11.8	11	173	215	273
DUTUML014	F		4.35	4.2	3.98	11.5	11.9	11.7	264	287	235
DUTUML015	M		3.99	4.22	3.94	13.6	14.1	13.4	312	298	185
DUTUML016	F		3.39	3.74	3.98	10.5	11	11.8	216	273	245
DUTUML017	M		6.41	6.1	6.15	14.9	15.1	15	222	205	258
DUTUML018	M		3.6	3.29	3.69	15.1	13.4	14.9	371	290	282
DUTUML019	M		4.9	4.1	4.21	15.3	15.1	15	285	289	299
DUTUML020	M		4.2	3.98	4	11.9	11.7	12.1	292	298	265
DUTUML021	F		4.15	3.8	3.72	10.9	12.3	12.1	390	346	289
DUTUML022	M		3.94	4.03	4.1	11.7	12.1	12	245	350	348
DUTUML023	M		3.41	3.5	3.38	14.2	13.6	13.4	270	256	215
DUTUML024	F		3.83	4.21	4.38	11.4	13.5	13	114	193	218
DUTUML025	M		4.67	5.26	4.91	14.2	15.3	15.1	266	274	201
DUTUML026	F		3.86	4.2	3.98	10.8	11.9	12.1	325	287	235
DUTUML027	F		3.37	3.29	4.2	10.5	10.7	11.2	360	367	369
DUTUML028	M		4.7	4.28	4.16	15.4	16.6	15.7	260	228	208
DUTUML029	M		4.78	5.04	4.34	14.3	14.5	12.3	237	251	240
DUTUML030	F		4.06	3.8	3.86	10.6	11.3	10.2	296	272	280
DUTUML031	M		3.97	3.84	3.91	15.3	14.5	13.8	215	153	223
DUTUML032	F		4.1	3.6	4.1	14.3	12.4	13.7	434	302	467
DUTUML033	F		3.84	3.72	3.82	10.9	12	12.1	313	271	236
DUTUML034	F		4.33	4	4.16	11.1	11.5	12	300	291	308
DUTUML035	F		4.28	4.15	4.22	14	13.5	13.6	204	171	225
DUTUML036	F		4.05	3.92	4.36	12.8	12.3	12.5	273	267	269
DUTUML037	M		3.91	3.96	3.94	13.6	13	13.8	196	189	182

			RCC	RCC	RCC	Hb	Hb	Hb	Plt	Plt	Plt
Participant code	**Gender**		**B**	**6**	**12**	**B**	**6**	**12**	**B**	**6**	**12**
DUTUML038	M		4.87	4.47	3.84	14.5	14.4	13.9	201	219	225
DUTUML039	M		4.93	5.24	4.93	15.6	16.6	15.9	276	273	304
DUTUML040	F		3.1	4.09	1.26	12.8	11.8	12.4	634	567	606
DUTUML041	F		4.66	4.49	4.52	13.8	12.8	13.1	307	208	245
DUTUML042	M		3.19	3.74	3.87	10.2	11	11.5	338	344	340
DUTUML043	F		3.39	2.92	4.2	11.7	10	12	386	477	452
DUTUML044	F		3.9	3.19	3.65	11.4	11.3	11	320	316	278
DUTUML045	F		3.29	3.44	3.45	13.4	12.5	13.3	250	212	261
DUTUML046	F		3.8	3.66	3.81	11.5	11.9	11.9	237	282	286
DUTUML047	M		3.93	4.33	4.34	13	13.5	13.5	291	318	331
DUTUML048	M		5.16	5.34	5.48	14.8	15.1	15.3	259	301	339
DUTUML049	F		4.42	4.39	4.65	12.3	12.1	12.9	266	320	274
DUTUML050	F		4.23	4.25	4.24	13.5	13.2	13.2	171	210	238
DUTUML051	F		4.14	3.3	3.7	12	9.2	11.8	177	373	339
DUTUML052	F		4.3	3.82	3.97	14.4	11.7	12.9	204	232	219
DUTUML053	M		3.96	3.63	4.41	15.3	14.2	14	391	309	303
DUTUML054	F		3	4.6	3.2	11	13.8	11.9	184	243	291
DUTUML055	F		4.93	4.8	5.19	15.8	16.2	15.8	201	196	215
DUTUML056	F		4.58	4.54	4.4	13.7	14.4	13.7	244	196	253
DUTUML057	F		3.77	3.68	3.74	13.1	12.4	12.5	187	195	235
DUTUML058	F		4	3.77	4.61	14.3	13.3	14.4	393	402	375
DUTUML059	F		3.97	4.31	3.69	12.8	13.5	12.2	321	302	392
DUTUML060	F		3.83	4.08	3.82	13.2	11.8	12.1	324	333	229
DUTUML061	F		3.96	4.35	4.58	12.3	13.2	13.3	429	418	519
DUTUML062	F		3.7	4.13	3.98	11.3	12.2	11.7	339	461	440
DUTUML063	F		4.21	4.08	4.39	11.9	11	11	290	249	337
DUTUML064	M		3.31	4.12	4.21	13.7	14.1	14.4	237	120	161
DUTUML065	M		4.33	4.64	4.65	12.9	12.7	13	257	254	297
DUTUML066	M		4.13	4.18	4.46	13	12.8	13.8	333	357	424
DUTUML067	F		3.69	3.16	4.08	11.8	11.7	13.2	312	312	315
DUTUML068	F		3.98	4.51	4.45	13.6	14.5	14.6	254	237	271
DUTUML069	F		2.92	3.05	3.31	12.4	12.5	13.3	314	305	346
DUTUML070	F		3.47	3.42	3.73	12	11.6	12.4	388	300	434
DUTUML071	F		3.11	4.17	4.07	12.4	13.3	13	279	302	243
DUTUML072	M		3.46	3.82	4.03	12.8	13.8	14.6	301	274	312
DUTUML073	F		2.35	2.37	3.44	8.8	11.8	10.6	241	275	343
DUTUML074	M		3.12	3.29	4.82	12.4	12.6	13.2	298	305	320
DUTUML075	F		3.78	3.45	3.58	11.8	11.7	12.2	180	196	192
DUTUML076	F		3.42	3.8	3.9	13.2	12.3	12.5	262	362	338

			RCC	RCC	RCC	Hb	Hb	Hb	Plt	Plt	Plt
Participant code	**Gender**		**B**	**6**	**12**	**B**	**6**	**12**	**B**	**6**	**12**
DUTUML077	M		3.31	4.45	4.26	12.4	14	12.8	223	235	252
DUTUML078	F		3.48	3.91	3.84	11.9	13	12.3	330	378	396
DUTUML079	F		4.46	4.41	4.52	13.1	12.5	12.6	232	216	259
DUTUML080	F		2.98	4.11	3.9	11.7	13.2	12.2	306	290	317
DUTUML081	F		3.08	3.06	4.36	11.7	12	12.2	338	265	300
DUTUML082	M		4.65	4.37	4.56	14.1	13.9	14.1	245	223	264
DUTUML083	F		3.52	2.87	3.58	11	10.3	12.1	313	324	395
DUTUML084	F		3.92	3.62	3.64	12.6	13.3	13.8	217	278	313
DUTUML085	F		3.02	3.19	3.09	11.7	12.3	12.5	269	229	225
DUTUML086	F		3.53	3.67	4.35	12	12	13.5	352	315	294
DUTUML087	F		4.1	4.58	4.62	13	12.8	13.4	443	259	281
DUTUML088	M		3.39	3.72	3.7	13.2	13.8	13.6	266	213	264
DUTUML089	M		4.66	4.76	4.78	13	14.7	14.8	388	354	272
DUTUML090	F		4.68	4.96	4.86	13.8	14.2	14.3	260	219	229
DUTUML091	F		4.29	4.34	4.55	13.2	12.6	13.2	335	352	366
DUTUML092	F		4.06	3.7	3.68	11.6	10	10.3	225	212	260