IMMUNOLOGICAL AND VIROLOGICAL RESPONSES IN HIGHLY ACTIVE ANTIRETROVIRAL THERAPY NAÏVE PATIENTS EXPOSED TO ISONIAZID PREVENTIVE THERAPY

By

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SUPERVISOR: Dr S Knight

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DEDICATION

To my late daughter, Juliana Mwendanalo Manda.

My Your Soul Rest in Eternal Peace dear Child

DECLARATION

I declare that immunological and virological responses in Highly Active Antiretroviral Therapy (HAART) naïve patients exposed to Isoniazid Preventive Therapy is my own work and that all the sources that I have used or quoted have been indicated and acknowledged by means of complete references.

SIGNATURE **DATE: 27th November 2009**

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IMMUNOLOGICAL AND VIROLOGICAL RESPONSES IN HIGHLY ACTIVE ANTIRETROVIRAL THERAPY NAÏVE PATIENTS EXPOSED TO ISONIAZID PREVENTIVE THERAPY

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ABSTRACT

OBJECTIVE: To compare immunological and virological outcomes in antiretroviral therapy naïve patients exposed to Isoniazid prevention treatment.

DESIGN: Quantitative, non-experimental retrospective cohort study.

METHODS: Medical records of antiretroviral naïve patients managed in the public sector from 1st January 2006 to 31st December 2006 were analysed.

RESULTS: Multivariate analysis of variance showed that each treatment group achieved statistically significant increases in $CD4^+$ cell count and viral load decay at each follow-up time point. Pairwise post hoc contrast tests showed patients in $NVP_{ipt\text{-}past}$ group and $EFV_{ipt\text{-}past}$ group to have superior immunological and virological outcomes respectively.

CONCLUSION: Prior exposure to Isoniazid preventive therapy resulted in better immunological and virological outcomes at each study follow-up time point. Overall, the immunological and virological group differences were not statistically significant. The null hypothesis is accepted.

KEY CONCEPTS Antiretroviral naïve; Antiretroviral therapy; Isoniazid preventive therapy; $CD4^+$ cell count; viral suppression; Nevirapine; Efavirenz; retrospective study; Multivariate analysis of variance, immunology, virological response.

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ACHAP	African Comprehensive HIV/AIDS Program
AIDS	Acquired Immune Deficiency Syndrome
ALT	Alanine Transaminase
ANC	Antenatal Care
AST	Aspartate transaminase
BBC	British Broadcasting Corporation
BCG	Bacillus Calmette Guerin
BOTUSA	Botswana United States of America
CBV	Combivir
CD4	Cluster Differentiation T- lymphocyte
CDC	Centres for Disease Control
CI	Confidence Interval
DNA	Deoxyribo Nucleic Acid
DOTS	Directly Observed Treatment Short course
EFV	Efavirenz
ELISA	Enzyme Linked Immune Sorbent Assay
ELISPOT	Enzyme Linked Immuno-Spot Assay
FDA	Food and Drug Administration
FTC	Emtracitabine
G8	Group of 8 nations
GDP	Gross Domestic Product
HAART	Highly Active Anti-Retroviral Therapy
HIV	Human Immunodeficiency Virus
HIV DR-TS	HIV Drug Resistance Threshold Survey
ID	Identification
IDCC	Infectious Disease Clinical Centre
IFA	Immune Fluorescence Assay
IMF	International Monetary Fund
INF	Interferon

Kg	Kilogram
Log	Logarithm
LTBI	Latent Tuberculosis Infection
MAC	Mycobacterium Avium Complex
MDR – TB	Multi-Drug Resistant Tuberculosis
mg	Milligram
mL	Millilitre
µL	Microliter
MMWR	Mortality and Morbidity Weekly Report
MTCT	Mother to Child Transmission
NRTI	Nucleoside Reverse Transcriptase Inhibitors
NNRTI	Non-Nucleoside Reverse Transcriptase
NtRTI	Nucleotide Reverse Transcriptase Inhibitors
NVP	Nevirapine
OI	Opportunistic Infection
UNAIDS	United Nations Programme on HIV/AIDS
PCR	Polymerase Chain Reaction
PEP	Post Exposure Prophylaxis
PrEP	Pre-Exposure Prophylaxis
PI	Protease Inhibitors
PMTCT	Prevention of Mother to Child Transmission
PEPFAR	Presidential Emergency Plan for AIDS Relief
QFT – G	QuantiFERON- Gold TB Test
RNA	Ribonucleic Acid
rRNA	reverse Ribonucleic Acid
RPR	Rapid Plasma Reagin
RR	Relative Risk
SPSS	Statistical Package for Social Scientist
STD	Sexually Transmitted Disease
STI	Sexually Transmitted Infection
TB	Tuberculosis
U & E	Urea & Electrolyte

UNICEF	United Nations Children Education Fund
VCT	Voluntary Counselling and Testing
WHO	World Health Organization
XDR – TB	Extremely Drug Resistance Tuberculosis

CHAPTER 1

ORIENTATION OF THE STUDY

1.1 INTRODUCTION

Tuberculosis (TB) is a common infectious disease caused mainly by Mycobacterium tuberculosis (Palomino, Leao & Ritacco 2007: 207). According to the World Health Organization (WHO), one–third of the world's population is currently infected with the tuberculosis bacillus (WHO Fact sheet No. 104 Revised March 2007). World Health Organization estimates that 9.27 million new cases of tuberculosis occurred in 2007 (139/100,000 population) compared with 9.24 million new cases (140/100,000 population) in 2006 (WHO/HTM/TB/2006.362). Of these 9.27 million new cases, 44% or 4.1 million (61/100,000 population) were smear-positive cases. India, China, Indonesia Nigeria and South Africa rank first to fifth in terms of the total number of incident cases. The report further states that of the 15 countries with the highest estimated tuberculosis incidence rates, 13 are in Africa (WHO Global Tuberculosis Control 2009 Report: 7).

Tuberculosis remains the most common opportunistic infection and leading cause of death in people living with Human Immunodeficiency Virus (HIV) in low and middle-income countries (Joint United Nations Programme on HIV/AIDS (UNAIDS) Report 2008: 143). In 2007, approximately 14 million people were co-infected with TB and HIV-1 worldwide and 70% of these people live in Africa. In some African countries, up to 75% of TB patients are co-infected with HIV and affected by the Acquired Immune Deficiency Syndrome (AIDS) (Lange, Schieferstein, Tossi & Gori 2007: 416). According to the World Health Organization, the global estimated number of HIV and tuberculosis or infected cases and deaths are now substantially higher than in previous years (WHO Global Tuberculosis Control Report 2009: 10).

HIV-related immune suppression involves much more than treatment with antiretroviral therapy as people living with HIV/AIDS are at an increased risk of a broad range of debilitating and life-threatening conditions. As a result of the synergistic relationship between HIV and tuberculosis, all people living with HIV/AIDS should be screened for active tuberculosis. After excluding active tuberculosis, it is recommended that treatment of latent tuberculosis infection with a 6 to 9 months course of preventive

therapy should be considered for all people living with HIV/AIDS (Joint United Nations Programme on HIV/AIDS (UNAIDS) Report 2008: 145).

1.2 THE CONTEXT OF THE RESEARCH PROBLEM: COUNTRY PROFILE OF BOTSWANA

1.2.1 HISTORICAL OVERVIEW

In the late 1800s Britain formed the protectorate of Bechuanaland, to prevent territorial encroachment of the Boers from the Transvaal or German expansion from South West Africa. In 1966 Bechuanaland became the independent Republic of Botswana, establishing a constitutional multi-party democracy and unitary government. Botswana is one of Africa's most stable countries and remains the continent's longest continuous multi-party democracy. The constitution upholds universal adult suffrage, a legislature, independent judiciary and executive presidency (British Broadcasting Corporation (BBC) Country Profile: Botswana 2007).

1.2.1.1 Geography

Botswana is a landlocked country situated in Southern Africa with a total land area of 582,000 km^2. The country shares borders with Namibia in the West and North, Zambia in the North, Zimbabwe in the North East and South Africa in the East and South. The climate ranges from semi-arid to sub-tropical. More than two thirds of land is arid and semi-arid with the Kalahari Desert dominating Southern and Western Botswana; the extreme south-west experiences semi-desert conditions, while eastern Botswana, though prone to drought, receives adequate rainfall to support viable arable farming. The mean annual rainfall ranges from 200 mm in the Southwest to 650 mm in the Northeast of the country (BBC Country Profile: Botswana 2007).

1.2.1.2 Population

The population of Botswana in 2007 was approximately 1.7 million people with a population density of 3/km^2 and annual growth rate of 2%. The population is mainly urban with 57% living in these areas. The percent population distribution according to the age structure is 0 to 14 years: 36%; 15 – 64 years: 59%; and 65^+ years: 5%. The dependency ratio is 68/100. There are 92 males per 100 females. The 2007 general fertility rate and total fertility rate was 108 and 3 respectively. The neonatal and infant mortality rates per 1000 live births are 46 and 86 and respectively. The population

crude birth and death rates are 30/1000 and 11/1000. The country records a maternal mortality ratio of 100/100,000 live births. About 79% of the adult population of Botswana was literate in 2007. Life expectancy at birth in 2007 was 42 years for males and 41 years for females (WHO: World Health Statistics 2007: 22).

1.2.1.3 Economic development

Through fiscal discipline and sound management, Botswana has transformed itself from one of the poorest countries in the world to a middle-income country with a per capita Gross Domestic Product (GDP) of more than US$10,900 in 2006 (BBC Country Profile: Botswana 2007). Botswana has maintained one of the world's highest economic growth rates since independence in 1966, though growth is projected to slow down to 3.5% in the longer term. The past decade has witnessed the mining sector forming an average of 35% of GDP, with diamonds constituting 94% of the sector's total exports. The mining sector is the main contributor to fluctuations in the GDP and growth rate over the past decade.

Diamond exports, Botswana's main source of foreign exchange, accounts for an average of 75% of the total annual exports over the past 10 years. Fiscal revenue also depends heavily on diamond mining receipts, which account for 95% of mineral revenues. Over the same period, mineral revenues accounted for 63% of the total tax revenues. The country is expected to remain the world's leading producer of diamonds well beyond the next decade (International Monetary Fund (IMF) Country Report No. 228/ 2007: 4). Other export minerals are copper and nickel. Tourism, financial services, subsistence farming, and cattle ranching are other important sectors that contribute to the national economy.

However, apart from the mining sector, growth in other sectors has been minimal. Growth in other sectors decelerated from an annual average of 7% from 1984/85 to about 1% in 1993/94. Growth has been particularly weak in the agricultural sector which declined from 10% of GDP in 1981 to 2% in 2004/05. Similarly the manufacturing and construction sectors recorded a reduction in the rate of growth from 13% to 3% and from 11 to 4% respectively over the decade to 2004/05. In contrast, the trade, hotels and restaurants sector seems relatively resilient. These sectors registered an average

annual growth of 7%, though this also represents a significant decrease from over 18% in the previous decade (IMF Country Report No. 228/2007: 22).

Botswana's economic success can also largely be attributed to the relative absence of corruption in the country. Botswana is ranked the least corrupt country in Africa and the 36th least corrupt country in the world by Transparency International (Corruption Perception Index 2008: 50).

Despite the economic success, the country faces serious challenges. These are multi-fold and include the high prevalence of HIV and AIDS, large numbers of tuberculosis notifications, unemployment, poverty and the need for economic diversification since diamonds represent only a finite resource (IMF Country Report No. 228/2007: 5). The total expenditure of GDP on social services has more than doubled from 1997/98 (Pula 1.4 billion) to 2004/05 (Pula 3.2 billion). Until recently, government provided free health care and education to its entire population. However, the rising number of social programme beneficiaries, the increasing share of social programme in total expenditure and the continuing HIV/AIDS related expenditure are exerting pressure on government finances (IMF Country Report No. 228/2007: 28).

The government still remains the largest employer whilst the mining industry accounts for only 3% of the formal employment. Unemployment has remained persistently above 20%, and the proportion of those unemployed is now rising, particularly among the youth. Unemployment is highest among unskilled youth at 61% for the age group 15 – 19 years and 46% for those 20 – 24 years of age. Unemployment is higher in urban than rural areas and female unemployment exceeded male unemployment by about 30%. The result of such high levels of unemployment is that about 1/3 of the population lives below the poverty datum line [US$ 2/day] (IMF Country Report No. 228/2007: 25).

Notwithstanding the free access to medications for treatment of HIV, TB and latent TB infection in Botswana, unemployment prevents access to health care largely through such factors as transport costs to the health facility. Unemployment can lead people to spend less on food resulting in malnutrition which increase people's vulnerability to opportunistic infections. Coupled with this, failure to follow dietary restrictions in accordance with the prescribed antiretroviral drugs as a result of food shortage can

lead to decreased viral suppression. Unemployment makes people to live in poor housing conditions which in itself are a major factor in causing such illnesses as TB.

The government of Botswana is currently working with multilateral agencies and regional programme initiatives that include HIV and AIDS and trade to alleviate government expenditure (IMF Country Report No. 228/2007: 22).

1.2.1.4 THE HEALTH CARE SYSTEM OF BOTSWANA

The growth of Botswana's health infrastructure has been extensive. The health system has successfully developed from a small and narrow hospital based service, serving a minority at independence to a broad based decentralised primary health care system where close to 90% of the population is now within a 8km radius of the nearest health facility (Master Health Facility List 2007: ii).

1.2.1.4.1 Healthcare structure and authorities

The healthcare delivery system in Botswana is based on a welfare system which aims to provide quality health care equitably. The Ministry of Health is the central government organ, with the portfolio responsibility for overall improvement and maintenance of national health. This ministry sets broad policy directions, goals and strategies for health development and delivery (Situational Analysis 2005: 7).

The health delivery system is composed of primary, secondary and tertiary levels. Primary level of health care consists of mobile clinic stops, health posts, clinics and primary hospitals which provide mainly preventive and curative services. The secondary level includes district hospitals whereas referral hospitals constitute the tertiary level of health and provides curative, rehabilitative, laboratory and radiological services, care provision.

The Ministry of Health runs secondary and tertiary health facilities while the Ministry of Local Government is in charge of primary health care facilities. The Ministry of Local Government has 15 local authorities (9 district councils, 4 town councils and 2 city councils) which are divided into 24 health districts for the delivery of primary health care services; preventive care, curative care and programs for the control of major public health diseases (Situational Analysis 2005: 7).

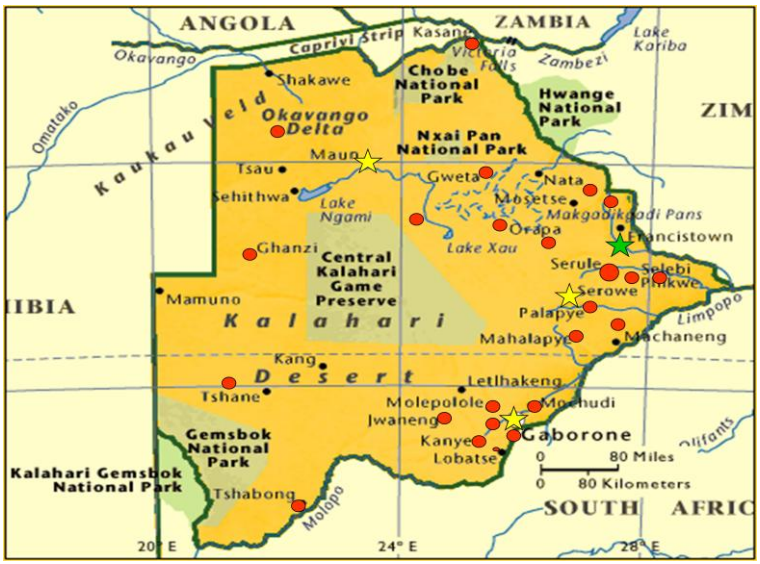
1.2.1.4.2 Health care facilities with specific reference to the provision of antiretroviral therapy

The health care system in Botswana contains a blend of both public and private sector in the areas of both health delivery and financing. The public health system in Botswana consists of all health facilities owned or supported by government, as well as facilities open to the public such as mine hospitals (Chandna 2005: 1).

According to the 2007 Master Health Facility List (2007: iv – v) there are 844 mobile stops, 338 health posts, 272 clinics, 17 primary hospitals, 14 district hospitals and 3 government referral hospitals. In Gaborone there is a private hospital as well as health care provided by several private medical practitioners.

The provision of antiretroviral therapy was initiated in the private clinics on a fee paying basis. Public provision of antiretroviral medication was originally implemented at the Princess Marina Hospital in Gaborone in 2002. By the end of 2003, 12 facilities were offering antiretroviral therapy in the country (Korte, Mazonde & Darkoh 2004: 8 - 9). Antiretroviral therapy treatment in Botswana can currently be accessed from primary, district and referral hospitals. These sites are supported by satellite clinics managed by the local authorities.

Botswana's roll out of antiretroviral therapy to 32 sites was accomplished through a step-wise approach. The initial 32 sites included a main Infectious Disease Care Clinic integrated into the existing hospital and linked to designated satellite clinics that conduct HIV screening and appropriate referrals. Currently, 32 main public antiretroviral therapy sites and 69 satellite clinics provide antiretroviral therapy (Bussmann, Rotz, Ndwapi, Baxter, Bussmann, Wester, Ncube, Avalos, Mine, Mabe, Burns, Cardiello, Makhema & Marlink 2008: 14).

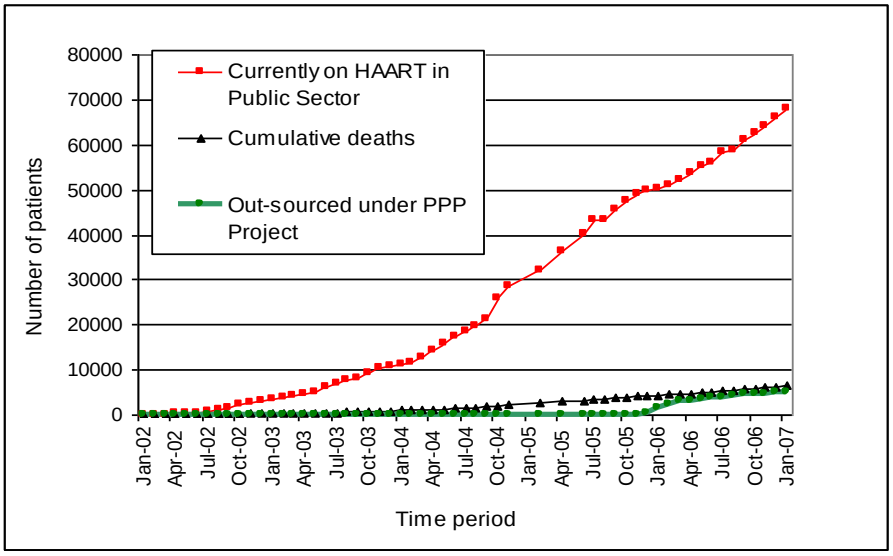


Source: MASA ARV program – Botswana

Figure 1.1 Map showing operational antiretroviral treatment sites – 2007.

The African Comprehensive HIV/AIDS Program (ACHAP) has been responsible for conducting and financially supporting the antiretroviral program since its inception. The organisation has provided critical human resources and logistical support and has facilitated and supported the establishment of a number of treatment sites (WHO Country Office for Botswana & WHO/AFRO 2005: 1 – 3). See Figure1.1 on this page.

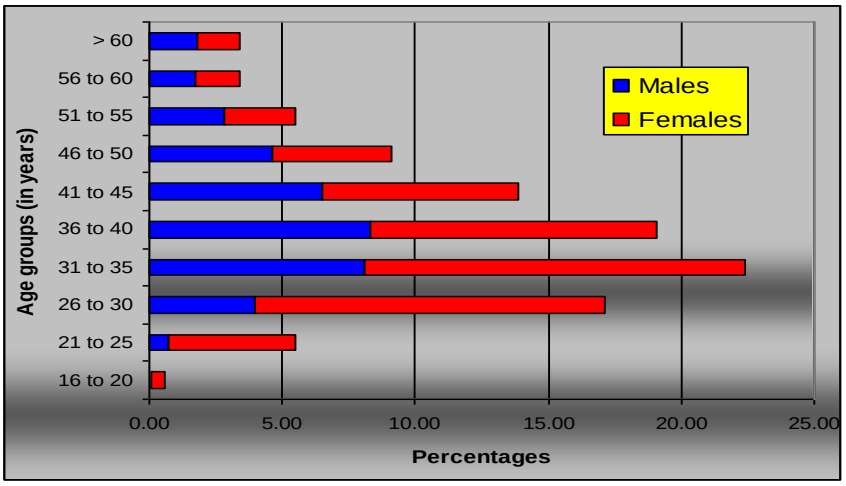
By the end of December 2007, 75,082 patients were recorded as being on antiretroviral therapy in the public sector, of which 45,723 (61%) were females. There were 6,267 (8%) children (≤ 12 years) on antiretroviral therapy in the public sector. The cumulative trend of patients initiated on antiretroviral therapy in Botswana has shown a steady increase since the program commenced in January 2002. See Figure 1.2 on page 9. The figure also shows the trend in the number of patients who have died while on antiretroviral therapy and those outsourced to the private sector under the auspices of a Public Private Partnership.



Source: MASA- ARV program - Botswana

Figure 1.2 Patients on HAART in Botswana: 2002 to 2007 (January).

Figure 1.3 on this page shows the age, gender and percentage distribution of patients on antiretroviral treatment in the country by the end of December 2007. More females than males were accessing antiretroviral therapy in Botswana with most of those accessing treatment being in the age range of 25 to 50 years.



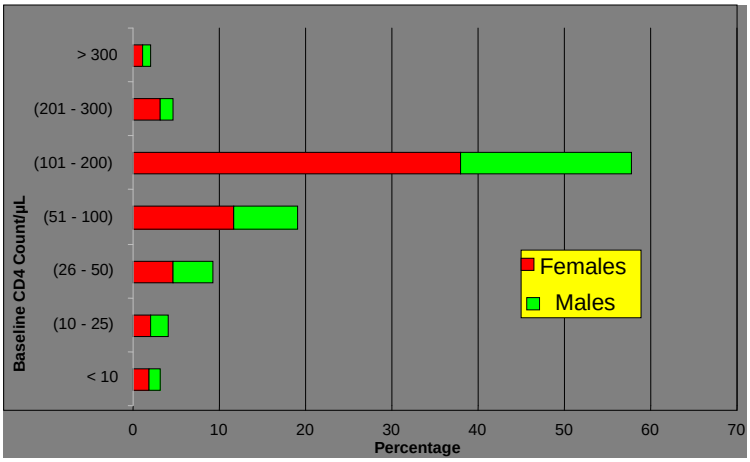
Source: MASA- ARV program –Botswana

Figure 1.3 Age and gender distribution of patients currently on antiretroviral treatment in public health sector (December 2007).

The rationale of outsourcing is to alleviate the work load and shorten the patient waiting time to initiation of antiretroviral therapy in public funded health facilities. A total of 8,336 patients benefited from care given by the private sector under the government out-sourcing program (Public Private Partnership). Another 9,514 were being treated in the private healthcare sector of the country funded by Medical Aid Schemes and the Work-place Treatment Programs. By the end of December 2007, Botswana had achieved among the highest coverage for delivering antiretroviral drugs to more than 90% of those who needed the medications (Joint United Nations HIV/AIDS Programme 2008: 193).

A cumulative total of 8,402 patients have died while on antiretroviral therapy since the beginning of the Antiretroviral Therapy (ART) program in 2002. By the end of December 2007, 496 (0.7%) patients who were eligible for antiretroviral therapy in the public sector had not been initiated on such treatment and comprise of those on the waiting list (MASA – the National ARV Program of Botswana 2007).

The gender and baseline $CD4^+$ cell count distribution at initiation of antiretroviral therapy is as shown in the Figure 1.4 that follows. More than 85% of patients initiate antiretroviral therapies with a $CD4^+$ range of 50 to 200 cells / µL. A higher percentage of females tend to commence antiretroviral therapy with a higher $CD4^+$ cell count of 100 to 200. Women have several entry points into the antiretroviral program including through prevention of mother to child transmission and family planning services, so HIV is diagnosed earlier. Males tend to present to the clinics when they are very ill and have lower $CD4^+$ cell counts of < 50 cells/µL (Muula, Ngulube, Siziya, Makupe, Umar, Prozesky, Wiysonge & Mataya 2007: 63).



Source: MASA- ARV program - Botswana

Figure 1.4 Gender and CD4$^+$ cell count distribution of patients currently on antiretroviral treatment in the public health sector (December 2007).

1.2.1.4.3 Health personnel

Botswana possesses a limited number of health personnel. Of the 800 doctors practising in the country, 700 are expatriates (Kgosisejo 2008: 16). At present there are 0.4 doctors and 2.65 nurses/1000 population which is well below the World Health Organization recommended ratios for doctor-patient and nurse-patient of 1: 250 and 1: 1000 respectively (World Health Report 2006: 190).

At each antiretroviral therapy intervention site, medical officers, nurses, social workers, laboratory and pharmacy technicians are involved in the management of patients. In addition, health education assistants (formerly family welfare educators) are employed to follow-up patients in the community.

The shortage of trained human resource personnel has proved to be a substantial challenge as the country continues to scale up the provision of antiretroviral therapy (WHO Country Office for Botswana & WHO/AFRO 2005: 1 – 3). Other development partners assisting the HIV and AIDS program are President's Emergency Plan for AIDS Relief (PEPFAR) and the Global Fund. In order to address the human resource shortage, the government has, through the Public-and Private-Partnership programme, engaged private practitioners to follow-up patients already stabilised on antiretroviral therapy. Furthermore, task shifting is occurring and nurses are being trained to

prescribe and dispense antiretroviral medication to ensure an equitable access to antiretroviral therapy.

1.3 RESEARCH PROBLEM

1.3.1 Tuberculosis/HIV co-infection as a public health

The interaction between TB and HIV is bidirectional. HIV infection is a known risk factor for both reactivation of latent TB infection and progressive primary disease. The lifetime risk to progression of active disease after TB infection is 10%. However in HIV- infected individuals; the annual risk of developing active tuberculosis is a 7 –10%. Tuberculosis is responsible for 13% of all adult deaths and 40% of deaths among people living with HIV/AIDS (Devi, Naorem, Singh, Singh, Prasad & Devi 2005: 220; National Tuberculosis Programme Manual 2007: 11 – 12).

Tuberculosis occurs early in HIV-infected persons when the $CD4^+$cell counts is >200 cells/ µL. During active TB in co-infected patients, macrophages and $CD4^+$ lymphocytes are activated by viable replicating mycobacteria and by pro-inflammatory cytokines. In this microenvironment of cellular activation, HIV replication is enhanced and spreads to uninvolved cells. Increased viral replication results in decline in $CD4^+$ cells, increase in viral RNA levels and an increased risk of opportunistic infections and survival (Whalen 2002: 1).

Treatment and management strategies for HIV and TB are discussed in more detail under section 2.4.1.4 on page 53. However, treating HIV/TB co-infected patients' posses a challenge resulting from drug-drug interactions between Rifampicin and non-nucleoside reverse transcriptase (NNTRI) and protease inhibitors (PIs). Rifampicin induces the synthesis of drug metabolizing enzymes (cytochrome P450 systems) with subsequent decrease in the half life of co-administered drugs metabolized by this enzyme system. Rifampicin has a modest effect on Efavirenz making the latter drug the preferred NNRTI when anti-tuberculosis regimens containing Rifampicin are used in adults. However, because of its teratogenecity, Efavirenz is contraindicated in the first trimester of pregnancy (da Silva & Ainsa 2007: 619).

Clinicians have possible options for antiretroviral therapy (ART) in patients with TB. These include deferring ART until TB treatment is completed; initiating ART after completion of the initial phase of anti-TB treatment and then using Ethambutol and Isoniazid in the continuation phase or treat TB with a Rifabutin-containing regimen Harries, Maher, Graham, Raviglione, Nunn, Gilks, Qazi, Weber, & van Praag 2004: 154.

1.3.1.1 The HIV/AIDS epidemic in Botswana

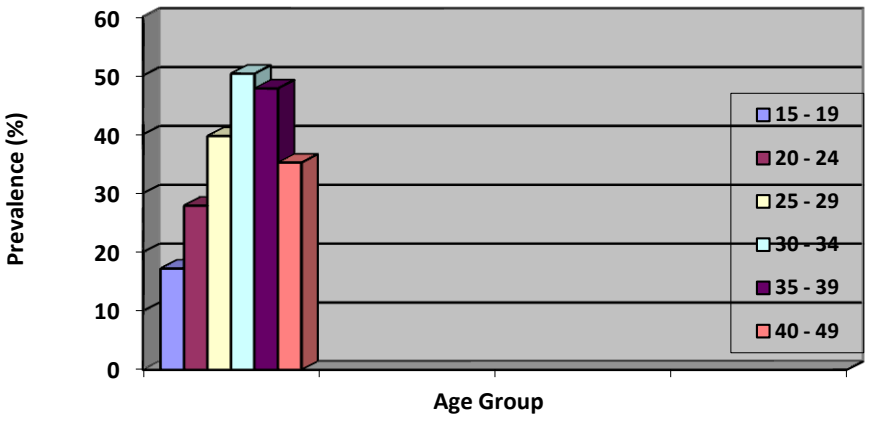
The first HIV/AIDS case in Botswana was diagnosed in 1985 and currently the country is experiencing one of the most severe HIV epidemics in the world with an estimated 300,000 [range 280,000 – 310,000] adults and children living with HIV/AIDS. The number of adults and children who have died of HIV/AIDS is estimated to be 11,000 [range 6,600 – 17,000] (Joint United Nations HIV/AIDS Programme 2008: 214,217).

The overall population prevalence of HIV between the ages 18 months and 68 years is 17.2% (Botswana Antenatal Second Generation HIV/AIDS Surveillance Technical Report 2007: 7). Unlike other countries in the region, rural areas have a higher prevalence than urban areas (Botswana Antenatal Second Generation HIV/AIDS Surveillance Technical Report 2007: 44).

More females (52%) than males (48%) are affected by the HIV epidemic. For every HIV-positive boy under the age of 14 years in the country, there are two HIV-positive girls of the same age group infected. The ratio rises to 1:3 in the age group 15 – 19 years, but converges towards a ratio of 1:1 in the older age groups (Botswana HIV Response Information Management System 2002: 8). The adjusted HIV sero-prevalence among 7,726 pregnant women aged 15 – 49 years attending antenatal clinics in the 22 health districts during the 2007 sentinel surveillance was 33.7% [range 21.4% - 49%] (Botswana Antenatal Second Generation HIV/AIDS Surveillance Technical Report 2007: 29).

Figure 1.5 on this page shows the adjusted age-specific HIV prevalence among pregnant women in Botswana during the 2007 sentinel surveillance. The figure shows the stepwise increase in the prevalence of the HIV/AIDS epidemic in the age cohorts and peaks in the age group 30 – 34 years before following a declining trend thereafter.

The prevalence of HIV in the 15 - 19 years age group is used as a proxy indicator for new infections in the population (Botswana Antenatal Second Generation HIV/AIDS Surveillance Technical Report 2007: 29 – 31).



Source: Botswana 2007 Sentinel Surveillance

Figure 1.5 Adjusted age-specific HIV prevalence among pregnant women attending antenatal services.

The principal mode of HIV transmission is through heterosexual sex. The key determining factors driving the HIV and AIDS epidemic in the country include the migratory pattern of wage workers, alcohol abuse, deterioration of traditional family structures that previously reinforced morality, ignorance about how HIV is transmitted and denial usually due to the stigma associated with infection. Other susceptibility factors are family separation resulting from transfers of married working couples to different areas, poverty, low status of women, high prevalence of sexually transmitted diseases, a high proportion of single parents and early parentage (Botswana HIV Response Information Management System 2002: 8).

Various structures have been instituted to ensure effective implementation of the HIV and AIDS national response. The structures currently in place include the National AIDS Council, Parliamentary Select Committee on HIV/AIDS, National AIDS Coordinating Agency, and sector committees, AIDS/Sexually Transmitted Infections (STI) Units of the Ministry of Health, Ministry of Local Government and District Multi-

Sectoral AIDS Committees (Botswana HIV Response Information Management System 2002: 8).

1.3.1.2 The tuberculosis epidemic in Botswana

Botswana has one of the highest tuberculosis notification rates in the world and consistently reported in excess of 590 cases/100,000 population annually since 2000. The Acute Respiratory Infection Surveys carried out in Botswana since 1956 have shown that the annual risk of TB infection declined from 5.8% in 1956 to 0.1% in 1989. This decline was paralleled by a major decline in tuberculosis notification rates from 506/100,000 in 1975 to 199/100,000 in 1989. However, during the early 1990s, tuberculosis notification began to rise again and reached a peak in 2002 with a notification rate of 623/100,000 and declined to approximately 500/100,000 in 2006.

The increase in the incidence of tuberculosis is a direct result of the increasing prevalence of HIV in the country. The proportion of tuberculosis patients who are co-infected with HIV in the country ranges between 60 and 86%. Tuberculosis is responsible for 13% of all adult deaths and 40% of deaths among people living with HIV and AIDS (National Tuberculosis Programme Manual 2007:12).

Successive drug susceptibility surveys have shown a progressive increase in the number of multi-drug resistance tuberculosis (MDR-TB) cases in the country. The prevalence of MDR-TB in both new and re-treatment cases is 0.8% and 10.4% respectively. Only two cases of extreme drug resistance tuberculosis (XDR-TB) have been notified to date (WHO/HTM/TB/2008.394: Annex 8 & 9).

1.3.1.3 Socio-economic consequences

Botswana's HIV prevalence had a concurrent impact in slowing down economic growth and poverty reduction. The occurrence of HIV-TB co-infection has compounded this impact. This has mainly been due to falling investment, reduction of skilled labour and losses in worker productivity as well as lower government revenue and higher spending (World Bank Report No. 22043-BT 2001: 17). The epidemic has become a serious developmental and social problem impacting negatively on various sectors.

At the macro-economic level the expenditure on health has sharply increased due to treatment and care of patients living with HIV/AIDS and TB/HIV co-infection. The

domestic and international expenditure on HIV/AIDS in 2007 was $229.458 (million US dollars) of which 88.8% was from domestic financing and the remainder from bilateral and multilateral financing (WHO Global Tuberculosis Control 2009:258).

Government is experiencing further budgetary pressure to replace losses in skilled and professional human capital. The productive sector has experienced an increase in morbidity and increased absenteeism which has resulted in high labour costs. The high mortality of skilled and professional human capital in the health care sector has added an additional overwhelming burden of care on the health care system (Botswana Second Medium Term Plan II 1997 – 2002: 19 - 21).

The socio-emotional burden of providing home and community based care has greatly affected households. There has been a demographic shift in population distribution towards children and elderly as the reproductive age group mortality increases. The care burden now rests on the young and elderly. The epidemic has driven households into deeper poverty as a result of loss of income support due to premature mortality of the young and economically active individuals. The observed trend has increased both the dependency ratio and demand for public social and welfare provision (Botswana Second Medium Term Plan II 1997 – 2002: 22).

At the social level, HIV and AIDS still remain stigmatised in the country, causing stressful situations for the infected persons and affected households. The consequence of stigmatisation has been the early withdrawal from the labour force by those infected thus adding to the economic burden on households and the Government (Botswana Second Medium Term Plan II 1997 – 2002: 22).

1.3.1.4 Treatment strategies adopted by the Government

Currently, in an effort to manage the dual HIV/AIDS and TB epidemic, the Botswana Ministry of Health has integrated the HIV/AIDS and TB programs to improve access to care and treatment.

1.3.1.4.1 Antiretroviral therapy

Botswana has been providing combination antiretroviral therapy free of charge to all patients who meet certain clinical criteria since January 2002. All HIV infected men and

women who qualify for antiretroviral therapy undergo adherence counselling before the antiretroviral medications are prescribed by a doctor.

The criteria of initiating antiretroviral therapy are indicated in table 1.1. Patients with $CD4^+$ cell counts ≤ 200 cells/μL are eligible for free antiretroviral therapy under the Botswana Antiretroviral Therapy Programme. In addition, those patients with $CD4^+$ cell count ≥ 200 cells/μL are monitored on a 3 to 6 monthly basis until they qualify to enrol for antiretroviral therapy. However, those with an AIDS defining illness are enrolled for therapy immediately. Very ill patients are also commenced on treatment once they have been stabilised.

Table 1.1 Indications for starting antiretroviral treatment in Botswana adult patients.

Category	CD4 cell count/μL	Recommendation
Symptomatic or TB	Any	Start treatment
Asymptomatic	≤200	Start treatment
Asymptomatic	≥200	Defer treatment Monitor $CD4^+$ cell count

Source: Botswana Guidelines on antiretroviral treatment. Version 2005

Doctors enrolling patients in the programme fill forms with baseline information on the following: past HIV-specific drug history; opportunistic infections; date of HIV test and location where it was conducted; the type of HIV test (rapid, PCR or ELISA), $CD4^+$ cell count; plasma viral load levels; current drug request; history concerning Isoniazid preventive therapy; pregnancy status for female patients; and physical examination findings. For all enrolled patients, a complete prospective profile of antiretroviral therapy is maintained, including the medications prescribed, the amount dispensed, the dose and the prescription refill dates.

The use of triple-drug combination antiretroviral therapy is mandatory under the Botswana National Antiretroviral programme. The current national guidelines for antiretroviral therapy recommend that all qualifying adults be initiated on fixed dose combination of Zidovudine and Lamivudine, co-formulated as Combivir plus either Efavirenz or Nevirapine, with Nevirapine being directly targeted for supply to all women of reproductive potential (Anabwani & Jimbo 2005:11).

In Botswana, as with most Southern African countries, proportionately more females are receiving combination antiretroviral therapy than men even when the higher HIV-1 prevalence among women is accounted for (Muula et al 2007: 63). The factors contributing to this disparity is that more females are accessing testing and blood screening and have better health seeking behaviours than males. In addition, women also access the treatment through the established prevention of mother to child transmission programme. There is a need to evaluate other factors that may be facilitating female access to antiretroviral therapy.

Monitoring therapy is undertaken by using viral load and $CD4^+$ cell counts (to assess treatment effectiveness), clinical examination (to assess toxicity, adherence, and clinical outcomes) and by blood chemistry and haematology (to assess drug toxicity). The recommended schedule for monitoring is shown in table 1.2 below (Anabwani & Jimbo 2005: 19).

Table 1.2 Recommended schedules for monitoring adult patients on antiretroviral treatment

	Baseline	2 weeks	1 month	3 months	6 months	12 months	Thereafter
Viral load	✓	-	-	✓	✓	✓	3 monthly
CD4$^+$ cell counts	✓	-	-	✓	✓	✓	3 monthly
Full Blood Count	✓		✓	✓	-	-	As indicated
U & E	✓	-	-	-	-	-	As indicated
AST/ALT	✓	✓	✓	✓	-	-	As indicated
RPR	✓	-	-	-	-	-	As indicated
Fasting lipid profile	✓	-	-	-	✓	-	As indicated
Monitoring OIs*, toxicity & adherence	✓	✓	✓	✓	✓	✓	3 monthly
Pap smear	✓	-	-	-	-	-	As indicated

Source: Botswana Guidelines on antiretroviral treatment. Version 2005

*OIs – Opportunistic Infections

The HIV Drug Resistance Threshold Survey (HIVDR – TS), conducted in 2005 and 2007 as part of the sentinel surveillance showed that the primary antiretroviral drug resistance prevalence is less than 5% in Gaborone and Francistown (Botswana Antenatal Second Generation HIV/AIDS Surveillance Technical Report 2007: 50 – 52). However, the countrywide primary antiretroviral drug resistance prevalence during the same period of this survey was unknown (Botswana Second Generation HIV/AIDS Surveillance Technical Report 2006: 57 - 60).

On average, national secondary antiretroviral drug resistance remains below 3% (Botswana Antenatal Second Generation HIV/AIDS Surveillance Technical Report 2007: 50 – 52).

1.3.1.4.2 Isoniazid preventive therapy

Globally, the provision of Isoniazid preventive therapy remains at very low levels. Only 27,056 people in 2006 – equivalent to less than 0.1% of the estimated 33 million estimated to be infected with HIV – have been treated with Isoniazid. Botswana accounts for 70% of the total number of people reported to be on Isoniazid preventive therapy globally in 2006 (WHO Report -Global Tuberculosis Control 2008:4).

The Botswana Isoniazid preventive therapy programme was introduced in 2001 as one of the components of the Botswana National Tuberculosis Programme to prevent tuberculosis among people living with HIV and AIDS. Currently, all districts offer Isoniazid preventive therapy to HIV infected individuals who are thoroughly evaluated with a user friendly screening algorithm to exclude active tuberculosis before commencing therapy. To date approximately 38,000 HIV positive patients have been screened for TB and 30,000 have been started on Isoniazid preventive therapy since 2001 (Botswana National Tuberculosis Program Evaluation Report 2006: 24). The exclusion criteria for Isoniazid preventive therapy include:

- Symptoms of active TB;
- Terminal AIDS;
- Active hepatitis;
- TB diagnosed and treated in the last 3 years;
- Clinical suspicion of extra-pulmonary TB;
- Prior Isoniazid intolerance or allergy;
- Habitual treatment defaulting;
- Pregnancy;
- Children <16 years of age, unless he/she is a close (household) contact of a sputum-positive adult; and
- Patients receiving repeat Isoniazid preventive therapy.

Once active TB has been excluded, self-administered daily Isoniazid at a dosage of 300mg/400mg for six months is prescribed regardless of $CD4^+$ cell count. Patients who weigh less than 30kg receive 10mg/kg with the same frequency and duration. Pyridoxine (Vitamin B6) is co-administered (25mg orally daily) for the prevention of peripheral neuropathy. Initiation of Isoniazid preventive therapy is done shortly after the diagnosis of HIV infection to avoid overlapping toxicities with antiretroviral therapy.

However, Isoniazid preventive therapy and antiretroviral therapy can safely be co-administered. Most antiretroviral treatment naïve patients are now either concurrently taking combination antiretroviral therapy and the Isoniazid preventative therapies or receiving the combination antiretroviral therapy after completing a course of Isoniazid Preventive Therapy. Patients returning for monthly refills are evaluated by the nurse or doctor who assesses unintentional weight loss, drug intolerance and side effects of Isoniazid. Patient adherence to therapy is assessed by means of pill counts and recorded in the Isoniazid Preventive Therapy register.

Patients with signs and symptoms of active tuberculosis discontinue Isoniazid preventive therapy and commence combination anti-tuberculosis therapy (National Tuberculosis Programme Manual 2007: 22).

Directly Observed Therapy – Short Course (DOTS) using a combination of 4 anti-tuberculosis drugs [Ethambutol, Rifampicin, Isoniazid and Pyrazinamide] for a minimum period of 6 months is the strategy employed for the treatment of active tuberculosis in the country. The DOTS strategy means that a health care worker, community volunteer or relative of a patient directly supervises the patient swallowing the medication. Direct Observation of Therapy was introduced in 1993 and occurs throughout the country (National Tuberculosis Programme Manual 2007:15; WHO Stop TB Strategy 2006).

Current available data in the country indicates a significant increase in resistance to anti-TB drugs among new cases from 0.2% ($n < 10$) in 1996 to 10.4% ($n = 123$) in 2007. However, the resistance to both Isoniazid and Rifampicin is relatively low as compared to other areas of the world with resistance to Isoniazid and Rifampicin of 4.5% and 2% respectively (WHO/HTM/TB /2008:394: 102, 130).

1.3.1.4.3 Research problem statement

HIV-related immune suppression involves much more than treatment with antiretroviral therapy as people living with HIV/AIDS are at an increased risk of a broad range of debilitating and life-threatening conditions. As a result of a synergistic relationship between HIV and tuberculosis, the preferred treatment for the management of HIV/TB co-infection is Isoniazid tuberculosis preventive and combination antiretroviral therapy.

The efficacy of prophylactic Isoniazid therapy to prevent TB in HIV-infected patients with latent TB infection has been demonstrated in several randomised clinical trials studies (Lim, Okwera, Manyanja-kizza, Ellner, Mugerwa & Whalen 2006: 180 - 181). Studies by Grant, Charambous, Feilding, Day, Corbett, Chaisson, De Cock, Hayes and Churchyard 2005: 2719 – 2725; Woldehanna and Volmink 2004: CD000171 have demonstrated that primary Isoniazid preventive therapy reduced incidence of TB by 38% - 46% among individuals with no history of TB prior to the study and in patients receiving placebo respectively (RR 0.64, 95% CI 0.51 – 0.81). This benefit was more pronounced in individuals with a positive tuberculin skin (RR 0.38, 95% CI 0.25 – 0.57) than in those who had a negative test (RR 0.83, 95% CI 0.58 – 1.18). However, there was no evidence that preventive therapy versus placebo reduced all-cause mortality (RR 0.95, 95% CI 0.85 – 1.06) although a favourable trend was found in people with a positive tuberculin test (RR 0.80, 95% CI 0.63 – 1.02).

A research by (Wilkinson, Kon, Newton, Meintjes, Davidson, Pasvol & Wilkinson 2006: 356) showed that Isoniazid increased the number of gamma- interferon (IFN-γ) producing T-cells (responsible for enhancing host immune response) when they analysed a cohort of *Mycobacterium tuberculosis*-infected people treated with Isoniazid and Rifampicin. In their study, recent immigrants to the United Kingdom with no clinical or serological evidence of HIV-1 infection, a normal chest radiography results and strongly positive tuberculin skin test results (Heaf grade≥3, equivalent to a Mantoux response of ≥15mm indurations) were recruited. Patients elected to receive preventive therapy for latent tuberculosis infection consisting of 12 weeks of Isoniazid (5mg/kg, up to 300mg daily) plus Rifampicin (10mg/kg, up to 600mg daily) or observation by serial radiographs. *In vitro* blood assay evaluation of the frequency of *Mycobacterium tuberculosis* antigen-specific IFN- γ-producing T cells by enzyme immunospot

(ELISPOT) was performed at enrolment, during treatment (2 – 6 weeks into treatment) and at the end of treatment (12 weeks).

Their findings were that IFN – γ-Spot Forming Cell counts tended to increase transiently, early during treatment for latent tuberculosis infection followed by a decrease toward the end of treatment in patients treated with Isoniazid and Rifampicin. The increase during treatment was significant when compared with response at both onset (p = 0. 006, student t test) and completion (p = 0.004) of preventive therapy. The difference between the IFN – γ-Spot Forming Cell counts at the beginning and end of treatment was not significant (p = 0.12) as the small sample size resulted in insufficient study power.

ELISPOT cultures tests were set up to further determine whether drug-induced disruption of actively dividing bacilli accounted for some initial increase in IFN – γ- Spot Forming Cell counts. There was a statistically significant difference between cultures treated with Isoniazid and Rifampicin (p = 0.0003). The researchers concluded that Isoniazid may contribute to the increased release of cell-associated antigens which become more accessible to circulating antigen-specific T cells in the peripheral blood. Production of IFN – γ has immune enhancing responses to bacterial, viral and tumour diseases (Palomino, Leao & Ritacco 2007: 164).

Many countries that lost the opportunity of introducing Isoniazid preventive therapy are now providing antiretroviral therapy that has shown to reduce the incidence of TB in the communities (Badri, Wilson & Wood 2002: 2062 – 2063; Santoro-Lopes, Felix de Pinho, Harrison & Achecter 2002: 545). Not only has highly active antiretroviral therapy reduced the incidence of TB but has also markedly reduced the mortality of HIV-1-infected patients (Egger, May, Chene, Phillips, Ledergerber, Dabis, Costagliola, d' Amino, de WF, Reiss, Lundgren, Justice, Staszewski, Leport, Hogg, Sabin, Gill, Salzberger & Sterne 2002: 125). The most important goals of antiretroviral therapy are maximal suppression of plasma viral load for as long as possible to delay the selection of drug resistance, to preserve $CD4^+$ cell count and to confer substantial clinical benefits (Panel on Antiretroviral Guidelines for adults and Adolescents 2008: 10).

Hung, Chen, Hsiao, Hsieh, Sheng & Chang (2005: 2618) conducted a prospective observational study to determine the survival and treatment responses to antiretroviral therapy between HIV-1-infected patients with active TB and those without TB. They found that increases in $CD4^+$ cell count (p = 0.70) and the proportion of patients achieving undetectable viral load [RR 0.93, 95%CI 0.65 – 1.34; p = 0.71] at four weeks of antiretroviral therapy were similar between the two groups. They conclude by saying that virological and immunological responses to highly active antiretroviral therapy of HIV-1-infected TB patients who were concurrently on treatment with anti-tuberculosis therapy and antiretroviral therapy were similar to those observed in non-TB patients. Similar findings were observed by Breen, Miller, Gorsuch, Smith, Ainsworth, Ballinger, Swaden, Cropley, Johnson and Lipman (2006: 1439) when they matched immunological (p = 0.73, signed rank test) and virological (p = 0.35, McNemar's test) responses in patients with HIV infection and TB who received antiretroviral therapy and anti-tuberculosis therapy concurrently with HIV-infected subjects without TB starting antiretroviral therapy.

Combination of two nucleoside reverse transcriptase inhibitors and either a non-nucleoside reverse transcriptase inhibitors or protease inhibitors have set the standard for highly active antiretroviral therapy. Manosuthi, Sungkanuparph, Vibgahgool, Rattanasiri, & Takkinstian (2004:107) compared Nevirapine vs. Efavirenz-based antiretroviral regimens in antiretroviral naïve patients with advanced HIV infection. Their definition of advanced HIV infection was a patient with $CD4^+$ cell count <100 cell /µL and viral load >100,000 copies/mL. They found that the probability of virological success estimated by the Kaplan-Meier survival method for the Nevirapine group at 3 months was 30.8% [95% CI: 16.7 (52.2%)] compared to 63.1% [95% CI: 44.7 (81.3%) at 4 months. The corresponding 3 and 4 months values for the Efavirenz group were 41.2% [95% CI: 25.8 (61%)] and 62.9% [95% CI: 45.7 (80.1%)] respectively. The median time to virological success (p = 0.678) and time to median increase in $CD4^+$ cell count of 100 cells/µL (p = 0.144) for the two groups were not statistically significant. After adjusting for baseline covariates of age, opportunistic infections and viral load using Cox proportional hazards, patients in the Nevirapine-based regimen had 25% [HR = 0.75, 95% CI: 0.37 – 1.15] less chance of virological success than patients who received Efavirenz-based regimen (p = 0.415). The conclusion drawn was that

Nevirapine and Efavirenz - based antiretroviral therapy regimens are effective and comparable in terms of immunological and virological outcomes.

Studies by (Hung et al 2005:2618; Breen et al 2006: 1439; Manosuthi et al 2004: 107) compared patients concurrently on antiretroviral and anti-tuberculosis therapies in patients with active TB to those without TB. However, research is lacking on the effect of Isoniazid preventive and Nevirapine and Efavirenz-based antiretroviral therapies on immunological and virological responses in HIV-1-infected individuals initiating antiretroviral therapy. Botswana, the first sub-Saharan African country to provide free public access to antiretroviral therapy for patients with HIV infection, with a countrywide Isoniazid preventive therapy program, provides a unique opportunity to study this phenomenon. This quantitative non-experimental retrospective cohort study will answer the following research question:

How do the immunological and virological treatment outcomes of HIV infected patients who completed Isoniazid TB prevention treatment prior to receiving either Nevirapine or Efavirenz-based combination antiretroviral treatment; compare with those HIV-infected who receive these treatments concurrently?

1.4 AIMS OF THE STUDY

1.4.1 Research purpose

The purpose of this investigation is to compare the immunological and virological responses of antiretroviral naïve HIV infected patients in the following four treatment groups: **$EFV_{ipt\text{-}current}$**, **$NVP_{ipt\text{-}current}$**, **$EFV_{ipt\text{-}past}$** and **$NVP_{ipt\text{-}past}$**. By pairing these four treatment groups against each other results in six possible comparison scenarios described under section 1.4.2.

1.4.2 Research questions

The following research questions are the focus of this study:

- How do patients concurrently taking Combivir + Nevirapine and Isoniazid Preventive Therapies **[$NVP_{ipt\text{-}current}$]** differ from those taking Combivir + Nevirapine

after completing Isoniazid Preventive Therapy [**$NVP_{ipt\text{-}past}$**] in terms of their $CD4^+$ cell counts and viral loads?

- How do patients concurrently taking Combivir + Efavirenz and Isoniazid Preventive Therapies **[$EFV_{ipt\text{-}current}$]** differ from those taking Combivir + Efavirenz after completing Isoniazid Preventive Therapy **[$EFV_{ipt\text{-}past}$]** in terms of their $CD4^+$ cell counts and viral loads?
- How do patients concurrently taking Combivir + Nevirapine and Isoniazid Preventive Therapies **[$NVP_{ipt\text{-}current}$]** differ from those concurrently taking Combivir + Efavirenz and Isoniazid Preventive Therapies **[$EFV_{ipt\text{-}current}$]** in terms of their $CD4^+$ cell counts and viral loads?
- How do patients taking Combivir + Nevirapine after completing Isoniazid Preventive Therapy **[$NVP_{ipt\text{-}past}$]** differ from those taking Combivir + Efavirenz after completing Isoniazid Preventive Therapies **[$EFV_{ipt\text{-}past}$]** in terms of their $CD4^+$ cell counts and viral loads?
- How do patients taking Combivir + Nevirapine after completing Isoniazid Preventive Therapy **[$NVP_{ipt\text{-}past}$]** differ from those concurrently taking Combivir + Efavirenz and Isoniazid Preventive Therapies **[$EFV_{ipt\text{-}current}$]** in terms of their $CD4^+$ cell counts and viral loads?
- How do patients concurrently taking Combivir + Nevirapine and Isoniazid Preventive Therapy **[$NVP_{ipt\text{-}current}$]** differ from those concurrently taking Combivir + Efavirenz after completing Isoniazid Preventive Therapies **[$EFV_{ipt\text{-}past}$]** in terms of their $CD4^+$ cell counts and viral loads?

1.4.3 Research hypothesis

The research hypotheses are:

- Null hypothesis - the immunological and virological responses will be equivalent in the four groups (H_o: $\mu_{EFVipt\text{-}current} = \mu_{NVPipt\text{-}current} = \mu_{EFVipt\text{-}past} = \mu_{NVPipt\text{-}past}$).
- Research hypothesis - there is inequality in immunological and virological responses among the four groups (H_o: $\mu_{EFVipt\text{-}current} \neq \mu_{NVPipt\text{-}current} \neq \mu_{EFVipt\text{-}past} \neq \mu_{NVPipt\text{-}past}$).

1.5 SIGNIFICANCE OF THE STUDY

Isoniazid preventive and antiretroviral therapies are key priority services for patients infected with HIV in Botswana. Treating patients for latent tuberculosis and HIV infection with the above mentioned therapies results in morbidity and mortality reduction. It is anticipated that this study will generate knowledge for clinicians treating and caring for HIV/AIDS patients and assist in addressing the interface of the TB and HIV epidemics as part of the health sector response to the intersecting epidemics.

In addition, the study findings can be used in implementing collaborative TB and HIV program activities.

1.6 DEFINITIONS OF KEY CONCEPTS

1.6.1 Combination antiretroviral therapy

Antiretroviral therapies are medications used for the treatment of infection with retroviruses, primarily HIV (Dybul, Fauci, Bartlett, Kaplan, Pau; Panel on Clinical Practices for Treatment of HIV 2002: 381 – 433). Combination antiretroviral therapy utilises several antiretroviral medications, typically three or four are taken in combination. The approach is known as Highly Active Anti-Retroviral Therapy (HAART) (Dybul et al 2002: 381 – 433).

A Nevirapine-based antiretroviral regimen is defined as a combination antiretroviral therapy containing Nevirapine as the preferred non-nucleoside reverse transcriptase inhibitor drug. Efavirenz-based antiretroviral regimen is defined as a combination antiretroviral therapy containing Efavirenz as the preferred non-nucleoside reverse transcriptase inhibitor drug (MASA - the National ARV program of Botswana 2008: 40).

1.6.2 HIV infected patient

HIV infection is defined as an infection caused by a retrovirus that can lead to Acquired Immunodeficiency Syndrome (AIDS), a condition in humans in which the immune system begins to fail, leading to life threatening opportunistic infections (Coffin, Haase, Levy, Montagnier, Oroszlan, Teich, Temin, Toyoshima, Varmus, Vogt & Weiss 1986: 10).

A patient is defined as an individual awaiting or receiving medical care and treatment; or the recipient of any of various personal services; or one that is acted upon (Encyclopaedia Britannica 2006, sv “patient”).

An HIV infected patient is an individual infected with HIV leading to Acquired Immune Deficiency Syndrome who awaits or receives medical care and treatment.

1.6.3 Immunological outcome

Immunological outcome refers to an increase in $CD4^+$ cell counts of 25 – 50 cells/ µL **OR** 5% percentage per year **OR** $CD4^+$ cell count/percentage drop of 50% from the highest recorded value (MASA - the National ARV program of Botswana 2008: 40).

$CD4^+$ cell count is defined as the number of $CD4^+$ cells that determine how well the immune system is working in people who have been diagnosed with HIV (Mellows, Margolick, Phair, Rinaldo, Detels, Jacobson & Munoz 2007: 2349).

1.6.4 Isoniazid preventative therapy

Isoniazid preventative therapy refers to the administration of Isoniazid to a maximum dosage of 400mg/day for the prevention of TB in HIV infected patients. In addition patients are also given pyridoxine 25mg/day to prevent the development of peripheral neuropathy (The Botswana Isoniazid Preventive Therapy: 2004:19). In this study a patient will be considered to be on Isoniazid preventive therapy after s/he has taken Isoniazid for at least 1 month and is still on therapy at the time of data collection. Patients who will have taken Isoniazid preventive therapy medication for six months and discontinued it will be considered to have completed therapy.

1.6.5 TB/HIV co-infected patient

A co-infected patient is an individual infected with both Human Immunodeficiency Virus and *Mycobacterium tuberculosis* (WHO/HTM/TB/2004.329: 37).

1.6.6 Virological outcome

Viral load refers to the number of virus particles per millilitre of blood by quantifying HIV RNA which is an indicator of viral replication (MASA - the National ARV program of Botswana 2008: 40).

Virological response can either be an increase or decrease in viral replication. In this study, virological response refers to a fall in viral load of 1 log ($1/10^{th}$) of the starting value by 3 months of HAART initiation **OR** viral load of < 400 copies/ml by 6 months after of antiretroviral combination treatment initiation **OR** viral load response can initially be suppressed to < 400 copies/ml or becomes detectable (i.e. > 400copies/ml) again at some time later in the future (MASA - the National ARV program of Botswana 2008::40).

1.7 RESEARCH SETTING

The study will be facility-based at six infectious disease clinical care centres. These facilities are Athlone hospital, Tsopeng, Area W, Botswelelo, Jubilee and Gerald Estates clinics.

1.8 RESEARCH DESIGN AND METHOD

1.8.1 Design

A quantitative, non-experimental and retrospective cohort design was used to describe concepts, identify possible relationships and describe the differences in immunological and virological outcomes in four treatment groups. By pairing these four treatment groups' results in six contrast combinations as described in the research questions.

1.8.2 Method

1.8.2.1 Study population and treatment allocation

Medical records of antiretroviral treatment naive patients exposed to Isoniazid preventive therapy enrolled between 1st January 2006 and 31st December 2006 were used for this study. The patients were initiated on nucleoside reverse transcriptase inhibitors Zidovudine and Lamivudine co-formulated as Combivir™. In addition, these patients had either completed or were still taking Isoniazid preventive therapy. A standard dose of pyridoxine is also given to patients on preventive therapy for the prevention of peripheral neuropathy resulting from Isoniazid use. The combination of antiretroviral therapy and exposure status to Isoniazid preventive therapy results in four treatment groups:

1. $NVP_{ipt\text{-}current}$
2. $NVP_{ipt\text{-}past}$

3. $EFV_{ipt\text{-}current}$
4. $EFV_{ipt\text{-}past}$.

1.8.2.2 Sample and sampling technique

A sample of two hundred medical records selected through purposive, non-probability sampling technique was used to select records meeting the study eligibility criteria. Refer to section 3.3.1.3 for the study eligibility criteria.

1.8.2.3 Data collection points and assays

A structured data collection approach using closed-ended (fixed-alternative) data collection instrument was used to record abstracted data from medical records. $CD4^+$ cell count and viral load at baseline (start of antiretroviral treatment) and weeks 12, 24 and 36 of treatment were abstracted.

The respective assays that were used to measure $CD4^+$ cell count and viral load are FACS Calibur™ flow cytometer (Becton Dickinson, San Jose, CA, USA) and Amplicor™ HIV-1 Monitor Assay (version 1.5) which uses quantitative reverse transcriptase – polymerase chain reaction analysis (Roche Diagnostic Systems, Branchburg, New Jersey, USA) and has a dynamic range of 400 – 750,000 HIV-1 RNA copies/mL (Botswana Guidelines on Antiretroviral Treatment: 2005: 3).

1.8.2.4 Statistical analysis

The researcher used descriptive and multivariate analysis of variance (MANOVA) to analyse and summarise data. Data was measured on the continuous and ratio scales. Tables and figures were used for the graphical presentation of the data. Data were analysed using SAS JMP statistical software version 8.0 (SAS Institute, Cary, NC, USA). Details on the research design and methods are described in chapter 3.

1.9 SCOPE OF THE STUDY

The study focuses on immunological and virological outcomes in antiretroviral naïve patients initiated on antiretroviral therapy and exposed to Isoniazid therapy. The study analysis will compare the four treatment groups as regards;

- The mean increase in $CD4^+$ cell count,
- Rate of immunological success and

- Time to and proportion of patients achieving virological success.

It is anticipated that the study results will guide clinical practice in the management of HIV patients initiated on antiretroviral therapy.

1.10 CONCLUSION

The rising incidence of tuberculosis in many regions of the world is closely linked to the Human Immunodeficiency Virus epidemic. Worldwide in 2007, approximately 14 million people were co-infected with TB and HIV-1 and 70% of these live in Africa (Lange et al 2007: 416). Isoniazid tuberculosis preventive and combination antiretroviral therapy constitute one of the strategies that national governments use to manage TB/HIV co-infection. The efficacy of both therapies in reducing patient morbidity and mortality has been demonstrated in several research studies, but there is a need to determine the effectiveness of various treatment regimens in the Botswana context.

In this chapter the researcher proposed a research study to investigate the immunological and virological treatment outcomes of HIV infected patients who completed Isoniazid TB prevention treatment prior to receiving either Nevirapine or Efavirenz-based combination antiretroviral treatment compared with those HIV-infected who receive these treatments concurrently.

The context and research problem were discussed in detail. The study aim, significance and definitions of key concepts used in the research were defined. The research setting and study design and methods to guide the conduct of the research were elaborated. The chapter concluded by laying out the structure of the dissertation. In the next chapter the researcher presents a report on the literature review.

1.11 STRUCTURE OF THE DISSERTATION

Chapter 1 of the dissertation discussed in detail the country profile of Botswana and how the government has responded to the challenges of the HIV and tuberculosis epidemics in the country. The main objective of this quantitative, non-experimental, retrospective cohort study was to compare immunological and virological outcomes in antiretroviral naïve patients exposed to Isoniazid preventive therapy. The study sample composed of eligible medical records of patients managed in the public health sector

from January 2003 to December 2006. Abstracted data from the records was recorded onto a developed data collection instrument and entered into the database for analysis using multivariate analysis of variance and pairwise post hoc contrast tests to compare the treatment outcomes of the groups.

Chapter 2 reviews literature related to the tuberculosis and HIV epidemics. It additionally presents an analysis of the literature on the management and treatment outcomes of HIV and tuberculosis in relation to the study topic.

In chapter 3 the empirical phase of the study is documented. It discusses the research design and methodology applied, strategies employed to enhance data quality and ethical considerations. Research findings of the statistical analyses and the study limitations are discussed in chapter 4. The interpretation of the findings, conclusions and recommendations are presented in chapter 5. Literature used in the conduct of this study is referenced and annexure are listed at the end of the dissertation.

CHAPTER 2

LITERATURE REVIEW

2.1 INTRODUCTION

Researchers rarely conduct research in an intellectual vacuum; their studies are undertaken within the context of an existing base of knowledge (Polit & Beck 2008: 105). Chapter 2 presents a review of literature on interventions against HIV infection which include TB prophylaxis and antiretroviral therapy. Delivering these interventions requires effective collaboration with HIV/AIDS programmes and promotes continuity of care for the patients. The researcher reviewed the theoretical and empirical literature to determine the background of the research problem and the methodological aspects of the studies done in relation to the research.

2.2 HUMAN IMMUNODEFICIENCY VIRUS INFECTION

2.2.1 Epidemiology

The discovery of HIV as the causative organism of Acquired Immunodeficiency Disease Syndrome (AIDS) was a major scientific breakthrough of the 20th century (Montagnier 2002: 1727; Gallo 2006: 72). Two types of viruses which infect humans (HIV-1 and HIV-2) have very similar structure and genome (Retroviridae 2006: 1). These viruses are thought to have respectively evolved from Simian Immunodeficiency Virus in chimpanzee (SIV_{cpz}) and Simian Immunodeficiency Virus in sooty mangabey (SIV_{mm}) after a series of mutations during the latter part of the twentieth century (Keele, van Heuverswyn, Li, Bailes, Takehisa, Santiago, Bibollet-Ruche, Chen, Wain, Liegois, Loul, Mpoudi, Ngole, Bienvenue, Depaporte, Brookfield, Sharp, Shaw, Peeters & Hahn 2006: 523). HIV-1 is more virulent, readily transmissible and responsible for the global epidemic than HIV-2 (Reeves & Doms 2002: 1253).

The HIV pandemic remains the most serious infectious disease challenge to public health since it was first described in homosexual men in USA in 1981(Gottlieb, Schroff, Schanker, Weisman, Fan, Wolf & Saxon 1981: 1425). In 2007, the globally estimated number of people living with HIV and AIDS was 33.2 million [30.6 – 36.1 million] of which 30.8 million [28.2 – 33.6 million] were adults, 15.4 million [13.9 – 16.6 million] women and 2.5 million [2.2 – 2.6 million] were children under the age of 15 years. Sub-Saharan Africa accounts for 68% (22.5 million) of all HIV cases in the world (UNAIDS/WHO 2007: 7). The epidemic in this region varies significantly in scale with

HIV prevalence in the age group 15 to 49 years ranging from less than 2% in some countries in the Sahel to above 15% in most of Southern Africa. South Africa has the largest number of HIV infected patients in the world followed by Nigeria (McNeil Jr. 2007: 1). In contrast, Oceania (Australia, New Zealand and Papua New Guinea) has the lowest number of adults and children infected with HIV with East Asia recording the lowest whereas Eastern Europe and Central Asia has the highest prevalence of adult HIV infections (UNAIDS/WHO 2007: 7).

New HIV infections in 2007 totalled 2.5 million [1.8 – 4.1 million] with adults accounting for 2.1 million [1.4 – 3.6 million] and children under the age of 15 years 420,000 [350,000 – 540,000 million]. Almost 32% of all these new HIV infections occurred in Southern Africa (UNAIDS/WHO 2007: 14). However, recent available data indicates that new infections in sub-Saharan Africa has declined from 2.2 million [1.7 – 2.7 million] in 2001 to 1.7 million [1.4 – 2.4 million] in 2007 and that the number of people living with HIV and AIDS has increased from 20.9 million [19.7 – 23.6 million] in 2001 to 22.5 million [20.9 – 24.3 million] in 2007 with almost 61% being women. During the same period the adult (15 to 49 years) HIV prevalence declined from 5.8% [5.5 – 6.6%] in 2001 to 5.0% [4.6 – 5.5%] in 2007 (UNAIDS/WHO 2007: 14).

The number of AIDS related deaths was 2.1 million [1.9 – 2.4 million] with 1.7 million [1.6 – 2.1 million] adults and 330,000 million [310,000 – 380,000 million] children under the age of 15 years. These deaths occurred mostly in low and middle income countries with inadequate access to prevention services and antiretroviral therapy. The sub-Saharan Africa region accounted for 76% of the global total and it is estimated that there are 11.4 million [10.5 – 14.6 million] orphans due to HIV/AIDS in this region (UNAIDS/WHO AIDS 2007: 7). This has greatly reduced life expectancy from 65 years to 48 years in 35 high prevalence African nations. However, a reversal in life expectancy is expected in those areas where antiretroviral therapy will be more widely available (UNAIDS 2006: 53).

On a positive note, the estimated number of persons living with HIV and AIDS in 2007 was 16% less compared with the estimate published in 2006 (39.5 million [34.7 – 47.1 million]). This reduction was as a result of declining prevalence of HIV infection in some

countries such as Angola, Kenya, India, Mozambique, Nigeria and Zimbabwe. However, with reduction of HIV-associated deaths resulting from the introduction of antiretroviral therapy, the prevalence of HIV is expected to increase (UNAIDS/WHO 2007: 6). Table 2.1 below summarises the global burden of HIV/AIDS epidemic in the WHO regions discussed in the above paragraphs.

Table 2.1 Summary of the regional HIV and AIDS statistics, 2007

Region	Adults and children living with HIV	Adults and children newly infected with HIV	Adult prevalence (%)	Adult and children deaths due to AIDS
Sub-Saharan Africa	22.5 million	1.7 million	5	1.6 million
Middle East and North Africa	380,000	35,000	0.3	25,000
South and South East Asia	4.0 million	340,000	0.3	270,000
East Asia	800,000	92,000	0.1	32,000
Oceania	75,000	14,000	0.4	1,200
Latin America	1.6 million	100,000	0.5	58,000
Caribbean	230,000	17,000	1.0	11,000
Eastern Europe and Central Asia	1.6 million	150,000	0.9	55,000
Western and Central Europe	760,000	31,000	0.3	12,000
North America	1.3 million	46,000	0.6	21,000
Total	33.2 million	2.5 million	0.8	2.1 million

Source: UNAIDS/WHO: AIDS Epidemic Update 2007

2.2.1.1 Pathogenesis

HIV infection begins with transmission of the virus mostly during unprotected sex or through exposure to contaminated blood and body fluids. Refer to section 2.2.1.2 for details on how HIV is transmitted. The HIV virus is a single-stranded RNA *Lentivirus* of the *retroviridae* family which are enveloped and have surface glycoproteins (gp120) responsible for viral entry into host cells (Stricher, Huang, Descours, Combes, Decker, Kwon, Lusso, Shaw, Vita, Kwong & Martin 2008: 511). It infects a variety of immune cells such as T-lymphocytes, macrophages and microglia cells (Berger & Alkhatib 2007:403) and entry into these target cells is through a primary receptor of the virus called the CD4 molecule and chemokine co-receptors (Clapham & McKnight 2002: 1809; Lusso 2006: 447). Macrophage (M-tropic) strains of HIV-1 use the CCR5 *β*-

chemokine receptor whereas T-tropic strains use the *α*-chemokine receptor CXCR4 for entry into target cells (Coakley, Petropoulous & Whitcomb 2005: 10). Both these chemokine receptors are used by dual-tropic HIV-1 strains (Jones & Nelson 2007: 391).

Possessing the CCR5-Δ32 mutation results in resistance to infection with the R5 virus as this stops HIV from binding to this co-receptor (Agrawal, Lu, Quingwen, Vanhorn-Ali, Nicolescu, McDermott, Murphy & Alkhatib 2004: 2278).

Upon entry into the cell, the virus is susceptible to a host of specific cellular antiviral immune defence mounted by the body to control its replication (Towers 2007: 40; Yeung, Benkirane & Jeang 2007: 74; Xie, Invernizzi, Richard & Wainberg 2007: 4226; Goila-Gaur 2008: 51). Failure of the host immunity to control viral replication results in the viral RNA genome being transcribed into double-stranded DNA by a virally encoded reverse transcriptase that is present in the virus particle. The resultant viral DNA migrates from the cell cytoplasm into the cell nucleus and integrates into one of the 46 chromosomes in the host cell with the help of a viral enzyme called integrase (Iordanskiy, Berro, Altieri, Kashanchi & Bukrinsky 2006: 4). Once inside the cell the virus can go into latency phase or multiply to produce virus particles that can infect other cells. This leads to a progressive decline in $CD4^+$ cell count which occurs by three mechanisms: direct killing of and increased apoptosis of infected cells and by $CD8^+$ cytotoxic lymphocytes killing infected $CD4^+$ cells. Decline in cell-mediated immunity up to a certain critical point results in the body being vulnerable to opportunistic infections (Jones & Nelson 2007: 391).

2.2.1.2 Transmission

Transmission of HIV infection is dependent on the viral load (infectiousness) of the index case and the susceptibility of the uninfected partner especially during acute HIV infection (Kamps & Hoffmann 2007: 24). People can contract HIV infections through the following possible ways:

1. Unprotected sexual intercourse, either homosexual or heterosexual with an infected partner. Heterosexual transmission is the leading cause worldwide.
2. Through contact with infected blood, blood products or other body fluids or tissues of infected persons. This can occur by injection or transfusion of

contaminated blood and blood products, sharing of unsterilized injection, tattooing and scarification equipment that has been used by someone who is infected

3. Mother to child transmission [during pregnancy, at birth and through breast feeding]. In Africa mother to child transmission of HIV has reversed decades of steady progress in child survival. This mode of transmission can be reduced by offering voluntary counselling and testing for HIV infection, breast feeding counselling and offering antiretroviral therapy (WHO/UNICEF/UNAIDS/UNFPA 2006: 1).

Due to hormonal changes, vaginal microbial ecology and a higher prevalence of sexually transmitted diseases, women are more susceptible to HIV-1 infection than men, (Clapham & McKnight 2001:47 – 48; Quinn & Overbaugh 2005: 1582; Centers for Disease Control and Prevention Fact Sheet 2007: 1 - 2).

The presence of a genital ulcer increases the risk of HIV infection by approximately 30 – 60% (Lorenzen & Graefe 2007:609). Though persons with gonorrhoea, Chlamydia and trichomoniasis infections have a lesser chance of infection, they are at increased risk due to local accumulations of lymphocytes and macrophage that serve as targets for HIV infection (Rothenberg, Wasserheit, St Louis & Douglas 2001: 411).

2.2.1.3 Diagnosis

There has been important progress in regards to laboratory diagnosis of HIV infection. This has evolved from the detection of virus-specific antibodies directed against certain viral antigens through detection of viral antigens such as p24 (the core protein of the virus) to more recently DNA-Polymerase Chain Reaction (PCR). The latter test can also quantify the concentration of viral nucleic acid (viral load) and has become indispensable in monitoring the effectiveness of antiretroviral therapy (Preiser & Korsman 2007: 50).

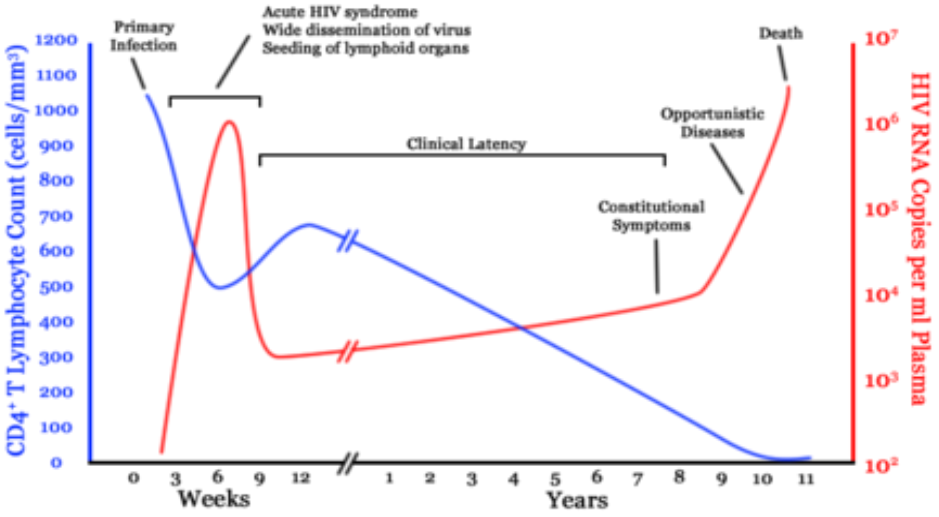
Today, a number of rapid HIV tests kits are available at point-of-care or bedside diagnostic tests. To make a diagnosis of HIV requires performing an Enzyme-Linked Immunosorbent Assay (ELISA) and verification with a confirmatory test such as Western Blot or Immunofluorescence Assay (Preiser & Korsman 2007: 42 – 44).

In Botswana, the ELISA test remains the “gold standard” of HIV diagnosis and is used for mass screening of individuals and blood donor specimens. Two rapid tests kits (Unigold™ and Determine™), run in parallel at the point-of-care are used for the diagnosis of HIV infection. Individuals are categorised as positive if both test kits used show a positive result. Discordant test kit results are confirmed by a double ELISA test (Routine HIV Testing 2008: iii). Testing patients for HIV infection is routine and on an “opt-out” basis. Opt-out HIV testing is provider initiated after notifying the patient that the test will be done and consent is inferred unless the patient declines. In the Botswana context where routine HIV testing is routine, all patients in a clinical setting are informed that they will be tested for HIV unless they explicitly refuse (Steen & de la Hoz Gomez 2005: 4).

The Centers for Disease Control and Prevention and WHO clinical staging can be used to diagnose HIV infection in settings with limited laboratory facilities. World Health Organization staging is widely used and is now recommended for classifying the clinical condition of paediatric, adolescent and adult patients. (See Annexure B & C for details on the WHO clinical staging and Centers for Disease Control and Prevention immunological and clinical staging in adolescents and adults (WHO 2007: 15; MMWR 1992 No.41 (RR-17)).

2.2.1.4 Clinical course

The course of HIV infection progresses through various stages: acute HIV syndrome, clinical latency and advanced HIV disease (AIDS) characterised by development of opportunistic infections (Kamps & Hoffman 2007: 26). The details of each clinical stage in relation to the timeline of $CD4^+$ cell count and viral load changes over time in untreated HIV infection is summarised in Figure 2.1 and explained below.



Source: HIV Medicine 2007 (http://www.HIVMedicine.com)

Figure 2.1 Timeline of CD4$^+$ cell count and viral load changes overtime in untreated HIV infection.

2.2.1.4.1 Acute HIV infection

Acute HIV-1 infection presents in 80 – 90% of infected individuals as a transient symptomatic illness and begins immediately after the virus enters the body and starts to multiply inside infected cells. The stage which usually takes between 2 – 6 weeks is characterised by very high viral load and an initial drop in CD4$^+$ cell count and the return of these cells to near-normal levels (Altfeld & Walker 2007: 33). The infection goes into the latency phase as the body's immune system succeeds in limiting viral replication. Symptoms during this stage may include fever, influenza-like illness, lymphadenopathy, sore throat, rash, muscle pains and sores in the mouth and gullet and others. Diagnosing acute HIV infection posses a major challenge to clinicians because individuals may experience all or none of these unspecific symptoms which clear between 7 – 10 days and rarely lasts longer than 14 days (Hecht, Busch, Rawal, Webb, Rosenberg, Swanson, Chesney, Anderson, Levy & Khan 2002: 1120). The benefits of providing antiretroviral therapy during this phase still remains controversial though recent evidence has shown that antiretroviral therapy is highly effective in reducing viral load and slowing down of extensive damage to the immune system (Hecht, Wang, Collier, Little, Markowitz, Margolick, Kilby, Daar, Conway & Holte 2006: 726; Kinloch-de loes. 2006: 721).

2.2.1.4.2 Asymptomatic stage

This is the longest stage of HIV disease and lasts between 10 to 15 years though in a few people it can be as short as one year. During this stage, viral replication is ongoing in lymphoid tissues destroying immune-system cells and persons exhibit few or no symptoms. Approximately 5% of people in the asymptomatic stage are either slow or long-term non-progressors (Kamps & Hoffman 2007: 26).

2.2.1.4.3 Advanced HIV disease (AIDS)

This stage is characterised by decline in $CD4^+$ cell count below a critical level of cell-mediated immunity and appearance of significant opportunistic infections. The symptoms during this stage may include; unexplained weight loss, body weakness, lymphadenopathy, joint pains, skin rashes, oral sores, aseptic meningitis and diseases of the respiratory tract such as sinusitis, bronchitis, otitis media, and pharyngitis (Altfeld & Walker 2007: 35). Persons with advanced disease develop severe opportunistic infections that can be viral, bacterial, protozoan or fungal in origin. In addition, tumours arising from various tissues are a common occurrence in patients with AIDS (Hoffmann 2007: 447). However, several studies by (Decker & Lazarus 2000: 65; Holmes, Losina, Walensky, Yazdanpanah & Freedberg 2003: 659; Feldman 2005:170) have found that not all patients with this condition experience these infections and tumours.

2.2.2.1 Treatment of HIV infection

2.2.2.1.1 Antiretroviral therapy

With no cure or vaccine in sight in the near future, the significant breakthrough in the battle against HIV/AIDS has been the introduction of antiretroviral drugs since 1996 (Barclay 2008: 1). Although antiretroviral drugs cannot eradicate HIV from infected cells (Chun, Nickle, Justement, Large, Semerjian, Curlin, O'Shea, Hallahan, Daucher, Ward, Moir, Mullins, Kovacs & Fauci 2005: 3250), the therapy has dramatically decreased morbidity and mortality in symptomatic and asymptomatic HIV-1-infected individuals and has in addition improved the general health and quality of life for those living with HIV and AIDS (Wood, Hogg, Yip, Harrigan, O'Shaughnessy & Montaner 2003: 712). Studies have shown that in the absence of antiretroviral treatment, HIV infection progresses to AIDS at a median time of 8 – 10 years with median survival time after developing AIDS significantly reduced to only 9.2 months (Morgan, Mahe, Mayanja, Okongo, Lubega &Whitworth 2002: 601; Kamps & Hoffmann 2007: 24).

The current recommended treatment for HIV infection is by combining at least three classes of antiretroviral drugs which is known as highly active antiretroviral therapy. These drugs are categorised based on the enzymatic and/or cellular processes of the virus during replication. Typically this combination is composed of two nucleoside/nucleotide analogue reverse transcriptase inhibitors plus either a non-nucleoside reverse transcriptase inhibitor or a protease inhibitor (Hoffmann & Mulcahy 2007:93).

The efficacy of antiretroviral therapy in suppressing viral replication and increasing $CD4^+$ cell counts has been demonstrated in several clinical studies (Dybul et al 2002: 382; Novitsky, Gilbert, Peter, McLane, Gaolekwe, Rybak, Thior, Ndung'u, Marlink, Lee & Essex 2003: 882; Manosuthi, Sungkanuparph, Vibgahgool, Rattanasiri & Thakkinstian 2004: 105). However, there is still controversy as to when to initiate antiretroviral therapy based on $CD4^+$ cell count and plasma viral load. The results of several observational studies and randomised clinical trials confirm that treatment should be started before the $CD4^+$ cell count falls below 200 cells/µL with an upper limit of 350 cells/µL (Grabar, Le Moing, Goujard, Leport, Kazatchkine, Costagliola & Weiss 2000: 401; Fauci 2007: 177). In resource limited settings where laboratory facilities for quantifying $CD4^+$ cell count is scarce, the WHO recommends that the basis for initiating treatment be WHO stages III and IV (WHO 2002: 25). The Working Group on Antiretroviral Therapy (2009: 18) recommends commencing therapy earlier and more aggressive treatment for children because AIDS progression is more rapid and less predictable than in adults.

Despite the evidence of the efficacy of antiretroviral drugs in improving clinical, immunological and virological outcomes in patients who take them, cost still remains the major limiting factor in accessing therapy in many countries of the developing world (Wirtz, Forsythe, Valencia-Mendoza & Bautista-Arrendondo 2009: S6).

2.2.2.1.2. Monitoring treatment outcomes

Clinical, immunological and virological outcomes are the basis of evaluating success of antiretroviral therapy in patients. Clinical success is said to have been achieved when there is a reduction in clinical endpoints such as AIDS-defining illnesses or death.

Increases in $CD4^+$ cell count of either 50, 100, ≥200 and 500/ µL is considered immunological success.

However, most clinicians use plasma viral load as a predictor of future clinical course than a change in $CD4^+$ cell count. Achieving viral suppression to 50 – 400 copies/mL by 12 weeks of therapy is the goal of therapy (Novitsky et al 2003: 882; Hoffmann & Mulcahy 2007: 166).

2.2.2.1.3 Factors affecting treatment success

Drug resistance, either primary or secondary is the most critical factor for antiretroviral therapy treatment failure. This is mainly due to suboptimal antiretroviral drug levels in the serum resulting from medication intolerance/side effects leading to non-adherence/persistence, inadequate potency or durability of the antiretroviral regimen, incorrect dosing of antiretroviral drugs and infection with a drug-resistant strain of HIV (Becker, Dezii, Burtcel, Kawabata & Hodder 2002: 21).

Fear of medication side effects such as lipodystrophies, dyslipidaemia, insulin resistance, diabetes mellitus,lactic acidosis and birth defects (Montessori, Press, Harris, Akagi & Montaner 2004: 229; Saitoh, Hull, Franklin & Spector 2005: 555) and psychosocial factors like poor access to medical care, inadequate social support, psychiatric disease, drug abuse, complexity of antiretroviral regimens, pill count burden, dosing frequency and meal restrictions also contribute to poor treatment success (Kleeberger, Phair, Strathdee, Detels, Kingsley & Jacobson 2001:83).

2.2.2.2 Prevention strategies, microbicides and vaccines

2.2.2.2.1 Vaccines

There is consensus in the research community that vaccination and immunoprophylaxis could prevent transmission of HIV though it will be at least a decade before phase 3 clinical trials are ready to begin (Esparza & Bhamarapravati 2000: 2061; Albrecht & Weitzel 2007: 517).

Current information on HIV vaccine as reported by *BBC News* (2007: 1) was by scientists at The National Institute of Allergy and Infectious Diseases who had exposed the weak spot on HIV's surface that could be a potential target for a vaccine development. Further reports on vaccine development were reported by researchers at the Heinrich Pette Institute of Experimental Virology and Immunology in Hamburg when

they modified a naturally occurring “Cre” recombinase to ‘Tre’ recombinase, an enzyme particle that can ‘see’ and splice out the virus inserted in the lymphocyte genome leading to its removal from the cell. Similar findings by scientists at Max Planck Institute for Molecular Biology and Genetics in Dresden were reported though they go further to report that in order to promulgate the enzyme in the body, the patient’ stem cells must be extracted, cured and re-injected for treatment to be effective (Paternity Testing Labs 2007: 1; Terra Daily 2007:1).

2.2.2.2.2 Microbicides

Anti-HIV vaginal microbicides have proved futile in preventing transmission of HIV. In some instances, women who received these microbicides were at greater risk of HIV infection than women in the control arm. This could result from microbicides that are toxic to the vaginal mucosa (Grant, Hamer, Hope, Johnston, Lange, Lederman, Lieberman, Miller, Moore, Mosier, Cshooley, Springer, Veazey, & Wainberg 2008: 532). To avoid such occurrence, scientists are currently formulating vaginal gels that have shown to be protective against Simian Immunodeficiency Virus in animal models through inhibition of CCR5 (Lederman, Veazey, Offord, Mosier, Dufour, Mefford, Piatak Jr., Lifson, Salkowitz, Rodriguez, Blauvelt & Hartley 2004: 485). Formulation of nucleoside (tide) and or non-nucleoside reverse transcriptase inhibitors as anti-HIV vaginal microbicides is on-going (Youle & Wainberg 2003: 938).

2.2.2.2.3 Pre-exposure and Post-exposure prophylaxis

The only known method of prevention is avoiding exposure to the virus. Pre-exposure prophylaxis is taking antiretroviral drugs by susceptible individuals on a prophylactic basis (Youle & Wainberg 2003: 937). Currently, a study by the Ministry of Health in collaboration with USA (BOTUSA) is under way in Botswana to determine if Tenofovir (TDF) and Emtricitabine (FTC) co-formulated as Truvada™ is an effective pre-exposure prophylaxis (PrEP) in reducing HIV transmission. However, many observers consider Pre-exposure prophylaxis as the most promising strategy for prevention of HIV transmission (Smith 2004: 16).

Post-exposure prophylaxis (PEP), which is believed to reducing the risk of HIV infection, is defined as taking antiretroviral medications immediately after exposure (MMWR 54 (RR02): 1). In Botswana, co-formulated Zidovudine (300mg)/Lamivudine

(150mg) is used for paediatric, adolescent and adult post-exposure prophylaxis (MASA - the National ARV program of Botswana 2008: 91).

2.2.2.2.4 Barrier methods

The use of physical barriers such as the latex condom is widely advocated to reduce sexually transmitted infections (STIs) and HIV. According to Holmes, Levine and weaver (2004: 455), condoms use reduces 90% of transmissions of HIV and sexually transmitted infections from an infected man to a non-infected woman. However, use of spermicides either alone or in conjunction with vaginal contraceptives like diaphragm, increases the male to female transmission of HIV due to increase in inflammation of the vaginal mucosa (Condom Brochure, FDA OSHI HIV STDs 2006).

Other prevention strategies include avoidance of breast feeding when it is acceptable, feasible, affordable, sustainable and safe, screening of blood for transfusion, practicing abstinence and mutual monogamy, universal precautions such as hand washing and safe disposal of infected material and used needles (WHO/HHS-CDC PMTCT Generic Training Package 2007: 9; Goodman 2004: 819; Singh, Darroch & Bankole 2004: 129).

2.2.2.2.5 Circumcision

This is another adopted armamentarium for prevention of HIV infection transmission primarily in males. Clinical trials conducted in Kenya, Uganda and South Africa have shown that male circumcision reduces heterosexual transmission of HIV infection by between 51 – 60% (Bailey, Moses, Parker, Agot, Maclean, Krieger, Williams, Campell & Ndinya-Achola 2007: 643; Gray, Kigozi, Serwadda, Makumbi, Watya, Nalugoda, Kiwanuka, Moulton, Chaudhary, Chen, Sewankambo, Wabwire-Mangen, Bacon, Williams, Opendi, Opendi, Reynolds, Laeyendecker, Quinn & Wawer 2007: 657; Auvert, Taljaard, Largarde, Sobngwi-Tambekou, Sitta & Puren 2005: e298). Efficacy of male circumcision in reducing the risk of heterosexually acquired HIV infection in men as demonstrated by previous observational studies and the three randomised clinical trials mentioned above resulted in the WHO and UNAIDS Secretariat recommend male circumcision as an important additional HIV prevention strategy (WHO/UNAIDS 2007: 4). In addition, recent evidence shows that circumcision also reduces heterosexual transmission of herpes simplex virus 2 (HSV-2) infections but perhaps not HIV (Roehr 2008: 1).

2.2.2.3 Economic impact

2.2.2.3.1 Gross domestic product

UNAIDS, the WHO and United Nations Development Programme have documented a correlation between decreasing life expectancies and lowering of gross national product from growth trajectory models of 30 sub-Saharan economies with HIV prevalence of 10% or more between 1990 and 2025. Their prediction is that annual economic growth in these countries will be between 0.56 and 1.47% lower, though the impact on gross domestic product per capita was less conclusive. Prior studies projected a contraction of Africa's per capita gross domestic product of 0.7% from 1990 – 1997 to 2000 with a further reduction of 0.3% anticipated in countries affected by the malaria epidemic (Bonnel 2000: 820). The current forecast is that the growth domestic product will undergo a further reduction of between 0.5 and 2.6% per annum in these countries (Greener 2002: 49). However, Bell, Devarajan & Gersbach (2003: 5) estimates that the net effect on the growth rates of per-capital growth domestic product will be modest as a result of the countervailing effect of increased labour productivity.

2.2.2.3.2 Human capital

HIV and AIDS retard economic growth by destroying human capital and UNAIDS has predicted outcomes for sub-Saharan Africa to the year 2025. Increased mortality in this region is expected to reduce the population of skilled personnel. The resultant labour force is expected to predominantly consists of unskilled young people with reduced productivity (UNAIDS 2006: 94). Absence from work, disability, workers compensation and increased employee turnover eventually lead to lower productivity (Goetzel, Ozminkowski, Baase & Billotti 2005: 760). In addition, HIV/AIDS weaken mechanisms that generate human capital through loss of income and death of supporting parents/guardians. Coupled to this, there is shrinking of the taxable base that greatly contributes to public expenditures for such sectors as education and health services (UNAIDS 2006: 93). Parallel to this is an increased expenditure for treating the sick, training of new staff to replace sick/ workers who have died and caring for AIDS orphans. At household level there is loss of income and increased spending on health care and funerals (Stover & Bollinger 2006: 2).

2.3 TUBERCULOSIS

Tuberculosis is caused by the bacterium *Mycobacterium tuberculosis*. The bacterium can be present in the body either as a latent infection or active TB. People infected with the HIV are at high risk of developing tuberculosis and this usually occur when the $CD4^+$ cell count drops to 300 – 400 cells /µL (Raviglione & O'Brien 2004: 953).

In HIV negative individuals, latent TB infection is effectively suppressed by the immune system and the lifetime risk of developing active disease is between 10 – 20%. However, in a person infected with HIV, the annual risk can exceed 10% (Palomino, Leao & Ritacco 2007: 269) and the case fatality if TB is untreated can exceed 50% (Onyebujoh & Rook 2004: 930).

2.3.1 Epidemiology

According to the World Health Organization, nearly 2 billion people, one–third of the world's population, have been exposed to the tuberculosis pathogen (WHO Fact Sheet 2007: No. 104). Globally 9.27 million new cases (139 per 100,000 population) occurred in 2007 and of these cases 44% or 4.1 million (61/100,000 population) were new smear-positive cases. During the same period, there were 1.37 million incident cases of TB/HIV co-infection and 456,000 deaths in this group of individuals (WHO Report – Global Tuberculosis Control 2009: 7). The African region has borne the brunt of TB/HIV co-infection and in some instances the proportion of those co-infected exceed 80% in newly diagnosed TB cases in some countries (Corbett, Marston, Churchyard & De Cock 2006: 927).

Table 2.2 below summarises the global epidemiological burden of tuberculosis by WHO region. As shown the African region bears the greatest burden in incidence, prevalence, and mortality and TB/HIV co-infection/100,000 population per year in the world. In the African region, Nigeria and South Africa had the highest estimated incidence and prevalence of TB. In contrast, the lowest estimated epidemiological burden of TB is in western pacific and Mediterranean regions. Overall India ranks the first in terms of the total number of incident cases (WHO Report – Global Tuberculosis Control 2009: 7).

Table 2.2: Estimated epidemiological burden of TB, 2007

	Incidence [a]				Prevalence [a]		TB Mortality		HIV prevalence in incident TB cases[b] (%)
	All forms		Smear-positive[b]		All forms		All forms		
WHO Region	Number (1000s)	Per 100 000 pop per year	Number (1000s)	Per 100 000 pop per year	Number (1000s)	Per 100 000 pop	Number (thousands)	Per 100 000 pop	
Africa	2879	363	1188	150	3766	475	735.0	93.0	38
America	295	32	157	17	348	38	40.9	4.5	11
Eastern Mediterranean	583	105	259	47	772	139	107.4	18.4	3.5
Europe	432	49	190	21	456	51	64.1	7.2	9.8
South East Asia	3165	181	1 410	81	4881	280	537.0	30.3	4.6
Western Pacific	1919	108	859	48	3500	197	291.0	16.8	2.7
Global	9273	139	4,062	61	13 723	206	1772.0	26.8	15

Source: Global TB Control |WHO Report 2009|page 7

[a]Incidence and prevalence estimates include TB in people with HIV

[b]Prevalence of HIV in incident TB cases of all ages

In Africa tuberculosis primarily affects adolescents and young adults whereas in Europe and United States older people are more affected (WHO Global TB control report 2007; 169).

The burden of multidrug-resistant TB (MDR-TB) and the recently described extensively drug-resistant TB is posing to be a major public health issue in many developing countries (Gandhi, Moll, Sturm, Pawinski, Govender, Lalloo, Zeller, Andrews & Friedland 2006: 1575). Primary resistance occurs in persons who are infected with a resistant strain of TB whereas secondary resistance (acquired resistance) can develop as a result of non optimum treatment, poor adherence or when less effective drug formulation are utilised. Resistance to Rifampicin and Isoniazid has been documented in nearly 90 countries and all WHO regions (WHO Report – Anti-tuberculosis drug resistance in the world 2008: 35). In 2004 there were 424 203 incident cases of multidrug-resistant TB which represents 4.3 % of all new and previously treated TB cases notified in 184 countries. The highest number of MDR-TB cases is in China, India

and Russian Federation accounting for 62% of the estimated global burden (Zignol, Hosseini, Wright, Weezenbeek, Nunn, Watt, Williams & Dye 2006: 479). China and India combined has approximately 50% of the global burden whereas the Russian Federation has a further 7% (WHO Report – Anti-tuberculosis drug resistance in the world 2008: 15).

Extensively drug-resistance TB (XDR-TB) which is MDR-TB strains with additional resistance to any flouroquinolones and to either capreomycin or kanamycin or amikacin has recently been described (Jassal & Bishal 2009: 19; Raviglione & Smith 2007: 656). As of February 2008, XDR-TB has been confirmed in 49 countries including all the G8 (group of eight) countries. The following countries in Southern Africa have reported some cases of XDR-TB: South Africa, Botswana, Mozambique, Lesotho, Namibia and Swaziland (WHO Stop TB 2008). The map of the world below shows countries that have reported at least a case of XDR-TB.

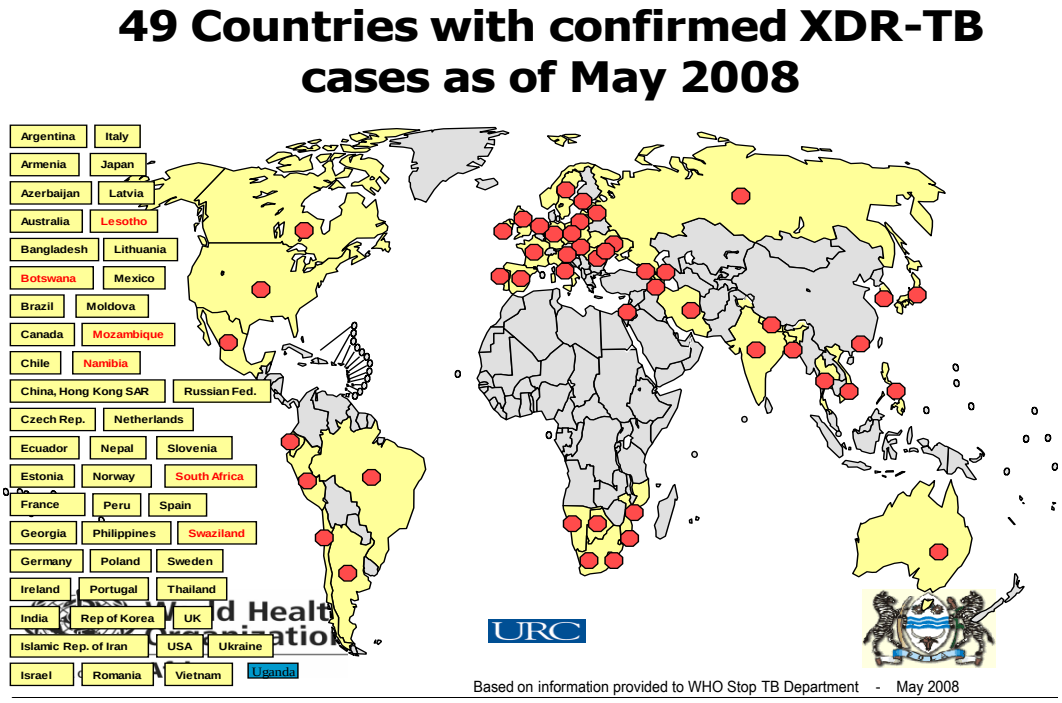


Figure 2.2 Countries with Extensive Drug Resistance Tuberculosis.

2.3.1.1 Risk factors

The risk of developing active depends on host immune defences and the number of inhaled bacilli (Davies, Yew, Ganguly, Davidow, Reichman, Dheda & Rook 2006: 291; Restrepo 2007: 436; Varaine, Henkens & Grouzard 2008: 16). These include among others:

- Host immune defences
- Age: risk is higher in children under 5 years and highest in less than six months old Older people over 60 years are also at higher risk
- Other diseases: HIV infection, clinical AIDS, cancer, diabetes
- Malnutrition
- Pregnancy
- Toxic substances and some medications: alcohol, tobacco, steroids and immunosuppressant's and
- Bacterial load which depends on proximity to infectious source, infectivity of the source and duration of exposure

2.3.1.2 Pathogenesis

Tuberculosis more often affects the lungs in part because inhalation in the commonest mode of infection and lung tissue provides a favourable environment for multiplication of the organism (Houben, Nguyen & Pieters 2006: 76). Inhalation of the TB bacterium for the first time gives rise to a primary lesion called *Ghon focus* characterised by an initial accumulation of macrophages that phagocytise the tubercle bacilli before the body mounts an immune response. Following development of cell-mediated immunity, there is more chemotaxis of macrophages to the infection site and containment of the tubercle bacilli (Kaufmann 2002: 54; Houben et al 2006: 82). Lymphocytes and specifically primed T-cells that secrete various lymphokines such as gamma interferon are responsible for necrosis of macrophages at the centre of the lesion. The further course of the infection is dependent on several factors including host immunity, infecting dose and virulence of the organism. In many patients hearing by fibrosis occurs. In few individuals, cavities with necrotic material containing living bacteria can be joined to the bronchi and later coughed up and passed on to contacts through droplet inhalation (Grosset 2003: 833 – 834; Schroder, Hertzog & Hume 2004: 163).

Spread from the primary complex to distant organs can occur through the blood stream though the heart, skeletal muscles, pancreas and thyroid are rarely affected (Agarwal, Malhotra, Awasthi, Kakkar & Gupta 2005: 29).

Miliary tuberculosis which is a more common disease in children and the elderly is life threatening and the outcome of acute respiratory distress syndrome caused by it has a case fatality rate ranging from 33% to 100%, which is much higher than respiratory distress caused by other diseases (Kim, Park, Kim, Kang, Shin, Park & Choi 2003: 359).

2.3.1.3 Transmission

Transmission of the tubercle bacillus is human-to-human by airborne spread from an infected person with pulmonary or laryngeal TB through aerosolized droplet nuclei after infected people cough, sneeze, spit, speak or even kiss. Transmission can only occur from people with active but not latent-TB. The probability of transmission from one person to another depends upon the number of infectious droplets expelled by a carrier, the effectiveness of ventilation, the duration of exposure, and virulence of the *M. tuberculosis* species. Patients with smear-positive sputum are by far the most contagious than those with only culture-positive and smear-negative sputum results. In addition, extrapulmonary forms of the disease are only contagious in exceptional circumstances. Children are less contagious because of weaker cough mechanics and they produce less sputum. Cutaneous and mucous inoculation is far less common (Centers for Disease Control and Prevention 2006; Varaine, Henkens & Grouzard 2008: 15).

2.3.1.4 Symptoms

Prolonged cough (> 2 weeks), sputum production, weight loss and night sweats are more specific symptoms of pulmonary TB. However, blood in sputum (haemoptysis) is the most characteristic sign. Other constitutional sign and symptoms include chest pains, anorexia, fatigue and moderate fever (Corbett, Zezai, Cheung, Bandason, Dauya, Munyati, Butterworth, Rusikaniko, Churchyard, Mungofa, Hayes & Mason 2010: 14); Varaine, Henkens & Grouzard 2008: 22). The other signs and symptoms will be dependent on the organ affected especially in extrapulmonary tuberculosis.

2.3.1.4.1 Diagnosis

A complete clinical assessment that includes a medical history, physical examination, a chest X-ray and laboratory tests is used to diagnose tuberculosis. Sputum smear microscopy continues to be the “gold standard” for the diagnosis of TB though it has low sensitivity. Culturing on Lowenstein-Jensen media is more sensitive but requires a more equipped and qualified laboratory (de Waard & Robledo 2007: 401). A positive Tuberculin Skin Test is of limited value to diagnose TB in high prevalence areas as it does not determine whether the TB is latent or active. This limitation is more pronounced in countries where TB immunization is a component of the routine childhood immunisation programmes (Rothel & Anderson 2005: 981; Kristi & Fiuza de Melo 2007: 513). Furthermore, approximately 30% of children with active TB have negative or doubtful tuberculin skin test when diagnosed (Varaine, Henkens & Grouzard 2008: 30). Limitations of the above tests have led to the development of new technologies that are more fast and accurate (Nahid, Pai & Hopewell 2006: 103; Franken, Timmermans, Prins, slootman, Dreverman, Bruins, van Dissel & Arend 2007: 447).

One such test is the QuantiFERON-TB Gold Test (QFT-G) that uses whole-blood in diagnosing latent and active *Mycobacterium* tuberculosis infection. This test is of limited value in severely immunosuppressed patients where it yields indeterminate or negative results (Brock, Ruhwald, Lundgren, Westh, Mathiesen & Ravan 2006: 56; Nahid, Pai & Hopewell 2006: 103). However, the ELISPOT test is not affected by HIV associated immunosuppression (Dheda, Lalvani, Miller, Scott, Booth, Johnson, Zumla & Rook 2005: 2038).

Lastly, the TB Polymerase Chain Reaction test is more rapid and reliable than the above mentioned tests. This test has a diagnostic sensitivity in culture and clinically confirmed tuberculosis of 76% and 81% respectively. Overall, diagnostic sensitivity for culture confirmed pulmonary and extra-pulmonary specimens is 82% and 72% respectively. The test can be more valuable in high burden countries where the need for rapid diagnosis is urgent in order to control transmission (Cheng, Yam, Hung, Woo, Lau, Tang & Yuen 2004: 281).

2.3.1.4.2 Management
2.3.1.4.3 Treatment

An active tuberculous lesion contains at least four distinct *M. tuberculosis* populations: actively dividing, slowly and sporadic multiplying and dormant bacilli. The principal of anti-tuberculosis therapy is therefore to use a combination of anti-TB drugs that acts on all these populations in order to prevent emergency of drug resistance. The five main drugs used as first-line treatment are: Isoniazid, Rifampicin, Ethambutol, Pyrazinamide and streptomycin. Their dosage is adjusted according to patient weight during the course of treatment which lasts a minimum of six months in new cases and as long as eight months for re-treatment cases (da Silva & Ainsa 2007: 593; Varaine, Henkens & Grouzard 2008: 57).

This is achieved by using directly observed treatment-short course (DOTS), an internationally recommended strategy to prevent transmission of *M. tuberculosis* and related deaths, to treat patients with active disease (Raviglione & Uplekar 2006: 952).

2.3.1.4.4 Prevention

- **BCG vaccine**

  Bacille Calmette-Guerin (BCG) vaccine is used in many countries as a TB preventive strategy and its efficacy ranges from 0 – 80%. The vaccine protect against the severe forms of the disease particularly TB meningitis and miliary TB. However, its protective effect wanes over time and the efficacy in adolescents and adults is highly variable (Anderson & Doherty 2005: 656; Martin, Bigi & Gicquel 2007: 341). Use of the vaccine in pregnant women and immunocompromised individuals is contraindicated (Centres for Disease Control and Prevention 2006).

- **Chemoprophylaxis**

  This is used for the treatment of the primary infection in order to sterilize the lesion and prevent development of active TB. It is recommended for: newborn infants of sputum smear-positive mothers, children under the age of five years, contacts of sputum positive patients and HIV infected patients after excluding active TB disease

The recommended regimens for prophylaxis are Isoniazid or Rifampicin, Rifabutin and Pyrazinamide or Rifampicin and Pyrazinamide for a period ranging from 6 to 9 months. The later regimen has hepatic complications and patients must be closely monitored for toxicities. Primary and secondary prophylaxis is respectively used after TB exposure in

patients with known HIV infection and in HIV patients who have completed curative TB treatment (MMWR 2003/52 (RR – 31: 735); Varaine, Henkens & Grouzard 2008: 98).

- **Controlling the spread of tuberculosis**

  General prevention measures must include: wearing of masks by attending health staff and infectious patients, health education of patients, visitors and family members on risk of TB transmission and how to avoid it and active case finding in high-risk groups like children, elderly, malnourished and immunocompromised individuals, contact tracing, regular supply of good quality medications and good drug adherence. Other measures include sunlight and ventilation to decontaminate the environment (Botswana National Tuberculosis Programme Manual 2007: 66).

2.4 TUBERCULOSIS AND HIV CO-INFECTION

2.4.1 Epidemiology

Tuberculosis and HIV are overlapping epidemics and the leading cause of infectious disease associated morbidity and mortality worldwide especially in resource-limited countries (Moore, Liechty, Ekwaru, Were, Mwima, Solberg, Rutherford & Mermin 2007: 713; Friedland, Churchyard & Nardell 2007: S1). Comparatively the synergy of the two diseases has increased incidence of TB in HIV-positive individuals to more than 8 times than in HIV-negative people with reported outbreaks mainly of mostly multi-drug resistant TB (Dye & Williams 2000: 8180; Corbett, Marston, Churchyard & De Cock 2006: 927). Similarly, the lifetime risk of an HIV-positive individual developing TB is 50% as compared to 5 – 10% in HIV- negative persons (Harries et al 2004: 37; Corbett et al 2006: 927). Patient initiated on antiretroviral therapy are still at risk of developing TB because this treatment does not fully restore immune function (Bonnet, Pinoges, Varaine, Oberhauser, O'Brien, Kabede, Hewison, Zacharia & Ferradini 2006: 1275 – 1276; Sutherland, Yang, Scriba, Odondo, Robinson, Conlon, Suttill, McShane, Fidler, McMichael & Dorrell 2006: 822).

Approximately 14 million people are co-infected with TB and HIV-1 worldwide with Africa bearing 70% of the burden. Sub-Saharan Africa is hardest hit with TB/HIV co-infection prevalence ranging from 24 – 79% (Lange, Schieferstein, Tossi & Gori 2007: 416). Table 2.3 below shows countries in sub-Saharan Africa with the highest prevalence of TB/HIV co-infection in descending order (Global Health Initiative 2003: 2).

Table 2.3: Estimated HIV positive prevalence among adult TB patients

Country	Prevalence (%)
Botswana	79
South Africa	60
Kenya	51
Mozambique	47
Ethiopia	29
Uganda	24

Source: Global Health Initiative

Recently TB/HIV co-infection has become more noticeable in Eastern Europe and Asia with countries in Western Europe and North America reporting a steady increase in prevalence and a declining incidence which has been attributed to readily availability of antiretroviral therapy (Girardi, Antonucci, Vanacore, Libanore, Errante, Matteeii, Ippolito & GISTA 2000: 1886; Surendram 2004: 323).

2.4.1.1 Interaction of HIV and tuberculosis

HIV significantly affects the progression of *M. tuberculosis* by depleting $CD4^+$ lymphocytes, cells responsible for cell mediated immunity. This leads to progression of latent or reactivation of TB to active TB disease in HIV-infected individuals. The risk is much higher after HIV-antibody seroconversion when the $CD4^+$ cell count is usually above 200. In turn, TB accelerates HIV progression by increasing viral replication and intensifying the immunodepression effect of HIV (Sonneberg, Glynn, Feilding, Murray, Godfrey-Faussett & Shearer 2005: 153; Sonneberg, Murray, Glynn, Shearer, Kambashi & Godfrey-Faussett 2001: 1687).

2.4.1.2 Clinical manifestations

The clinical manifestations of TB in the early stages of HIV infection are similar to those explained in section 2.3.1.4 on page 47 and on the clinical course of HIV. However, in advanced HIV disease, symptoms such as cough and haemoptysis are often absent. In addition, atypical presentation with smear negative sputum, disseminated and extrapulmonary forms become more common with advanced disease (Varaine, Henkens & Grouzard 2008: 43).

2.4.1.3 Diagnosis

Diagnostic steps in the management of the TB/HIV-infected patient are similar to those used for a HIV-negative patient as described in section 2.3.1.4.1 on page 47 and 48 and in section 2.2.1.3 on HIV infection. However, it is important to note that the role of tuberculin skin test in TB/HIV co-infected individuals is of limited value as the test gives false negative results especially in patients with $CD4^+$ cell count of less than 200/µL (Fisk, Hon, Lennox, Reyn, & Horsburg Jr 2003: 1102). Use of more specific and sensitive tests like ELLISPOT and QuantiFERON for diagnosis of TB in HIV- infected individuals is advised. Like the tuberculin skin test, these ELISA tests are of limited value in severely immunocompromised individuals (Ferrara, Losi, D'Amico, Roversi, Piro, Meacci, Meccugni, Dori, Andreani, Bergamini, Mussini, Rumpianesi, Fabbri & Richeldi 2006: 1328).

2.4.1.4 Managing HIV and TB co-infection

2.4.1.4.1 Treatment

TB/HIV co-infected patients have high morbidity and mortality if not treated promptly and it is advised that treatment for tuberculosis takes precedence over initiating antiretroviral therapy (Harries et al 2004: 153; Apers, Lyden, Worodia & Colebunders 2005: 1209). Furthermore, treatment for TB in HIV patients follows the usual TB regimens as described in section 2.3.1.4.3 on page 48 though considerations should be made of replacing Rifampicin with Rifabutin due to drug-drug interaction between antiretroviral therapy and anti-TB drugs. It is for this reason that patients with $CD4^+$ cell count of 100 – 200/µL can delay start antiretroviral therapy by at least two months whereas those with $CD4^+$ cell count of > 200/µL can initiate antiretroviral therapy after completing anti-TB therapy. However, there is an exception in patients with $CD4^+$ cell count of < 100/µL where simultaneous treatment of both infections is indicated because of high risk of mortality (Harries et al 2004: 153; Hammer, Saag, Montaner, Schooley, Jacobson, Thompson, Carpenter, Fischi, Gazzard, Gatell, Hisch, Katzenstein, Richman, Vella, Yeni & Volberding 2006: 829).

2.4.1.4.2 Preventive treatment

- **Isoniazid Preventive Therapy**

The WHO and UNAIDS recommended in 1998 that Isoniazid preventive therapy be offered to HIV infected patients either presenting with or at risk of latent TB infection (Godfrey-Faussett 2003: 3) and its efficacy in preventing active TB disease has been

demonstrated in several randomised controlled studies (Lim et al 2006:178). Preventive therapy with Isoniazid for 6 – 9 months is considered adequate to prevent active disease (American Thoracic Society and Centers for Disease Control 2000: S222).

Studies have shown that preventive therapy lowers the incidence of TB by 70 – 90% in HIV and AIDS infected patients though this treatment has no effect on HIV progression to AIDS or mortality in settings with high prevalence of tuberculosis (Quigley, Mwinga, Hosp, Lisse, Fusch, Porter & Godfrey-Faussett 2001: 219; Woldehanna & Volmink 2004: Issue 1. Art No: CD000171; Lim et al 2006: 178). However, a study by (Wilkinson et al, 2006: 356) showed that treating latent TB infection with Isoniazid resulted in an increase in number of *M. tuberculosis* specific IFN-γ (gamma interferon) producing T cells. In another study by (Pai, Joshi, Dogra, Mendiratta, Narang, Dheda & Karanti 2006: 4) T cell IFN-γ responses remained persistently high for 10 months after completion of Isoniazid treatment though there was a modest decline in the average IFN-γ responses. The interferon molecules interfere with viral replication and it is possible that this immune response effect may enhance antiretroviral therapy (Schroder, Hertzog, Ravasi & Hume 2004: 163) and up-regulate both innate and adaptive immunity by activation of $CD4^+$ cells (Taniguchi & Takaoka 2001:378; Schroder et al 2004: 163).

- **Antiretroviral treatment**

Antiretroviral therapy also reduces the incidence of tuberculosis by approximately 80% in HIV infected individuals without having to use Isoniazid (Miranda, Morgan, Jamal, Laserson, Barreira, Silva, Santos, Wells, Paine & Garrett 2007: 4) and the greatest effect is among individuals with the lowest $CD4^+$ cell counts (Badri et al 2002: 2062). Despite this efficacy, tuberculosis remains persistently higher (2 – 10% per year) among patients on antiretroviral therapy than among HIV negative individuals (Santoro-Lopes et al 2002: 545). Of remarkable significance is that antiretroviral therapy leads to reductions in morbidity and mortality in HIV infected people and improves the outcome of treatment for tuberculosis (Harries et al 2004: 137; Burman & Jones 2001: 7; Akksilp, Karnkawinpong, wattanaamornkiat, Viriyakitja, Monkongdee, Sitti, Rienthong, Siraprapasiri, Wells, Tappero & Varma 2007: 1005).

2.4.1.4.3 Combination anti-tuberculosis and antiretroviral treatment

Whereas antiretroviral therapy significantly increases $CD4^+$ cell counts and reduces plasma viral load, anti-tuberculosis treatment has no such effect during treatment of active TB in co-infected patients (Morris, Martin, Bredell, Nyoka, Sacks, Pendle, Page-Shipp, Karp, Sterling, Quinn & Chaisson 2003: 1970). Comparative studies by (Hung et al 2003: 2619) and more recently (Breen et al 2006: 1439) have demonstrated no statistically significant differences in virologic, immunologic and clinical outcomes to antiretroviral therapy between TB/HIV co-infected patients concurrently treated with anti-tuberculosis and antiretroviral therapy and non-tuberculosis control group on antiretroviral therapy only. However, an observational cohort comparing the non-nucleoside reverse transcriptase inhibitors (Efavirenz vs. Nevirapine), showed that Efavirenz was associated with superior virological outcomes than Nevirapine [Hazard ratio (HR 1.52; 95% CI 1.24 – 1.86)] (Nachega, Hislop, Dowdy, Gallant, Chaisson, Regensberg & Maartens 2008: 2120). This is in contrast to a retrospective study by (Shipton et al 2009: 362) which showed no significant statistical differences when patients on Nevirapine and Efavirenz were stratified according to tuberculosis treatment exposure status.

Concurrent co-administration of antiretroviral and anti-tuberculosis therapies is associated with adverse effects due to drug-drug interactions between Rifampicin and Protease or non-nucleoside reverse transcriptase inhibitors (Seden, Back & Khoo 2009: 6). Patel A, Patel K, Patel J, Shah, Patel B and Rani (2004: 1168) conducted an observational longitudinal cohort study that compared the safety and effectiveness of concomitant use of Rifampicin-containing anti-TB treatment and Efavirenz in antiretroviral naïve TB/HIV co-infected patients with active TB disease to those without tuberculosis. The nucleoside reverse transcriptase inhibitor backbone used was either Zidovudine and Lamivudine or Stavudine and Lamivudine. They found that Efavirenz-based antiretroviral therapy co-administered with Rifampicin in HIV/TB co-infected patients resulted in an immunologic response that was comparable to that of the group not receiving Rifampicin. The adverse effects profiles were comparable except that patients on Rifampicin had a higher incidence of hepatitis ($p < 0.0001$). More recently, similar findings were reported by (Manosuthi, Mankatitham, Lueangniyomkul, Chimsuntorn & Sungkanuparph 2008: 296) in their retrospective cohort study when they

compared standard dose Efavirenz vs. Nevirapine in antiretroviral regimens among TB/HIV co-infected patients who received Rifampicin.

2.5 CONCLUSION

Chapter 2 discussed the relevant literature review that guided the study. The researcher has discussed the epidemiology, pathogenesis, transmission, clinical manifestation, diagnosis and prevention of TB and HIV. The chapter has described the literature on the interaction of TB and HIV and reviewed various studies that have been conducted to study the phenomenon. The various studies reviewed in this chapter guided the researcher on the methodological approaches used in this study. Chapter 3 describes the research design and method adopted by the researcher to study the immunological and virological outcomes of patients accessing antiretroviral therapy.

CHAPTER 3

RESEARCH DESIGN AND METHODS

3.1 INTRODUCTION

Research design is an overall plan for addressing research questions, including specifications for enhancing the research rigor. It spells out the basic strategies that researchers adopt to develop evidence that is accurate and interpretable (Polit & Beck 2008: 203). The purpose of this study is to compare the immunological and virological responses in patients concurrently receiving either Nevirapine or Efavirenz based antiretroviral and Isoniazid preventive therapies to those taking either Nevirapine or Efavirenz based antiretroviral therapy after completing Isoniazid preventive therapy.

This conduct of this quantitative research and the method for data collection is detailed in this chapter. The chapter concludes by elaborating on the measures which the researcher applied to enhance data quality and ensure that the relevant ethical principles were adhered to. It is the view of the researcher that the results generated from this research will provide an insight into the treatment options that a clinician taking care of TB/HIV co-infected patients can use to enhance the immunological and virological outcomes.

3.2 RESEARCH DESIGN

This is a quantitative, non-experimental and retrospective cohort design. Quantitative research is conducted to test theory or hypotheses by describing variables, examining relationships among variables and determining cause-and-effect interactions between variables (Burns & Grove 2003: 18 – 19). This study followed the quantitative paradigm because the researcher followed a formal, objective, and systematic process to generate quantifiable data about the immunological and virological outcomes of patients on antiretroviral therapy and Isoniazid preventive therapy. In addition, all the research variables used in the study are measurable and quantifiable to enable the researcher to determine the relationships between the independent (age, gender, Isoniazid exposure status and antiretroviral drug regime) and dependent ($CD4^+$ cell count and viral load) variables and to compare the outcomes in different treatment groups.

It is non-experimental in that the researcher did not intervene by manipulating the independent variable but only collected data from pre-existing patient's medical records. The researcher's main aim was to observe, describe and document aspects of a phenomenon as it naturally occurs.

It is retrospective because the researcher linked immunological and virological outcomes of antiretroviral therapy at the present moment to determinants that occurred in the past. The researcher began by looking at the dependant variables ($CD4^+$ cell count and Viral load) and examined whether it was correlated with one or more antecedent variables without any intervention.

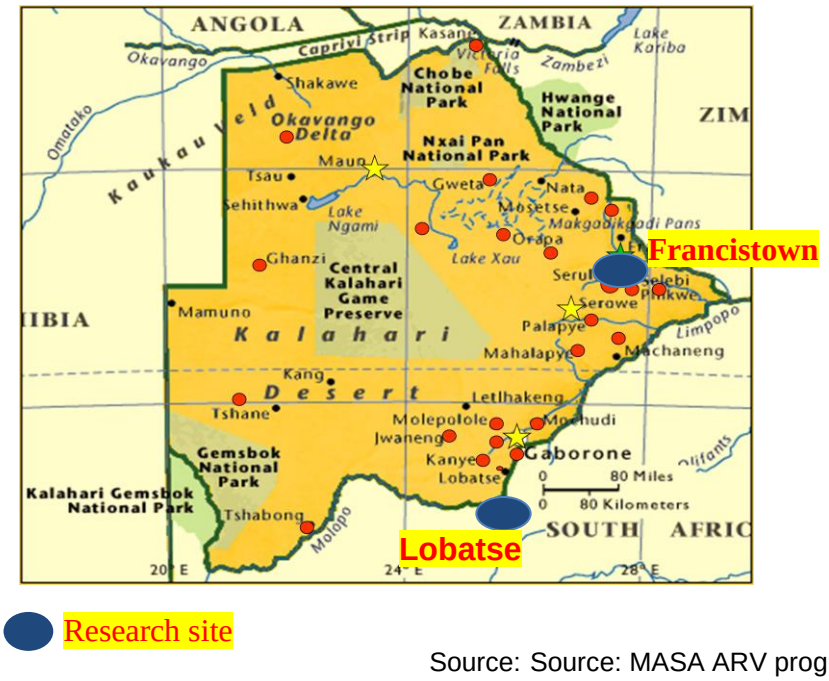
3.3 RESEARCH METHODS

Burns and Grove (2003:27) describe the quantitative research methodology as "a formal, objective, rigorous, systematic process for generating information about the world". Quantitative methods involve objective measurement of data from patients and utilising such information to describe variables and analyse their relationships.

In this section, the methods are outlined by referring to sample selection, data collection, data management and data analysis. Design validity and the ethical considerations of the study are discussed in detail at the end of the chapter.

3.3.1 Research setting

The setting refers to the place/s where the research was conducted. The settings were one hospital and five primary health care clinics specialising in the provision of antiretroviral therapy. Four sites are located in the city of Francistown: Jubilee, Area W, Botswelelo and Gerald Estates. The other two sites (Athlone hospital & Tsopeng clinic) are located in Lobatse town. All the five primary health care clinics are managed by the district health teams and form an integral part of all the elements of primary health care. In addition to serving as a district referral hospital for Lobatse town, Athlone hospital provides care for HIV patients. Figure 3.1 below shows the map of Botswana and the geographic location of the mentioned study sites.



Source: Source: MASA ARV program -Botswana

Figure 3.1 Map showing the location of study sites

3.3.1.1 Population

A research population is the entire aggregate of cases in which a researcher is interested. It is sometimes useful to make a distinction between the accessible and target population. The accessible population is the aggregate of cases that conform to designated criteria and that are accessible as subjects of the study. Meanwhile the target population can be defined as the aggregate of cases about which the researcher would like to generalise the findings (Polit & Beck 2008: 337 - 338).

The study population comprised of nine hundred and eleven (911) medical records of HIV-infected, antiretroviral naïve patients enrolled in the antiretroviral program from 1st January 2006 to 31st December 2006 meeting eligibility criteria, and receiving care in public-sector HIV treatment facilities according to Botswana's 2005 Antiretroviral Treatment Guidelines.

3.3.1.2 Study sample

A sample can be defined as a subset of a population selected to participate in a study (Polit & Beck 2008: 765). It has the same distribution of characteristics as the population from which it is drawn. There are several approaches to determining the sample size. These include using a census for small populations, imitating a sample size of similar

studies, using published tables, and applying formulas to calculate a sample size (Israel 2003: 3).

A total of two hundred (200) medical records of patients that met the selection criteria stated below were used for data analysis.

3.3.1.3 Sampling technique

Sampling is the process of selecting a portion of the population to represent the entire population so that inferences about the population can be made (Polit & Beck 2008: 339).

Purposive sampling technique was used to select medical records for this study. The technique was chosen because the researcher had specific predefined groups to include in the sample based on inclusion and exclusion criteria that suited the purpose of the study. Sampling for proportionality was not the researchers' primary concern. The medical records of patients were grouped as:

Group 1: HIV infected patients receiving Combivir™ + Efavirenz antiretroviral therapy **co-administered** with Isoniazid preventive therapy [$EFV_{ipt\text{-}current}$].

Group 2: HIV infected patients receiving Combivir™ + Nevirapine antiretroviral therapy **co-administered** with Isoniazid preventive therapy [$NVP_{ipt\text{-}current}$].

Group 3: HIV infected patients receiving Combivir™ + Efavirenz antiretroviral therapy **after** completing Isoniazid preventive therapy [$EFV_{ipt\text{-}past}$].

Group 4: HIV infected patients receiving Combivir™ + Nevirapine antiretroviral therapy **after** completing Isoniazid preventive therapy [$NVP_{ipt\text{-}past}$].

Inclusion criteria

Medical records included in the study sample met the below listed criteria:

- Males or females of ≥12 years of age;
- Non-pregnant women;
- Had a recorded $CD4^+ \leq 200$ cells/ µL at initiation of antiretroviral therapy;

- Received the Botswana recommended first line antiretroviral therapy (Combivir™ & Efavirenz or Combivir™ & Nevirapine) for 36 weeks;
- Completed Isoniazid preventive therapy for a period of 6 months or on Isoniazid preventive therapy for ≥ 1 month and initiated on antiretroviral therapy;
- Demonstrated a ≥80% drug adherence rate for both Isoniazid and antiretroviral drugs and
- Contain a cross reference in the patient record to countercheck the completeness and accuracy of the results.

Exclusion criteria

Medical records were excluded from the study sample if:

- They were for HIV infected patients who were treated for TB prior to receiving Isoniazid preventive therapy;
- Initiated on combination anti-tuberculosis therapy during the course of the follow-up interval;
- HIV positive patients were not exposed to Isoniazid preventive therapy;
- There was a switch of Combivir + Nevirapine or Combivir + Efavirenz to other combination antiretroviral therapy regimens before completion of the 36 weeks period of follow-up;
- Concurrent opportunistic infections and malignancies were present;
- There are missing variables of interest (e.g. gender, age, laboratory results);
- Illegible recordings were done;
- A female got pregnant during the course of follow-up; and
- Medical records with restricted access by authorities.

3.3.1.4 Outcome measures

The primary outcomes measures for the study were:

- The mean increase in $CD4^+$ cell count;
- The rate of immunological success [an increase of $CD4^+$ cell count of ≥ 50 cells/ µL over the baseline value]; and
- Time to and proportion of patients achieving virological success [viral load ≤400 copies/mL] after initiation of antiretroviral therapy.

Data for calculating these measures were abstracted from medical records at initiation of antiretroviral therapy and at 12, 24 and 36 weeks of treatment follow-up.

3.3.2 Data collection

3. 3.2.1 Data sources

The data sources used in this study were primary medical records of patients kept at the study sites. These records and reports are prospectively generated by knowledgeable and competent personnel. Primary medical records have a unique patient identifier that can be used to construct a longitudinal history of medical care utilization for each patient. The medical records contain routinely-collected clinical data and included patient treatment charts, drug adherence charts, care plan statements, laboratory reports, patient registers and print outs of recordings of bio-physiological measures. These documents contain information on age, gender, date of HIV screening and antiretroviral therapy initiation, history of opportunistic infections and concurrent medical conditions, virological and immunological responses, therapy-related to prevention of mother to child transmission of HIV, post exposure prophylaxis, antiretroviral drugs, Cotrimoxazole and Isoniazid prophylaxis.

Data abstracted from pre-existing medical records and documents are advantageous because they permit the researcher to examine trends of a phenomenon under study over time especially when data is collected repeatedly. In addition, it eliminates problems stemming from people's awareness of and reaction to the study during data collection which may introduce bias (Polit & Beck 2008: 368).

The major drawback of using medical records for research is that the investigator may be unaware of the records' limitations. These limitations may arise when the researcher does not have a good understanding of the forms and filing systems used including keeping track of possible changes in record-keeping procedures and inaccurate data entries. Lastly, gaining access to institutional medical records can be very challenging (Polit & Beck 2008: 368).

3.3.2.2 Approach and method

Structured data collection involves the collection of data using formal instruments and protocols that dictate what to collect and how to record the needed information (Polit & Beck 2008: 433). It involves formulating a set of questions to be answered in a specified sequence with predetermined response options to the researcher and the people providing the data during data collection. Applying this principal yield accurate, valid and meaningful data that is maximally effective in answering research questions (Polit & Beck 2008: 414).

Data relevant to the research was abstracted from medical records of patients kept at the study treatment sites onto a developed structured data collection form (Annexure A).

3.3.2.3 Development and testing of the data collection instrument

According to Polit and Beck (2008: 425), designing a high-quality structured data collection instrument is challenging and time consuming. To design an accurate instrument, researchers must carefully analyse the research requirements and attend to minute detail. Once data needs have been identified, related constructs should be clustered into separate areas of questioning. Arrangement of the instrument must be made in such a way as to minimise bias.

In this study the data collection instrument was developed after reviewing relevant literature on the phenomenon under study and with due consideration of the research problem, specific research questions and research hypotheses. Before designing the instrument, a thorough conceptualisation of the construct was done to ensure that the instrument captured the full pre-determined information so that the collected and recorded information was reliable. Such conceptualisation came from a variety of sources such as first-hand knowledge, consultation with experts and exhaustive literature review. A small-scale /pilot test of the instrument was conducted at Tsopeng satellite clinic prior to data collection. The purpose of the pilot study was to evaluate the appropriateness and quality of the instrument. The pilot study did not lead to modification of the data collection tool or study procedures. The results from the pilot test were not included in the final analysis of the data.

3.3.2.3.1 Characteristics of the data collection instrument

The data collection instrument comprised of closed-ended. Each category on the instrument was assigned a numerical value and responses are coded. This according to Polit and Beck (2008: 414) ensures standardised recording, accuracy and usefulness of data for subsequent statistical data analysis. All variables on the data collecting instrument are measured at the highest possible rating scale. See table 3.1 below for the variables used and their level of measurement in this study. No patient identifiers were recorded on collection forms and databases created for this study.

Table 3.1: The research variables and levels of measurement

VARIABLE	**CATEGORY**	**LEVEL OF MEASUREMENT**
INDEPENDENT		
Age	Continuous numeric	Ratio
Gender	Binominal categorical	Nominal
Exposure status to Isoniazid preventive Therapy (current or past)	Binominal categorical	Nominal
Antiretroviral combination	Binominal categorical	Nominal
Medication dosage	Continuous numeric	Ratio
Medication frequency	Continuous numeric	Interval
DEPENDANT		
$CD4^+$ cell count	continuous numeric variable	Ratio
Viral load	continuous numeric variable	Ratio

3.3.2.3.2 Structure of the instrument

The data collection instrument is segmented into three sections (see Annexure A). The sections in the observational schedule are:

Section A: Biographical data (identification number, date of recording, study site, age, gender and Isoniazid preventive status)

Section B: Treatment (Medication, dosages and frequency)

Section C: Baseline and follow up $CD4^+$ cell count and viral load results

3.3.2.3.3 Reliability of the instrument

The reliability of a quantitative instrument is a major criterion for assessing its quality and adequacy. Reliability is the consistency of your measurement or the degree to which an instrument measures the same way each time it is used under the same conditions with the same participants (Polit & Beck 2008: 452). Sequentially grouping similar questions under the same section of the instrument as mentioned in section 3.3.2.1.4 on this page was a measure of ensuring reliability in this research. Coupled to this, the consistent results from doctors and nurses who evaluated the instrument during the pilot test conducted at Tsopeng satellite clinic provided a good estimate of the instruments reliability. In addition, only the researcher was responsible for entering abstracted data onto the instrument during the conduct of the actual research and by so doing increased the chances of consistency in the results.

3.3.2.3.4 Validity of the instrument

The second important criterion for evaluating a quantitative instrument is its validity. Validity is the degree to which an instrument measures what it is supposed to measure (Polit & Beck 2008: 459; De Vos 2002: 166). This was ensured by applying the best fit between the conceptual and operational definitions of a variable. Consultation with doctors and nurses providing antiretroviral therapy patient care on the appropriate sample of questions that measured the construct domain of the study was done to ensure instruments validity. In addition, use of medical records eliminated the Hawthorne or placebo effect that might have influenced the researcher and participant responses about the desired outcomes (Polit & Beck 2008: 300).

3.3.2.4 Data collection process

The researcher moved from site to site collecting data. At each study site, all medical records of patients initiated on antiretroviral therapy from 1st January to 31st December 2006 were serially arranged according to the initiation date. The researcher then critically scrutinized each medical record according to the eligibility criteria to determine the sample size at each site. Thorough cross referencing between primary clinical case notes and computer-based medical records was conducted before a medical record was classified as ineligible.

To minimize the chance of error, the researcher independently reviewed the selected medical records and manually abstracted data. Data from each patient's medical record was identified by a serial number that linked it to and permits easy access back to the source material for checking and to obtaining additional data if required.

Abstracted data included patient's sex, age, exposure status of Isoniazid preventive therapy status [current or past], antiretroviral treatment regimen, $CD4^+$ cell count, viral load level, dose frequency of every antiretroviral and Isoniazid preventive therapy medications at baseline. In addition, $CD4^+$ cell count and viral load follow-up data at 12, 24 and 36 weeks were abstracted onto the observation schedule form. Abstracted data was later entered into SAS JMP statistical software version 8.0 database (SAS Institute, Cary, NC, USA).

3.3.2.4.1 Data management

Data management involves various clerical and administrative tasks such as logging in forms, reviewing data for completeness and legibility, retrieving pieces of missing information and assigning identification numbers. Another task involves selecting statistical software for data analysis (Polit & Beck 2008: 642).

Most of the data on the data collection form was pre-coded with a numeric code for easy entry into the computer so as to create a data set for analysis. Before entering data into the statistical software, it was checked for completeness to verify entries and any errors corrected. After entering data into the computer, the data sheet was printed and the entered codes visually compared with entries on the data collection instrument in order to identify inconsistencies and completeness.

Finally, the researcher cross tabulated the independent with the dependant variables described on page 55 to check for missing data. Data cleaning was performed to track for outliers and consistency checks carried out to detect coding or misreported information before data analysis.

3.4 INTERNAL AND EXTERNAL VALIDITY OF THE STUDY

Design validity is the determination of whether the study provides a convincing test of the framework propositions (Burns & Grove 2003: 198). Thus, it is an inductive estimate to which conclusions about cause-effect-relations are likely to be true in view of the measures used, the research setting and the whole research design. Design validity can be used to strengthen quantitative research designs, including enhancing rigor by minimizing biases and controlling extraneous variables (Polit & Beck 2008: 286). According to Burns and Grove (2003: 199), four types of design validity are described: statistical conclusion validity, internal validity, construct validity and external validity.

3.4.1 Internal validity

Gay and Airasian (2000:345) describe internal validity as the "condition that observed differences of the dependent variable are a direct result of the independent variable, not some other variable". Internal validity is threatened when plausible rival explanations cannot be eliminated. The researcher must develop strategies that will rule out other plausible presumed causes of the effect.

The following measures enhanced internal validity of the research:

- Restriction was used to limit the study to records that meet eligibility and exclusion criteria.
- By selecting all records that meet the criteria eliminates biases resulting from differential selection of records.

- Use of patients records eliminates reactivity to experimental arrangements (Hawthorne effect) in that patients were not aware that such a study will be undertaken hence rendering the study as inconspicuous as possible.

3.4.1.1 Statistical conclusion validity

This concerns the validity of inferences that there truly is an empirical relationship or correlation between the presumed cause and effect (Polit & Beck 2008: 286). The researcher must provide the strongest possible evidence that the relationship is *real* and the intervention truly caused the outcome. The researcher must be aware of making false conclusions after statistical analyses. Multivariate analysis of variance (MANOVA) and posthoc tests were used to control for significant covariates at baseline. The

researcher was mindful of not running into the risk of inferring causal relationships as the researcher did not have any control over the independent variables. (Polit & Beck 2008: 272).

3.4.1.2 External validity

External validity is the extent to which study findings can be generalised beyond the sample used in the study (Burns & Grove 2003: 200). It is also the extent to which the results of a study can be generalised to and across populations, settings and times (Johnson & Christensen 2000: 200). It also concerns inferences about the extent to which relationships observed in a study hold true over variations in people, conditions and settings as well as over variations in treatment and outcomes (Polit & Beck 2008: 301).

This study used medical records of a heterogeneous sample of patients which can attest that the study results can be replicated for various subgroups in the sample. By selecting medical records from divergent sites ensured a broader representation of the study population and a broad-based variability in the dimensions considered important for the study. Furthermore, the setting in which the study was conducted is representative of the clinical settings in which the findings will be applied.

An important primary issue in external validity is to know whether there is constancy of a relationship (or constancy of causation) and not whether the magnitude of the effect is constant (Polit & Beck 2008: 302).

3.4.1.3 Ethical considerations related to data collection

Research ethics refers to the moral principles governing research from inception through to completion and publication of results and beyond the study itself (Burns & Grove 2003: 186). The trust in research rests on the trust in the integrity of researchers and the reliability of the results of their scientific work. The outcome and interpretation of their research can be verified by the scientific community, but cannot be verified by the public for which the new knowledge is intended. Therefore, if science is to remain trustworthy, researchers must observe basic moral principles in their work, and must be people of integrity and honesty (Academy of Sciences of the Czech Republic 2006).

3.4.1.4 Protecting the rights of the institutions involved

Permission to conduct the research involving patient records was approved by University of South Africa postgraduate research committee and the office of the Human Research Development Committee (Ministry of Health, Botswana). These committees determined the acceptability of the proposed research based on guiding applicable law and standards of professional conduct and practice. See annexure D and E for the letters requesting and granting permission for the study to be conducted. Permission to gain access to medical records at the study sites was approved by the authorities at the study sites. Authorities at the study site were assured that any information that the researcher was coming across during the conduct of the research will not be divulged to any interest groups that could jeopardize the patients' welfare in society and the concerned institution (Babbie & Mouton 2001: 251).

3.4.1.4.1 A**utonomy**

This can be defined as the right to self-determination in research participation (Neave 2001: 1). Conduct of the research at the study sites and access to medical records was approved by authorities after informing them about the research and allowing them to choose whether to participate. In research involving abstraction of data from medical records, authorities act on behalf of the patients for reviewing proposed research plans before data can be abstracted. The researcher abstracted data from primary and secondary medical records of patient on antiretroviral therapy at the study sites and no interaction with patients was made during the conduct of the research. Authorities at the study sites were asked to specify their own restriction on the access to and use of their data.

3.4.1.4.2 Confidentiality and anonymity

Based on the right to privacy, the research participant has the right to anonymity and the right to assume that the data collected will be kept confidential. Confidentiality is the researcher's management of private information shared by the participant (Burns & Grove 2003: 172). Polit and Beck (2008:180) describe anonymity as the most secure means of protecting confidentiality and occur when even the researcher cannot link the participants to their data.

The researcher undertook the following steps to ensure that a breach of confidentiality and anonymity did not occur:

- Remove all explicit patient identifiers to ensure anonymity of abstracted medical data while maintaining the integrity of the medical records.
- Assign an identification number matching each abstracted data.
- Enter no identification information onto the observation schedule and into the computer.
- Store research data in locked cabinets and secured databases.
- Report research findings in the aggregate to disguise the person's identity.
- Limited access to the abstracted data to the researcher and those who were involved in analysing the data.

3.4.1.4.3 Beneficence

One of the fundamental ethical principles in research is that of beneficence which imposes a duty on the researchers to minimise harm and to maximise benefits (Polit & Beck 2008: 170). Such an assessment is designed to determine whether the risk/benefit ratio is acceptable. Though the researcher did not directly interact with the patients, there was still a potential risk of invading a patients' privacy while accessing the data. The benefits of this study outweigh the risks by contributing to the existing body of knowledge on the management of patients exposed to Isoniazid preventive therapy with the roll out of antiretroviral therapy.

3.4.1.4.4 Scientific integrity on the part of the researcher

The goal of research is to generate sound scientific knowledge, which is possible only through the honest conduct, reporting and publication of quality research (Burns & Grove 2003: 187). Scientific integrity has been characterised as a commitment to truthfulness, to personal accountability, and to vigorous adherence to standards of professional conduct (e.g., accuracy, fairness, collegiality, transparency) (Warner & Roberts 2004: 381).

Research misconduct is defined by United States Department of Health and Human Services (2005: 42 CFR Part 93, Subpart A), as a fabrication, falsification or plagiarism

in proposing, performing, or reviewing research, or in reporting research results. According to Burns and Grove (2003: 188), an act of research misconduct requires a significant departure from the acceptable practice of the scientific community for maintaining the integrity of the research record.

To maintain scientific integrity and eliminate the possibility of scientific misconduct and plagiarism, the researcher took the following steps:

- Recognise and intentionally disseminate the principles of reliable, trustworthy scientific practice in the scientific community and refuse all scientific dishonesty and infringement of the principles of scientific research.
- Stand resolutely against the non-ethical and inappropriate use of scientific knowledge.
- Carry out research in such a way that society, the environment and cultural values are not threatened.
- Observe principles of scientific work when obtaining, selecting and assessing scientific data, and at the same time take into account the specificity of this study.
- Account for the precision and objectivity of this research and recognise the limits of research methods used.
- Be responsible for the completeness and verifiability of the results in the dissertation for their undistorted interpretation.
- Refrain from falsifying or fabricating primary and secondary data sources which the researcher abstracted data from during the conduct of the research.
- Preserve primary data and documentation used during the study.
- Acknowledge the scientific contributions of predecessors and colleagues to the question studied to which the dissertation is linked directly.
- When citing ideas, words, processes, findings and results obtained by other authors, a clear reference was made to the respective source.
- Cite important results which are contrary to my own results and conclusions.
- Adhere to a fair and open relationship between the researcher and the supervisors.

- Disclose any potential conflicts of interest in the conduct of the research to the University without fail.
- Be accountable for any breach in confidentiality of medical data.

3.5 CONCLUSION

This chapter discussed the research steps and procedures that guided this study. The quantitative, non-experimental and retrospective research design guided this study. Research method including data sources, research population and setting, sampling technique, sample size and data collection approach were described. The data collection process and management, data collection instrument validity and reliability were also described. How the research internal and external validity is ensured was explained and the chapter concluded by discussing ethical considerations related to the research.

The next chapter presents the data analysis and discusses the findings in relation to the study. Research results are compared and contrasted to some studies discussed in the literature review chapter of this dissertation.

CHAPTER 4

DATA ANALYSIS, PRESENTATION AND FINDINGS

4.1 INTRODUCTION

The purpose of this study was to investigate the immunological and virological responses in antiretroviral therapy naïve patients exposed to Isoniazid preventive therapy categorized into four groups:

1. Group 1: Patients concurrently taking Combivir + Efavirenz and Isoniazid preventive therapies [$EFV_{ipt\text{-}current}$];
2. Group 2: Patients concurrently taking Combivir + Nevirapine and Isoniazid preventive therapies [$NVP_{ipt\text{-}current}$];
3. Group 3: Patients taking Combivir + Efavirenz after completing Isoniazid preventive therapy [$EFV_{ipt\text{-}past}$]; and
4. Group 4: Patients taking Combivir + Nevirapine after completing Isoniazid preventive therapy [$NVP_{ipt\text{-}past}$].

The specific objectives of this study are to compare the four study groups with regards to:

- The mean increases in $CD4^+$ cell counts;
- Rate of immunological success [an increase of $CD4^+$ cell count of ≥ 50 cells/ µL over the baseline value] ; and
- Time to and proportion of patients achieving virological success [viral load ≤400 copies/mL after initiation of antiretroviral therapy.

The tested null hypothesis is ($H_{o:}$ $\mu_{EFVipt\text{-}current} = \mu_{NVPipt\text{-}current} = \mu_{EFVipt\text{-}past} = \mu_{NVPipt\text{-}past}$).

This study answered the following six specific research questions using the post hoc pairwise contrast analysis:

1. How do patients concurrently taking Combivir + Nevirapine and Isoniazid Preventive Therapies **[$NVP_{ipt\text{-}current}$]** differ from those taking Combivir + Nevirapine

after completing Isoniazid Preventive Therapy [**$NVP_{ipt\text{-}past}$**] in terms of their $CD4^+$ cell counts and viral loads?

2. How do patients concurrently taking Combivir + Efavirenz and Isoniazid Preventive Therapies **[$EFV_{ipt\text{-}current}$]** differ from those taking Combivir + Efavirenz after completing Isoniazid Preventive Therapy **[$EFV_{ipt\text{-}past}$]** in terms of their $CD4^+$ cell counts and viral loads?
3. How do patients concurrently taking Combivir + Nevirapine and Isoniazid Preventive Therapies **[$NVP_{ipt\text{-}current}$]** differ from those concurrently taking Combivir + Efavirenz and Isoniazid Preventive Therapies **[$EFV_{ipt\text{-}current}$]** in terms of their $CD4^+$ cell counts and viral loads?
4. How do patients taking Combivir + Nevirapine after completing Isoniazid Preventive Therapy **[$NVP_{ipt\text{-}past}$]** differ from those taking Combivir + Efavirenz after completing Isoniazid Preventive Therapies **[$EFV_{ipt\text{-}past}$]** in terms of their $CD4^+$ cell counts and viral loads?
5. How do patients taking Combivir + Nevirapine after completing Isoniazid Preventive Therapy **[$NVP_{ipt\text{-}past}$]** differ from those concurrently taking Combivir + Efavirenz and Isoniazid Preventive Therapies **[$EFV_{ipt\text{-}current}$]** in terms of their $CD4^+$ cell counts and viral loads?
6. How do patients concurrently taking Combivir + Nevirapine and Isoniazid Preventive Therapy **[$NVP_{ipt\text{-}current}$]** differ from those concurrently taking Combivir + Efavirenz after completing Isoniazid Preventive Therapies **[$EFV_{ipt\text{-}past}$]** in terms of their $CD4^+$ cell counts and viral loads?

4.2 DATA ANALYSIS

Data analysis refers to organising, presenting, interpreting and summarising and communicating numeric information (Mouton 2001: 108; Polit & Beck 2008: 556). Descriptive statistics was used to describe the characteristics of the sample from which the data were collected and values obtained from the measurement of variables.

Analytical statistics using multivariate analysis of variance (MANOVA) was used to answer the research questions and to verify hypotheses. The *F*-statistic for **time** factor or within – group effect (differences at 12, 24 and 36 weeks follow-up) was computed to indicate whether across all the groups, the mean immunological and virological

outcomes differed over time. Another *F*-statistic for **treatment** or between – groups effect (differences among groups) was computed to test whether across all follow-up time periods the immunological and virological outcomes differed among the four comparison groups. Finally, the **time*treatment** interaction effect was tested to determine whether group differences varied across time.

One of the core underlying assumptions in the multivariate analysis of variance (MANOVA) procedure is that of sphericity (a special case of the assumption of group homogeneity), that checks whether the variance/covariance matrix of the observed data follows a particular pattern. In order to test sphericity, the Mauchly's test which tests for the equivalence of the hypothesised and the observed variance/covariance patterns was computed. The significance of the Mauchly's test [χ^2 (5) = 13.572, p = 0.019] on group effects immunological outcomes indicated that the results of the MANOVA *F*-statistic needed to be adjusted because of the violations of the assumption of equivalence which could lead to Type I errors. Adjusted Huynh-Feldt (H – F) epsilon was chosen as a test of significance to interpret the findings of the analysis because the Greenhouse – Geisser (G – G) epsilon (ε = 0.992) tends to underestimate epsilon when the epsilon is greater than 0.70 hence lead to rejection of the null hypothesis. In contrast, the Mauchly's test when computing virological outcomes was not violated and as such the multivariate test statistic Wilk's lambda test was used to interpret the results since it the most commonly used and gives exact *F* statistic values.

The Turkey-Kramer Honest Square Difference posthoc analyses were performed after the initial statistical analysis to identify which groups were significantly different in terms of immunological outcomes. Contingency analysis by group mosaic plots and contingency tables were used to calculate the time and proportion of patients achieving undetectable viral load (<400 copies/mL) at each follow-up time point.

Associations between categorical variables were assessed by Chi-Square (χ^2) test and proportions (%) where appropriate. Only the effects of the age covariate that was statistically significant at baseline was adjusted for in the regression analysis. The effect of gender on immunological and virological outcomes could not be tested in the

multivariate analysis because abstracted medical records in the $NVP_{ipt\text{-}past}$ group did not contain records for the male gender.

The 95% level of confidence (95% CI) and a probability two-sided value of $p < 0.05$ were used as the definition of significance to compare groups. Data were analysed by a professional statistician using SAS JMP statistical software version 8.0 (SAS Institute, Cary, NC, USA).

The study findings are discussed in accordance with the objectives and questions outlined above and presented in the form of tables, graphs and figures.

4.3 RESEARCH RESULTS

4.3.1 Baseline characteristics of the study population

Nine hundred and eleven medical records of antiretroviral naïve patients initiated on antiretroviral therapy between 1st January 2006 and 31st December 2006 were abstracted. Of these, 711 (78%) were excluded from the sample for seven main reasons. One hundred and eighty nine (21%) were not actually given Isoniazid preventive therapy, 182 (20%) were excluded for having another opportunistic infection other than TB or an intercurrent malignancy, 137 (15%) had missing or illegible data recorded in the patient record, 91 (10%) switched from one antiretroviral regimen to another, 45 (5%) developed TB while on Isoniazid preventive therapy, 40 (4%) became pregnant during follow–up and 27 (3%) were recorded as having had prior TB therapy before Isoniazid preventive therapy [Figure 4.1] on page 78.

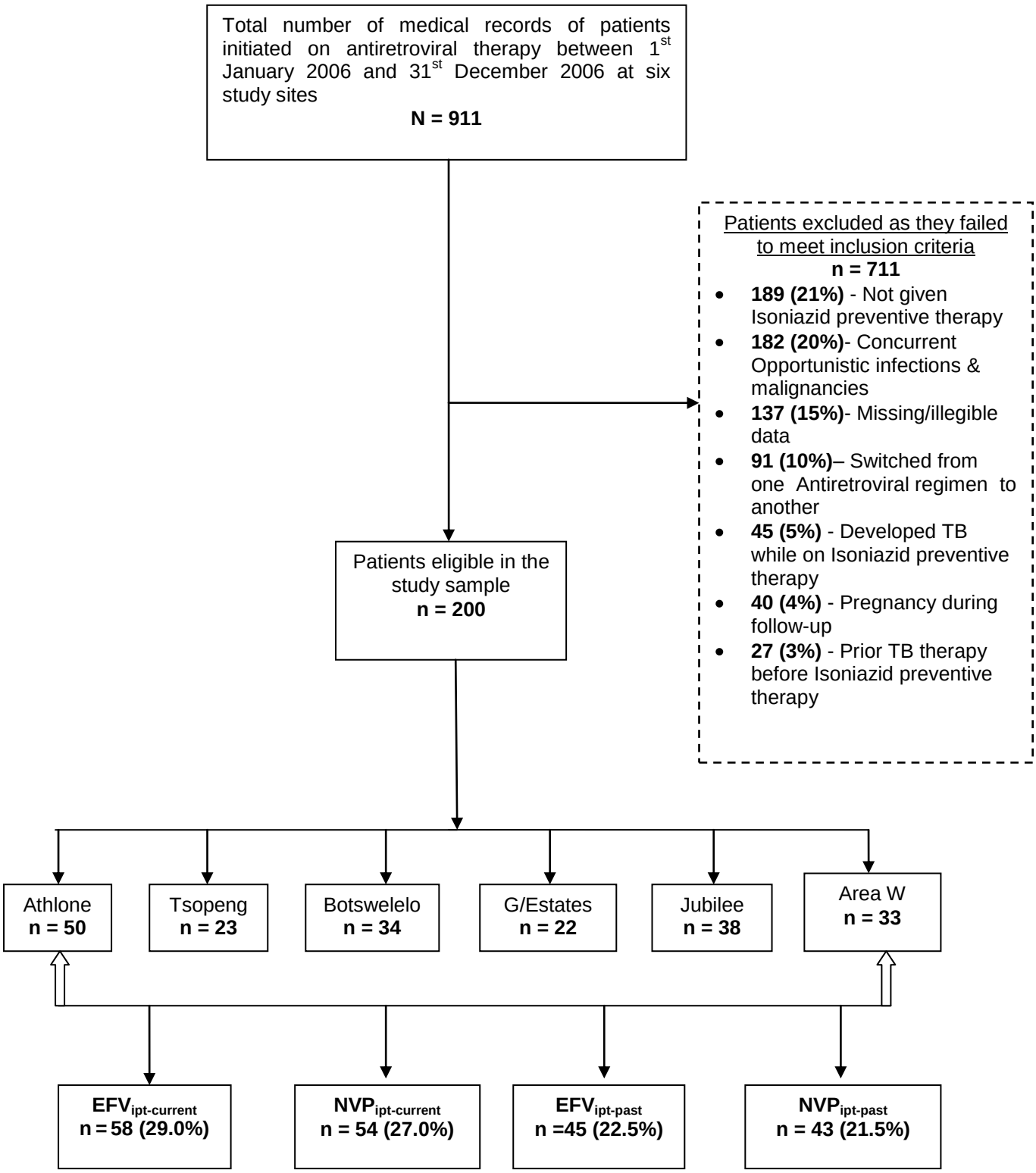


Figure 4.1 Flow chart showing breakdown of patients initiated on antiretroviral therapy between 1st January 2006 and 31st December 2006 by treatment group at six study sites

Of the remaining 200 (22%) patient medical records, 50 (25%) were from Athlone hospital, 23 (12%) Tsopeng, 34 (17%) Botswelelo, 22 (11%) Gerald Estates, 38 (19%) Jubilee and 33 (16%) Area W clinics. The 200 medical records were categorized into the following four treatment groups; 58 (29%) $EFV_{ipt\text{-}current}$, 54 (27%) $NVP_{ipt\text{-}current}$, 45 (22.5%) $EFV_{ipt\text{-}past}$ and 43 (21.5%) $NVP_{ipt\text{-}past}$ (Table 4.1).

Table 4.1 Number of patients in different antiretroviral regimen and Isoniazid preventive therapy status groups from the six study sites, (N = 200)

Category	$EFV_{ipt\text{-}current}$	$NVP_{ipt\text{-}current}$	$EFV_{ipt\text{-}past}$	$NVP_{ipt\text{-}past}$	TOTAL
Athlone Hospital	11	11	14	14	**50**
Area W	7	7	9	10	**33**
Botswelelo	19	6	7	2	**34**
Gerald Estates	4	13	1	4	**22**
Jubilee	12	10	11	5	**38**
Tsopeng	5	7	3	8	**23**
TOTAL	**58 (29.0%)**	**54 (27.0%)**	**45 (22.5%)**	**43 (21.5%)**	**200**

The mean age of patients in group $EFV_{ipt\text{-}current}$ was 42 years (range 29 to 63 years), 15 (11.4%) were females and 43 (62.3%) males, that in group $NVP_{ipt\text{-}current}$ was 31 years (range 21 to 43 years), 51 (38.9%) females and 3 (4.3%) males and in group $EFV_{ipt\text{-}past}$ was 42 years (range 28 to 56 years), 22 (16.8%) were females and 23 (33.3%) males. Lastly, the mean age in group $NVP_{ipt\text{-}past}$ was 32 years (range 20 to 47 years), 43 (32.8%) were females and zero (0%) males. There were statistically significant differences in gender and age distribution among the groups. Overall there was a predominance of females initiated on antiretroviral therapy. This probably reflects the gender proportions of the adult population infected with HIV in Southern Africa and that women have comparatively several entry points into HIV care and support programs than men (Muula et at 2007: 63). Women access HIV testing through prevention of mother to child transmission, antenatal care, and family planning and maternal and child health programs.

Of the 131 females, 94 (71.8%) were initiated on Nevirapine – based treatment ($NVP_{ipt\text{-}current}$ and $NVP_{ipt\text{-}past}$) whereas 66 (95.7%) males out of 69 were in the Efavirenz – based

groups (Table 4.2). Gender dominance in either the Nevirapine – based treatment or Efavirenz – based treatment groups is consistent with Botswana National Antiretroviral Treatment Guidelines. Efavirenz – based treatment regimens is associated with teratogenecity when administered to women of reproductive age groups (Botswana Guidelines on Antiretroviral Treatment 2005; 11).

The baseline immunological and virological characteristics of the study groups are similarly summarized in (Table 4.2). The ranking order for $CD4^+$ cell count from the highest to the lowest was [$NVP_{ipt\text{-}past}$ = 131 cells/µL, $EFV_{ipt\text{-}past}$ = 123 cells/µL, $NVP_{ipt\text{-}current}$ = 122 cells/µL and $EFV_{ipt\text{-}current}$ = 104 cells/ µL. Similarly, the ranking order of viral load from lowest to highest was [$NVP_{ipt\text{-}past}$ = 247,236 copies/mL, $NVP_{ipt\text{-}current}$ = 363,898 copies/mL, $EFV_{ipt\text{-}past}$ = 371,412 copies/mL and $EFV_{ipt\text{-}current}$ = 376,916 copies/mL. There is an inverse proportion between $CD4^+$ cell count and viral load in the groups. Patients in the $NVP_{ipt\text{-}past}$ group had the highest $CD4^+$ cell count and lowest viral load unlike those in the $EFV_{ipt\text{-}current}$ who had the lowest $CD4^+$ cell count and highest viral load at baseline. The other groups somewhat came in between these extremes. Despite these differences at baseline, the results show that there were no statistically significant differences in $CD4^+$ cell count and viral load among the four groups.

Table 4.2 Baseline characteristics of patients initiated on antiretroviral therapy in the four study groups, (N = 200)

Category	$EFV_{ipt\text{-}current}$ n = 58	$NVP_{ipt\text{-}current}$ n = 54	$EFV_{ipt\text{-}past}$ n = 45	$NVP_{ipt\text{-}past}$ n = 43	Totals	*p* value*
Female	15 (11.4%)	51 (38.9%)	22 (16.8%)	43 (32.8%)	131	<0.001
Male	43 (62.3%)	3 (4.3%)	23 (33.3%)	0 (0%)	69	<0.001
Mean Age in years (range)	42 (29 – 63)	31 (21 – 43)	42 (28 – 56)	32 (20 – 47)	36.8 (24.5 – 52.2)	<0.001
$CD4^+$ (cells/μL)†	104 (2.0)	122 (2.1)	123 (2.1)	131 (4.8)	120 (2.1)	0.086
Viral load (copies/mL)†	376,916 (5.6)	363,898 (5.6)	371,412 (5.6)	247,236 (5.4)	339,868 (5.5)	0.155

†Mean $CD4^+$ cell count & VL (Log values in parentheses). *For significance at 95% CI: overall (4 groups) $p < 0.05$.

4.3.2 Mean increase in $CD4^+$ cell count

Multivariate analysis of variance (MANOVA) test **without** taking age and gender covariates into consideration were computed. Analysis was conducted to determine within – group and between – group effects. Within - group effect compare treatment effect by observing changes in outcomes within each group across treatments.

Between – group effect shows the comparison about the effect of the treatments (Table 4.3 (a – d)). Sphericity is a mathenmatical assumption of homogeneity of variance in repeated measures analysis of variance designs. Mauchly's sphericity test for within – group immunological outcomes in this analysis indicated that the assumption of sphericity had been violated (Chi –Square = 13.572, $p = 0.019$). Therefore the degrees of freedom were corrected using Huynh – Feldt estimates of sphericity (epsilon = 0.988) (Table 4.3a). Huynh – Feldt correction was chosen to interpret the findings of the analysis because the Greenhouse – Geisser correction factor has been shown to be too conservative and at times fails to detect a true difference between group means.

Table 4.3a Test of homogeneity of variance on immunological outcomes in patients initiated on antiretroviral therapy between 1st January 2006 and 31st December 2006 at the six study sites

Within – Groups Effect	Mauchly's Criterion	χ^2	*df*	*p* – value*	Epsilon	
					Huynh-Feldt	Greenhouse-Geisser
Mean CD4$^+$ cell count	0.933	13.572	5	0.019	<0.001	<0.001

*Mauchly's sphericity test

The results in Table 4.3b show that there was a significant main effect of time on mean increase in CD4$^+$ cell count in the four treatment groups (F [3,194] = 152.324, p = 0.001). It can be concluded that mean CD4$^+$ cell count increased with time in each of the treatment group. However, the F value associated with the interaction between time and treatment group was not statistically significant (F [9, 588] = 1.545, p = 0.129). The conclusion to be drawn from the result confirms the null hypothesis ($H_{o:}$ $\mu_{EFVipt\text{-}current}$ = $\mu_{NVPipt\text{-}current}$ = $\mu_{EFVipt\text{-}past}$ = $\mu_{NVPipt\text{-}past}$) which states that there will be no differences in immunological outcomes among the four treatment groups.

Table 4.3b Results of the effect of time and interaction between time and group on immunological outcomes in patients initiated on antiretroviral therapy between 1st January 2006 and 31st December 2006 at the six study sites

Within-Group Effect	F-Statistic	Numerator *df*	Denominator *df*	*p*-value*	Epsilon	
					Huynh-Feldt	Greenhouse-Geisser
Time	152.324	3	194	<0.001	<0.001	<0.001
Time*Group	1.545	9	588	0.129	0.130	0.132

*Multivariate analysis of variance (MANOVA)

Following the multivariate tests of significance for within-groups effects, an analysis of between-groups effects was computed as shown in Table 4.3c below. The F value and associated p value for the test of group effect is statistically significant. We can therefore conclude that a statistically significant difference exists between treatment

groups on their overall mean increase in $CD4^+$ cell count (F [3,196] = 4.053, p = 0.008). However, this result does not tell us which treatment groups were statistically different. Therefore a post hoc test at each follow-up time was computed to identify which particular groups were significantly different from which other groups in terms of immunological outcome. (See section 4.3.2.1 below).

Table 4.3c Result for between – groups treatment main effect on immunological outcomes in patients initiated on antiretroviral therapy between 1st January 2006 and 31st December 2006 at the six study sites

Between–Group Effect	SS value†	F-Statistic	Numerator *df*	Denominator *df*	*p*-value*
Intercept	6.661	1305.489	1	196	<0.001
Group	0.620	4.054	3	196	0.008

†Sum of Squares

*Multivariate analysis of variance (MANOVA)

4.3.2.1 Post hoc tests

Between-group differences were identified using Tukey-Kramer Honest Square Difference post hoc tests. Computing post hoc tests has an advantage of localising groups which have a statistically significant different overall *F*-statistic. In addition, the computed 95% Confidence Intervals provide further insight into the variability of plausible mean differences in $CD4^+$ cell counts between the observed groups. The results of the post hoc pairwise contrasts test at baseline and 12, 24 and 36 weeks of follow-up are summarised in Table 4.5a – d below.

Tables 4.4a and 4.4b summarise the results showing the p - values and 95% Confidence Intervals for immunological outcomes and treatment effect at baseline and 12 weeks of treatment follow-up. The main effect of all the six pairwise comparisons was not statistically significant. The results show equivalence in $CD4^+$ cell count at baseline and during the 12 weeks treatment follow-up in the four groups.

Table 4.4a Mean $CD4^+$ cell count differences at baseline between treatment groups of patients initiated on antiretroviral therapy at the six study sites

Source ($CD4^+$ cell count)	Level ($CD4^+$ cell count)	-Level ($CD4^+$ cell count)	Mean difference (95 % CI)[a]	*p* – value*
Group	$NVP_{ipt\text{-}past}$ (131)	$EFV_{ipt\text{-}current}$ (104)	0.338 (-0.023 – 0.699)	0.076
	$NVP_{ipt\text{-}past}$ (131)	$NVP_{ipt\text{-}current}$ (122)	0.232 (-1.430 – 0.599)	0.357
	$EFV_{ipt\text{-}past}$ (123)	$EFV_{ipt\text{-}current}$ (104)	0.228 (-0.128 – 0.584)	0.349
	$EFV_{ipt\text{-}past}$ (123)	$NVP_{ipt\text{-}current}$ (122)	0.122 (-0.240 – 0.484)	0.817
	$NVP_{ipt\text{-}past}$ (131)	$EFV_{ipt\text{-}past}$ (123)	0.110 (-0.273 - 0.492)	0.879
	$NVP_{ipt\text{-}current}$ (122)	$EFV_{ipt\text{-}current}$ (104)	0.105 (-0.234 - 0.445)	0.852

*The mean difference is significant at $p < 0.05$ level. **$CD4^+$ cells/µL in parenthesis.

[a] Adjustment for multiple comparisons: Tukey – Kramer

Table 4.4b Mean $CD4^+$ cell count differences at 12 weeks of treatment follow-up between treatment groups of patients initiated on antiretroviral therapy at the six study sites

Source ($CD4^+$ cell count)	Level ($CD4^+$ cell count)	-Level ($CD4^+$ cell count)	Mean difference (95 % CI)[a]	*p* – value*
Group	$NVP_{ipt\text{-}past}$ (252)	$EFV_{ipt\text{-}current}$ (209)	0.283 (-0.025 – 0.591)	0.084
	$NVP_{ipt\text{-}past}$ (252)	$EFV_{ipt\text{-}past}$ (211)	0.242 (-0.084 – 0.568)	0.223
	$NVP_{ipt\text{-}current}$ (248)	$EFV_{ipt\text{-}current}$ (209)	0.214 (-0.076 – 0.503)	0.226
	$NVP_{ipt\text{-}current}$ (248)	$EFV_{ipt\text{-}past}$ (211)	0.172 (-0.137 – 0.481)	0.473
	$NVP_{ipt\text{-}past}$ (252)	$NVP_{ipt\text{-}current}$ (248)	0.070 (-0.243 - 0.382)	0.939
	$EFV_{ipt\text{-}past}$ (211)	$EFV_{ipt\text{-}current}$ (209)	0.041 (-0.263 - 0.345)	0.985

*The mean difference is significant at $p < 0.05$ level. **$CD4^+$ cells/µL in parenthesis.

[a] Adjustment for multiple comparisons: Tukey – Kramer

Differences in increase in $CD4^+$ cell count between – groups started appearing at 24 weeks of treatment follow-up and continued until study endpoint as shown in tables 4.5c and 4.5d on page 81. Of the six pairwise contrasts, only the $NVP_{ipt\text{-}past}$ vs. $EFV_{ipt\text{-}current}$ was significant (Table 4.4c).

The results shows that mean increases in $CD4^+$ cell count were higher in the $NVP_{ipt\text{-}past}$ group at 24 weeks of follow- up and persisted until 36 weeks treatment follow-up. In addition, statistically significant pairwise contrast between $NVP_{ipt\text{-}current}$ vs. $EFV_{ipt\text{-}current}$ was observed at 36 weeks treatment follow-up time point**.** The result shows that patients in the $NVP_{ipt\text{-}current}$ group achieved the highest $CD4^+$ cell count increase than those in the $EFV_{ipt\text{-}current}$ group (Table 4.5d). Generally patients in the $NVP_{ipt\text{-}past}$ and $NVP_{ipt\text{-}current}$ had better immunological outcomes during the follow-up time periods.

Table 4.4c Mean $CD4^+$ cell count differences at 24 weeks of treatment follow-up between treatment groups of patients initiated on antiretroviral therapy at the six study sites

Source ($CD4^+$ cell count)	Level ($CD4^+$ cell count)	-Level ($CD4^+$ cell count)	Mean difference (95 % CI)[a]	*p* – value*
Group	$NVP_{ipt\text{-}past}$ (277)	$EFV_{ipt\text{-}current}$ (217)	0.263 (0.017 – 0.509)	0.031*
	$NVP_{ipt\text{-}past}$ (277)	$EFV_{ipt\text{-}past}$ (234)	0.217 (-0.044 – 0.478)	0.140
	$NVP_{ipt\text{-}current}$ (261)	$EFV_{ipt\text{-}current}$ (217)	0.185 (-0.046 – 0.416)	0.165
	$NVP_{ipt\text{-}current}$ (261)	$EFV_{ipt\text{-}past}$ (234)	0.139 (-0.108 – 0.385)	0.466
	$NVP_{ipt\text{-}past}$ (277)	$NVP_{ipt\text{-}current}$ (261)	0.078 (-0.172 - 0.328)	0.849
	$EFV_{ipt\text{-}past}$ (234)	$EFV_{ipt\text{-}current}$ (217)	0.046 (-0.196 - 0.289)	0.960

*The mean difference is significant at $p < 0.05$ level. **$CD4^+$ cells/µL in parenthesis.

[a] Adjustment for multiple comparisons: Tukey – Kramer

Table 4.4d Mean $CD4^+$ cell count differences at 36 weeks of treatment follow-up between treatment groups of patients initiated on antiretroviral therapy at the six study sites

Source ($CD4^+$ cell count)	Level ($CD4^+$ cell count)	-Level ($CD4^+$ cell count)	Mean difference (95 % CI)[a]	*p* – value*
Group	$NVP_{ipt\text{-}past}$ (305)	$EFV_{ipt\text{-}current}$ (228)	0.284 (0.041 – 0.526)	0.015*
	$NVP_{ipt\text{-}current}$ (289)	$EFV_{ipt\text{-}current}$ (228)	0.234 (-0.006 – 0.478)	0.042*
	$NVP_{ipt\text{-}past}$ (305)	$EFV_{ipt\text{-}past}$ (246)	0.232 (-0.025 – 0.489)	0.093
	$NVP_{ipt\text{-}current}$ (289)	$EFV_{ipt\text{-}past}$ (246)	0.182 (-0.061 – 0.425)	0.216
	$EFV_{ipt\text{-}past}$ (246)	$EFV_{ipt\text{-}current}$ (228)	0.052 (-0.188 - 0.291)	0.944
	$NVP_{ipt\text{-}past}$ (305)	$NVP_{ipt\text{-}current}$ (289)	0.050 (-0.196 - 0.296)	0.953

*The mean difference is significant at $p < 0.05$ level. **$CD4^+$ cells/µL in parenthesis.

[a] Adjustment for multiple comparisons: Tukey – Kramer

There are several factors that affect immunological and virological outcomes in patients whilst on antiretroviral therapy as already mentioned in section 2.2.2.1.3 on page 39. These result mainly from fear of medication side effects, being infected with a drug-resistant strain of HIV, psychosocial factors, age and gender (Kleeberger et al 2001: 83; Becker et al 2002: 21; Montessori et al 2004: 229; Saitoh et al 2005: 555; Nicastri, Leone, Angeletti, Palmisano, Sarmati, Chiesa, Geraci, Vella, Narciso, Corpolongo & Andreoni 2007: 728; The Collaboration of Observational HIV Epidemiological Research Europe (COHERE) study group 2008: 1465). In this study only the effect of the age covariate of on immunological and virological outcomes was analysed. Absence of males in the $NVP_{ipt\text{-}past}$ group could have introduced bias in the gender covariate analysis hence it was not computed.

Multivariate modelling adjusting for the age covariate that was statistically significant at baseline was analysed. The findings of a statistically significant Mauchly's sphericity test (Chi-Square = 13.777, $p = 0.017$) shown in Table 4.5a violates the assumption of group mean equivalence hence the Huynh – Feldt estimate of sphericity (epsilon = 0.992) was used to correct for the adjustment.

Table 4.5a Test of homogeneity of variance after adjusting for the age covariate on immunological outcomes in patients initiated on antiretroviral therapy between 1st January 2006 and 31st December 2006 at the six study sites

Within – Group Effect	Mauchly's Criterion	χ^2	*df*	*p* – value*	Epsilon	
					Huynh-Feldt	Greenhouse-Geisser
Mean $CD4^+$ cell count	0.931	13.778	5	0.017	<0.001	<0.001

*Mauchly's test of sphericity

The within – group analysis summarised in Table 4.4b on page 83 shows that the *F* value and associated *p* value for test of time effect was statistically significant even after adjusting for the age covariate. This shows that treatment group mean ages were not a factor in mean increases in $CD4^+$ cell count over time. The analysis of the interaction effect between time and group (F [9,585] = 1.204, p = 0.290 and between time, group and age (F [3,193] = 0.994, p = 0.436) was not statistically significant. The result suggests that there is no interaction among these variables to influence the overall mean increase in $CD4^+$ cell count.

Table 4.5b Results of the effect of time, interactions between time and group and time, group and age after adjusting for the age covariate on immunological outcomes in patients initiated on antiretroviral therapy between 1st January 2006 and 31st December 2006 at the six study sites

Within- Group Effect	F-Statistic	Numerator *df*	Denominator *df*	*p*-value*	Epsilon	
					Huynh-Feldt	Greenhouse-Geisser
Time	6.338	3	193	0.000	,0.001	<0.001
Time*Group	1.204	9	585	0.290	0.290	0.292
Time*Group*Age	0.994	3	193	0.396	0.436	0.433

*Multivariate analysis of variance (MANOVA)

The analysis of between – groups (treatment effect) in Table 4.4c below show that the treatment group effect was statistically significant even after adjusting for the age factor (F [3,195] = 4.315, p = 0.006). Finally, the test result of the age factor on mean increase in $CD4^+$ cell count between the treatment groups was not statistically significant (F [1,195] = 1.757, p = 0.187). Age by itself did not seem to influence the mean increase in $CD4^+$ cell count between the treatment groups.

Table 4.5c Result for between – groups treatment main effect after adjusting for the age covariate on immunological outcomes in patients initiated on antiretroviral therapy between 1st January 2006 and 31st December 2006 at the six study sites

Effect	Sum of Squares value	F - Statistic	Numerator *df*	Denominator *df*	*p* – value*
Intercept	0.136	26.445	1	195	<0.001
Group	0.066	4.315	3	195	0.006
Age	0.009	1.757	1	195	0.187

*Multivariate analysis of variance (MANOVA)

Figure 4.2 below illustrates the mean increases in $CD4^+$ cell count of patients on antiretroviral therapy in each treatment group at different follow-up time points from the baseline. Patients in all treatment groups achieved progressive increases in $CD4^+$ cell count with the highest mean increase at study endpoint being in patients in the $NVP_{ipt\text{-}past}$ group. This group was followed by those in $EFV_{ipt\text{-}past}$, $NVP_{ipt\text{-}current}$ and $EFV_{ipt\text{-}current}$ respectively. These group differences paralleled differences in pre-treatment $CD4^+$ cell count at initiation of antiretroviral therapy. Patients concurrently on Isoniazid preventive and antiretroviral therapy had lower $CD4^+$ cell count increases.

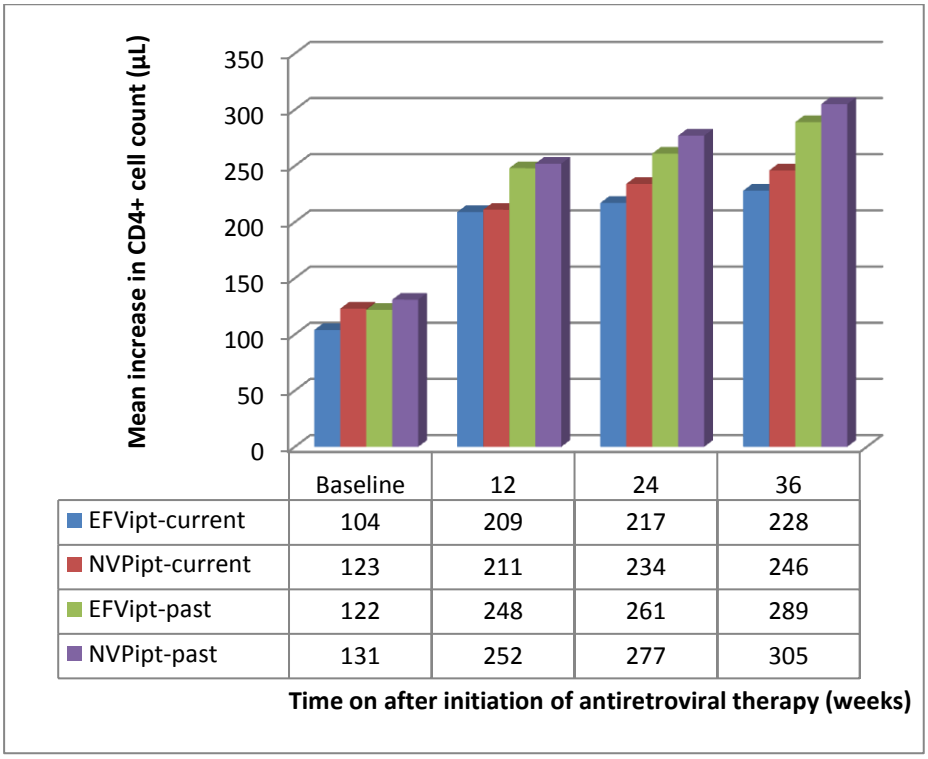


Figure 4.2 Mean increases in $CD4^+$ cell count from baseline to different follow-up time points in antiretroviral naïve patients in the four study groups after initiation of antiretroviral therapy

4.3.2.2 Rate of immunological success from baseline to 36 weeks treatment follow –up in four treatment groups

In this study the immunological success is defined as an increase in $CD4^+$ cell count of ≥50 cell/µL relative to the baseline value at different times in the study. There was a progressive increase in the proportion of patients achieving immunological success in treatment groups $EFV_{ipt\text{-}current}$, $EFV_{ipt\text{-}past}$ and $NVP_{ipt\text{-}current}$. Patients in the $NVP_{ipt\text{-}past}$ had a rapid improvement in imminent logical function that peaked at 12 weeks and showed a decline at 24 weeks before showing a steady upward trend at 36 weeks of antiretroviral therapy (Figure 4.3).

The figure shows that throughout the course of follow-up the highest proportion of patients with immunological success were in the group $NVP_{ipt\text{-}past}$. However, at study endpoint the proportion of patients in this group was equivalent to that in the $NVP_{ipt\text{-}current}$. Overall, the proportion of patients achieving immunological success had the

following group ranking order ($NVP_{ipt\text{-}past}$ > $NVP_{ipt\text{-}current}$ > $EFV_{ipt\text{-}current}$ > $EFV_{ipt\text{-}past}$). Treatment groups which had higher baseline $CD4^+$ cell count maintained a higher nadir in the proportion of patients achieving immunological success.

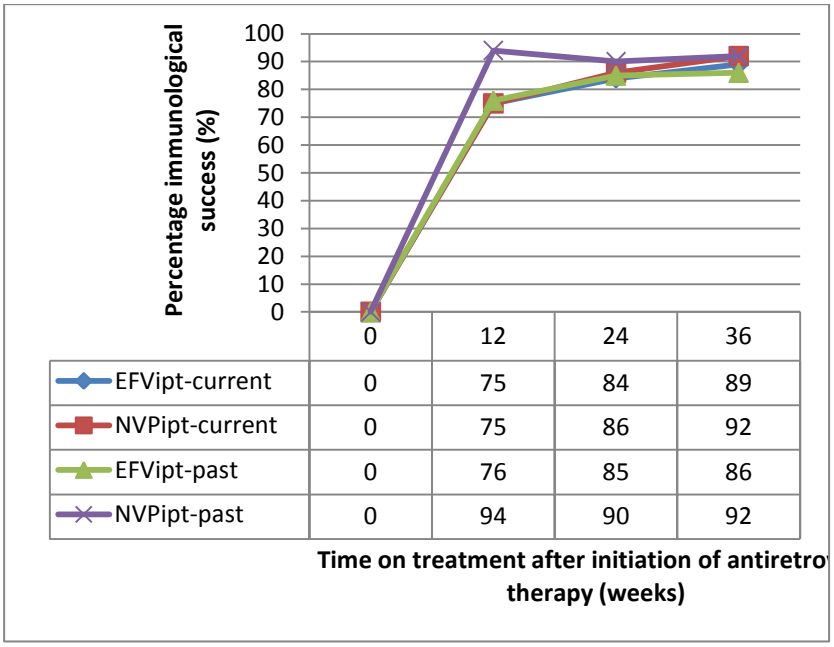


Figure 4.3 Proportion of patients in each treatment group achieving immunological success at 12, 24, and 36 weeks after initiation of antiretroviral therapy

4.3.3 Time to and proportion of patients to virological success

Table 4.6a shows the result of the Mauchly's sphericity test for the within – group analysis computed for equivalence in virological outcomes among the four treatment groups. The finding of non-significance (Chi-Square = 1298.222, *p* = 1.556) corresponds to concluding that the assumptions of equivalence had been met and therefore there was no need correcting for sphericity. The multivariate test statistic Wilk's Lambda was used to interpret test results since it the most commonly used and gives exact *F* statistic values.

Table 4.6a Test of homogeneity of variance on virological outcomes in patients initiated on antiretroviral therapy between 1st January 2006 and 31st December 2006 at the six study sites

Within – Group Effect	Mauchly's Criterion	χ^2	*df*	*p* – value*
Mean viral load	0.001	1298.222	5	1.556

*Mauchly's test of sphericity

Table 4.6b shows the summary of the test result involving within-group independent variables of time effect and interaction effect between time and group on virological decay. Since the *F* – statistic for time effect is statistically significant (*F* [3,194] = 106.531, *p* <0.001), we can conclude there was a decrease in viral load with time in each of the treatment groups. In the instance of the interaction effect between time and group, the *F* value associated with the multivariate test is not statistically significant (*F* [9,472.3] = 0.944, *p* = 0.259). The drawn conclusion is that decrease in viral load with time was not dependent upon the treatment group effect thereby confirming the null hypothesis of equivalence among the four treatment groups ($H_{o:}\ \mu_{EFVipt\text{-}current} = \mu_{NVPipt\text{-}current} = \mu_{EFVipt\text{-}past} = \mu_{NVPipt\text{-}past}$).

Table 4.6b Results of the effect of time and interaction between time and group on virological outcomes in patients initiated on antiretroviral therapy between 1st January 2006 and 31st December 2006 at the six study sites

Within- Group Effect	Value	F-Statistic	Numerator *df*	Denominator *df*	*p*-value*
Time	1.647	106.531	3	194	<0.001
Time*Group	0.944	1.256	9	472.3	0.259

*Wilk's Lambda *F* statistic

Similarly, the treatment group effect was not statistically significant (*F* [3,196] = 2,503, *p* = 0.060) on the overall decrease in viral load when computing the between – groups tests (Table 4.6c).

Table 4.6c Result for between – groups treatment main effect on virological outcomes in patients initiated on antiretroviral therapy between 1st January 2006 and 31st December 2006 at the six study sites

Between–Group Effect	SS value†	F-Statistic	Numerator *df*	Denominator *df*	*p*-value*
Intercept	1.631	319.750	1	196	<0.001
Group	0.038	2.503	3	196	0.060

†Sum of Squares
*Multivariate analysis of variance (MANOVA)

Table 4.7a shows the non-significant (Chi-Square = 1293.668, *p* = 1.556) Mauchly's sphericity test result for within – group analysis after adjusting for the age covariate. Once again the assumptions of equivalence have been met and there was no need to correcting for sphericity. The Wilk's Lambda *F* statistic values was used to interpret test findings of the results.

Table 4.7a Test of homogeneity of variance after adjusting for the age covariate on virological outcomes in patients initiated on antiretroviral therapy between 1st January 2006 and 31st December 2006 at the six study sites

Within – Group Effect	Mauchly's Criterion	χ^2	*df*	*p* – value*
Mean viral load	0.001	1293.668	5	1.556

*Mauchly's test of sphericity

Table 4.7b shows a statistically significant *F* value and associated *p* value for time effect for within – group analysis after adjusting for the age covariate. We conclude by interpreting that treatment group mean ages were not a factor in viral load decay over time. The analysis of the interaction effect between time and group (*F* [9,469.86] = 1.134, *p* = 0.337) and between time, group and age (*F* [3,193] = 1.092, *p* = 0.354) was not statistically significant. The result suggests that there is no interaction among these variables to influence the overall viral load decay.

Table 4.7b Results of the effect of time, interactions between time and group and time and age after adjusting for the age covariate on virological outcomes in patients initiated on antiretroviral therapy between 1st January 2006 and 31st December 2006 at the six study sites

Within-Group Effect	Value	F-Statistic	Numerator *df*	Denominator *df*	*p*-value*
Time	0.063	4.036	3	193	0.008
Time*Group	0.949	1.134	9	469.86	0.337
Time*Group *Age	0.017	1.092	3	193	0.354

*Wilk's Lambda *F* Statistic

Finally the between – groups (treatment effect) analysis in Table 4.4c show that the treatment group effect (*F* [3,195] = 2.156, *p* = 0.094) and age effect (F [1,195] = 0.006, p = 0.939) was not statistically significant even after adjusting for the age factor (*F* [3,195] = 4.315, *p* = 0.006). We conclude that age had no effect on virological decay.

Table 4.7c Result for between – groups treatment main effect after adjusting for the age covariate on virological outcomes in patients initiated on antiretroviral therapy between 1st January 2006 and 31st December 2006 at the six study sites.

Effect	Sum of Squares value	F - Statistic	Numerator *df*	Denominator *df*	*p* – value*
Intercept	0.049	9.618	1	195	0.002
Group	0.033	2.156	3	195	0.094
Age	2.976	0.006	1	195	0.939

*Multivariate analysis of variance (MANOVA)

Table 4.7 below shows that by 12 weeks of treatment follow-up the proportion of patients achieving undetectable viral load had not reached a 100% in each of the treatment group. The table shows that the highest proportion of patients with undetectable viral load at this follow-up time point was in the $EFV_{ipt\text{-}past}$ group.

By 24 weeks of treatment follow-up, the picture changed with 100% of patients in the groups $EFV_{\mathbf{ipt\text{-}current}}$ and $EFV_{\mathbf{ipt\text{-}past}}$ reaching undetectable viral load. This persisted until the study endpoint. Although patients in the NVP – based treatment groups did not

achieve 100% viral suppression by study endpoint, there was a progressive increase in the proportion to that effect. At all follow-up time point's group differences were not statistically significant as reported by the Pearson Chi-Square (χ^2**)** test.

Table 4.8 The proportion of patients by group achieving undetectable viral load (< 400 copies/mL) at 12, 24 and 36 weeks of treatment follow-up, N = 200.

Time on treatment	Treatment groups				
	$EFV_{ipt\text{-}current}$ n= 58	**$NVP_{ipt\text{-}current}$ n = 54**	**$EFV_{ipt\text{-}past}$ n = 45**	**$NVP_{ipt\text{-}past}$ n = 43**	***p* – value***
12 weeks	55 (94.8%)	51 (94.4%)	43 (95.6%)	41 (95.4%)	0.079
24 weeks	58 (100.0%)	51 (94.4%)	45 (100.0%)	42 (97.7%)	0.132
36 weeks	58 (100.0%)	53 (98.2%)	45 (100.0%)	42 (97.7%)	0.532

*Pearson Chi-Square

4.4 OVERVIEW OF RESEARCH FINDINGS

Data for two hundred eligible antiretroviral naïve patients enrolled in the antiretroviral program between 1st January 2006 and 31st December 2006 at the six study sites was summarised and analysed. At baseline statistically significant differences in age and gender was observed among the groups. However, the $CD4^+$ cell count and viral load were similar across all four groups.

Multivariate analysis of variance (MANOVA) was used to analyse the mean increase in $CD4^+$ cell count, rate of immunological success and time to and proportion of patients in each group achieving viral suppression. The independent variables were age, time on treatment, and treatment group and interaction effect between time, age and group. The dependent variables were the $CD4^+$ cell count and viral load outcomes. Because the Mauchly's tests for within – group variable showed a violation of the assumption of sphericity when analysing effects on mean increases in $CD4^+$ cell count, the Huynh – Feldt correction was used to interpret these results.

Each treatment group showed statistically significant mean increases in $CD4^+$ cell count and viral load decay from baseline to study endpoint (**time effect**). However, the **treatment effect** (between – group differences) on immunological outcomes were varied across the groups. Mean increases in $CD4^+$ cell count among the groups when

arranged in descending order took the following rankings [$NVP_{ipt\text{-}past}$ > $NVP_{ipt\text{-}current}$ > $EFV_{ipt\text{-}current}$ > $EFV_{ipt\text{-}past}$]. In contrast, the ranking order for viral load suppression was [$EFV_{ipt\text{-}past}$ > $EFV_{ipt\text{-}current}$ > $NVP_{ipt\text{-}past}$ > $NVP_{ipt\text{-}current}$].

Post hoc tests were performed to isolate between-groups differences in mean increases in $CD4^+$ cell count and showed differences between $EFV_{ipt\text{-}current}$ vs. $NVP_{ipt\text{-}past}$ at 24 weeks treatment follow-up which persisted until study endpoint. At 36 weeks treatment follow-up time point, differences were observed between $EFV_{ipt\text{-}current}$ vs. $NVP_{ipt\text{-}current}$. Throughout the period of follow-up there were no statistically significant differences in the proportion of patients in each of the four groups achieving virological success as reported by the Pearson Chi-Square (χ^2).

The analysis of the interaction effect between time and treatment for $CD4^+$ increase and viral load outcomes was no statistically significant confirming the hypothesis of equivalence ($H_{o:}\ \mu_{EFVipt\text{-}current} = \mu_{NVPipt\text{-}current} = \mu_{EFVipt\text{-}past} = \mu_{NVPipt\text{-}past}$). Adjusting for the age covariate at baseline had no effect on virological success and increases in $CD4^+$ cell count.

4.5 CONCLUSIONS

The study has shown that each treatment group achieved statistically significant mean increases in $CD4^+$ cell count and viral load decay at each follow-up time point. Post hoc analyses showed that patients in the $NVP_{ipt\text{-}past}$ had superior immunological outcomes. In contrasts, the highest proportion of patients achieving virological success was in the $EFV_{ipt\text{-}past}$ treatment group. Prior exposure to Isoniazid preventive therapy resulted in better immunological and virological outcomes at each study follow-up time point. However, the four groups were statistically equivalent in $CD4^+$ cell count and viral load outcomes at study endpoint confirming the null hypothesis ($H_{o:}\ \mu_{EFVipt\text{-}current} = \mu_{NVPipt\text{-}current} = \mu_{EFVipt\text{-}past} = \mu_{NVPipt\text{-}past}$) which states equivalence among the groups.

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CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 INTRODUCTION

The efficacy of Isoniazid monotherapy exposure status on immunological and virological outcomes in patients initiated on either a Nevirapine or Efavirenz-based antiretroviral therapy regimen has not been well established. The aim of this facility-based retrospective analysis study was to investigate the immunological and virological responses in highly active antiretroviral therapy naïve patients exposed to Isoniazid preventive therapy in a country with a high coverage of both therapies. The multivariate analysis of variance (MANOVA) was used to analyse the main effect of each factor and the interaction between them (time, time*group and time*group*age) and to test the null hypothesis in the research objectives. Post hoc pairwise contrast analysis tested the research questions.

5.2 RESEARCH DESIGN AND METHOD

A quantitative, non-experimental and retrospective cohort design was used to conduct this research. The design was chosen to test both the null and research hypotheses using descriptive and analytic statistics and not to infer any cause-and-effect relationships.

The study was facility - based conducted at six antiretroviral therapy treatment centres in Botswana. The data sources for the study were primary medical records of patients retained at the study sites and initiated on combination antiretroviral therapy from 1stJanuary 2006 to 31st December 2006.

A total of 911 medical records of patients initiated on antiretroviral therapy during this period were abstracted onto structured data collection tool that was developed and tested prior to data collection. Through purposive sampling technique, only 200 (22%) of these records met the inclusion criteria and were included in the data analysis.

5.3 SUMMARY AND INTERPRETATION OF THE RESEARCH FINDINGS

5.3.1 Mean increase in $CD4^+$ cell count

Each treatment group showed statistically significant mean increases in $CD4^+$ cell count from baseline to study endpoint (time effect) ($p < 0.001$). The analysis of the interaction effect between time and treatment (time*treatment) was not statistically significant (H – F $\varepsilon = 0.988$, $p = 0.1296$) confirming the null hypothesis ($H_{o:}\ \mu_{EFVipt\text{-}current} = \mu_{NVPipt\text{-}current} = \mu_{EFVipt\text{-}past} = \mu_{NVPipt\text{-}past}$). Adjusting for the age covariate at baseline had no effect on increases in $CD4^+$ cell count. The findings of equivalence in immunological outcome of the comparative groups serve to validate findings of previous studies by (Hung et al 2003: 2619; Breen et al 2006: 1439; Manosuthi et al 2008: 296) that demonstrated no statistically significant differences in virologic, immunologic and clinical outcomes to antiretroviral therapy between TB/HIV co-infected patients concurrently treated with anti-tuberculosis and antiretroviral therapy and non-tuberculosis control group on antiretroviral therapy only.

5.3.1.1 Post hoc tests

Tukey Kramer post hoc tests were used to answer the six research questions with regards to group differences in immunological outcome as shown when computing the between-groups effects in the multivariate analysis of variance. The results show no statistically significant differences in $CD4^+$ cell count increase between the comparative groups at baseline and 12 weeks of treatment follow-up. These findings at short period of follow-up are similar to a study by (Hung et al 2003: 2619) that assessed immunological outcomes at 4 weeks of antiretroviral treatment. However, by 24 weeks of treatment follow-up, this study found a statistically significant difference in $CD4^+$ cell count increase between the groups $EFV_{ipt\text{-}current}$ vs. $NVP_{ipt\text{-}past}$ with the latter group achieving the highest mean increase ($p = 0.031$). Differences between these two groups persisted until the 36 weeks follow-up time point ($p = 0.051$).

In addition, by 36 weeks treatment follow-up between-groups differences were also observed in the $EFV_{ipt\text{-}current}$ vs. $NVP_{ipt\text{-}current}$ ($p = 0.042$). Patients in the $NVP_{ipt\text{-}current}$ had the highest mean $CD4^+$ cell count increase. The results show that irrespective of the Isoniazid exposure status, patients in the Nevirapine-based antiretroviral regimes had

superior immunological outcomes. This is in contrast to a retrospective study conducted by Shipton, Wester, Stock, Ndwapi, Gaolathe, Thior, Avalos, Moffat, Mboya, Widenfelt, Essex, Hughes & Shapiro (2009: 362) that compared HIV-infected adults exposed and not exposed to TB treatment with similar baseline viral load levels and $CD4^+$ cell counts following antiretroviral therapy initiation. Their findings showed that there were no statistically significant differences in immunological (p = 0.80) and virological (p = 0.28) responses in patients on antiretroviral therapy only and those exposed to TB treatment 12 months following antiretroviral therapy. In addition, there were no differences in virological and immunological outcomes when the Nevirapine and Efavirenz-based antiretroviral therapy regimens were stratified according to TB treatment exposure status. Similar findings were obtained in a randomized, open-label pilot study undertaken by Nunenz, Soriano, Martin-Carbonero, Barrios, Barreiro, Blanco, Garcia-Benayas and Gonzalez-Lahoz (2002: 188 – 189) to explore antiviral activity and tolerability of Nevirapine and Efavirenz in patients exposed to antiretroviral therapy only.

Differences in $CD4^+$ cell count increase between Nevirapine and Efavirenz – based treatment groups found in this study can be explained by the fact that patients in Nevirapine – based treatment groups comparatively initiated antiretroviral therapy with the higher baseline $CD4^+$ cell count and maintained a high nadir increase in $CD4^+$ cell count throughout treatment follow-up. This is supported by recent evidence that has shown that initiating antiretroviral therapy at a higher $CD4^+$ cell count results in better response to therapy and preservation of immune function leading to improved prognostic outcomes (Kitahata, Gange, Abraham, Merriman, Saag, Justice, Hogg, Deeks, Eron, Brooks, Rourke, gill, Bosch, Martin, Klein, Jacobson, Rodriguez, Sterling, Kirk, Napravnik, Rachlis, Calzavara, Horberg, Silverberg, Gebo, Goedert, Benson, Collier, Van Rompaey, Crane, McKaig, Lau, Freeman, Moore, for the NA-ACCORD Investigators 2009: 1819). However, the contrasting results from this study shows that a larger randomized controlled study is needed to further demonstrate significant differences between Nevirapine and Efavirenz in patients on antiretroviral therapy and exposed to Isoniazid preventive therapy.

5.3.2 Rate of immunological success from baseline to 36 weeks

In this study the immunological success was defined as an increase in $CD4^+$ cell count of ≥50 cell/μL relative to the baseline value. There was a statistically significant (p < 0.001) progressive increase in the proportion of patients achieving immunological success in all the four treatment groups. At study endpoint, the proportion of patients achieving immunological success had the following ranking order ($NVP_{ipt\text{-}past}$ > $NVP_{ipt\text{-}current}$ > $EFV_{ipt\text{-}current}$ > $EFV_{ipt\text{-}past}$). The overall proportions of patients per treatment group achieving immunological success were no statistically significant.

5.3.3 Time to and proportion of patients achieving virological success

Each treatment group achieved statistically significant decrease in viral load with time (F [3,194] = 106.531, p <0.001). Comparing the interaction effect between time and treatment group showed no statistically significant differences [Wilks' Lambda (λ) = 0.944, F (9,472.3) = 1.2558; p = 0.2588] and this remained so even after adjusting for the age covariate at baseline [Wilks' Lambda (λ) = 0.949, F (9,469.86) = 1.1339; p = 0.3369]. This was also true when between – groups test analysis showed no statistically significant results (F [3,196] = 2,503, p = 0.060).

The proportion of patients achieving virological success was not statistically significantly different as shown by the Pearson Chi-Square (χ^2) test result despite a higher proportion of patients in the $EFV_{ipt\text{-}past}$ treatment group. These findings are similar to studies by (Hung et al 2003: 2619; Breen et al 2006: 1439; Manosuthi et al 2008: 296) that showed virological equivalence between Efavirenz and Nevirapine. In contrast, an observational cohort study by (Nachega et al 2008: 2117 – 25) that compared the non-nucleoside reverse transcriptase inhibitors (Efavirenz vs. Nevirapine) showed Efavirenz to have superior virological outcomes than Nevirapine [Hazard ratio (HR 1.52; 95% CI 1.24 – 1.86)]. Differences in baseline viral load had no effect on virological outcome in this study.

5.4 CONCLUSIONS

The conclusion to be drawn from this study after multivariate analysis of variance is that exposure status to Isoniazid preventive therapy in patients on antiretroviral therapy had no statistically significant effect on immunological and virological outcomes of the comparative groups. These findings confirm the null hypothesis (H_o: $\mu_{EFVipt\text{-}current} = \mu_{NVPipt\text{-}current} = \mu_{EFVipt\text{-}past} = \mu_{NVPipt\text{-}past}$) which states immunological and virological equivalence in the four treatment groups. However, computing the pairwise *posthoc* contrast analyses showed that patients initiating antiretroviral therapy with **prior exposure to Isoniazid therapy** had better immunological ($\mathbf{NVP_{ipt\text{-}past}}$) and virological ($\mathbf{EFV_{ipt\text{-}past}}$) outcomes.

5.5 RECOMMENDATIONS

Based on the results of pairwise *post hoc* contrasts analyses, patients should complete Isoniazid preventive therapy before initiating antiretroviral therapy in order to benefit from better outcomes. This study only suggests causality and but fails to determine causal pathways related to immunological and virological outcomes in patients with prior exposure to Isoniazid preventive therapy and initiating antiretroviral therapy. To conclusively determine the causal-effect pathway, a randomised clinical trial is recommended to answer the question of whether prior exposure to Isoniazid or the antiretroviral regimen itself is responsible for these group differences.

5.6 CONTRIBUTIONS OF THE STUDY

Prior studies have shown that Isoniazid increases the number of gamma- interferon (IFN-γ) producing T-cells responsible for enhancing host immune response in patients being treated for latent tuberculosis (Wilkinson *et al.* 2006: 356). This study has shown that prior exposure to Isoniazid preventive therapy results in better immunological and virological outcomes. It remains to be determined whether gamma- interferon (IFN-γ) acts additively/synergistically with antiretroviral therapy to better immunological and virological outcomes.

5.7 LIMITATIONS OF THE STUDY

The study is limited by its retrospective design that involved secondary analysis of clinical data gathered primary not for research purposes but for clinical patient management. As such, the researcher could not control for some of the confounding covariates such as the effect of pill burden, patient psycho-social issues, and optimum time to initiation of antiretroviral therapy after completing Isoniazid therapy, specificity and sensitivity of assays used to monitor treatment outcomes. The other limitation of this observation study is that it only suggests causality but fails to determine causal patterns with any degree of certainty and the more reason why clinicians should continue to provide Isoniazid preventive and antiretroviral therapies to eligible patients as it has been shown in prior studies to have other added benefits of reducing the incidence of tuberculosis in HIV infected persons.

Thirdly, this study did not evaluate the effect of the time period between completion of Isoniazid therapy and initiation of antiretroviral therapy in order to achieve better immunological and virological outcomes. Fourthly, the possibility effect of having zero males in the $NVP_{ipt\text{-}past}$ group to detect immunological and virological differences among the comparative groups cannot be excluded. A further limitation is that these findings cannot be directly generalised to settings other than the clinical sites despite the fact that all patients in the study received an equivalent standard of care. However, data suggests that exposure to Isoniazid therapy results in better immunological and virological outcomes in addition to the already known benefit of reducing the incidence of tuberculosis in HIV infected patients.

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ANNEXURE A

OBSERVATIONAL SCHEDULE

SECTION A: BIOGRAPHICAL DATA

1 Identification number: | | | |

2 Date of recording: | D | D | M | M | Y | Y |

3. Research Site: _____

4 Age _____ Years **Office use** A4 |

5 Gender: Male 1, Female 2 **Office use** A5 |

6 Isoniazid preventive therapy status: Current 1, Past 2 **Office use** A6 |

SECTION B: TREATMENT

Medication: **Office use**

Combivir + Nevirapine antiretroviral therapy **after** completing Isoniazid	1
Combivir + Nevirapine + Isoniazid + Pyridoxine	2
Combivir + Efavirenz **after** completing Isoniazid	3
Combivir + Efavirenz + Isoniazid + Pyridoxine	4

B1 |

NB: Combivir™ is a combination of Zidovudine and Lamivudine

Dosage:

Zidovudine (300mg) + Lamivudine (150mg)] + Nevirapine (200mg)	1
Zidovudine (300mg) + Lamivudine (150mg) + Nevirapine (200mg) + Isoniazid (300mg) + Pyridoxine (25mg	2
Zidovudine (300mg) + Lamivudine (150mg) + Efavirenz (600mg)	3
Zidovudine (300mg) + Lamivudine (150mg) + Efavirenz (600mg) + Isoniazid (300mg) + Pyridoxine (25mg)	4

Office use	
B2	

SECTION C TREATMENT OUTCOMES

				Office use	
1	CD4 count at 12 weeks:	_____	cells/µL	C1	
2	CD4 count at 24 weeks:	_____	cells/µL	C2	
3	CD4 count at 36 weeks:	_____	cells/µL	C3	
4	Viral load at 12 weeks:	_____	virus particles per ml	C4	
5	Viral load at 24 weeks:	_____	virus particles per ml	C5	
6	Viral load at 36 weeks:	_____	virus particles per ml	C6	

ANNEXURE B

ADULT/ADOLESCENT WHO CLINICAL STAGING*

Clinical Stage 1: ASYMPTOMATIC

Asymptomatic

Persistent generalised lymphadenopathy

Clinical stage 2: MODERATE DISEASE

Unexplained moderate weight loss <10% of baseline weight

Recurrent upper respiratory infections (sinusitis, otitis media, tonsillitis, pharyngitis)

Herpes zoster

Angular cheilitis

Recurrent oral ulceration

Popular pruritic eruptions

Seborrhoeic dermatitis

Fungal nail infections

Clinical Stage 3: ADVANCED DISEASE

Unexplained weight loss 10% of baseline

Unexplained chronic diarrhoea of more than one month

Unexplained persistent fever (>37.6°C, intermittent or constant) for more than one month

Persistent oral candidiasis

Oral hairy leukoplakia

Pulmonary TB (current)

Severe bacterial infections (e.g. pneumonia, empyema, pyomyositis, meningitis, bone/joint infection, bacteraemia)

Acute necrotising ulcerative stomatitis, gingivitis or periodontitis,

Unexplained anaemia (<8g/dl), neutropaenia (<0.5 × 109 per litre) thrombocytopenia (<50 × 10^9 per litre)

Clinical Stage 4: SEVERE DISEASE

HIV wasting syndrome

Pneumocystis pneumonia

Severe recurrent bacterial pneumonia

Chronic HSV infection (orolabial, genital, anorectal of more than one month or visceral at any site)

Oesophageal candidiasis (or candidiasis of trachea, bronchi or lungs)

Extrapulmonary tuberculosis

Kaposi's sarcoma

Cytomegalovirus infection (retinitis or infection of other organs)

Central nervous system toxoplasmosis

HIV encephalopathy

Extracryptococcosis including meningitis

Disseminated non-tuberculous mycobacterial infection

Progressive multifocal leukoencephalopathy

Chronic cryptosporidiosis (with diarrhoea)

Chronic isosporiasis

Disseminated mycosis (coccidiomycosis or histoplasmosis)

Recurrent non-typhoidal Salmonella bacteraemia

Lymphoma (cerebral or B-cell non-Hodgkin's or other solid HIV-associated tumours)

Invasive cervical cancer

Atypical disseminated leishmaniasis

Symptomatic HIV-associated neuropathy and cardiomyopathy

*Adapted from WHO case definitions of HIV for surveillance and revised clinical staging and immunological classification of HIV-related disease in adults and children - 2007

ANNEXURE C

REVISED CDC CLININAL AND IMMUNOLOGICAL STAGING FOR ADOLESCENT AND ADULTS*

IMMUNOLOGICAL STAGING

Category 1: ≥500 cells/µL

Category 2: 200 – 499 cells/µL

Category 3: < 200 cells/µL

CLINICAL STAGING

Category A

Asymptomatic HIV infection

Persistent generalised lymphadenopathy

Acute HIV infection with accompanying illness or history of acute HIV infection

Category B

Bacillary angiomatosis

Oral candidiasis

Fever or diarrhoea of more than one month

Oral hairy leukoplakia

Herpes zoster (≥ 2 distinct episodes or multi-dermatome)

Peripheral neuropathy

Vulvovaginal candidiasis (persistent and recurrent)

Pelvic inflammatory disease (complicated with tubo-ovarian abscess)

Cervical dysplasia or carcinoma in situ

Category C

Candidiasis (bronchi, trachea and lungs)

Oesophageal candidiasis

Invasive cervical cancer

Coccidiomycosis (disseminated or Extrapulmonary)

Cytomegalovirus infection (retinitis or outside liver, spleen and lymph nodes)

HIV-related encephalopathy

Herpes simplex virus infection of more than one month (bronchitis, pneumonitis or oesophagitis)

Disseminated or Extrapulmonary histoplasmosis

Isosporiasis (with diarrhoea) for than one month

Kaposi's sarcoma

Lymphomas (Burkitts, Immunoblastic and primary brain)

Mycobacterium avium complex

Mycobacterium tuberculosis

Pneumocystis carinii (*jirovici*) pneumonia

Recurrent pneumonia

Progressive multifocal leukoencephalopathy

Recurrent salmonella septicaemia

Cerebral toxoplasmosis

HIV wasting syndrome

Adapted from the revised CDC classification system for HIV-infected adolescents and adults (MMWR 1992/41 (RR-17)

ANNEXURE D

Ministry of Health

Republic of Botswana

Application for Approval of Human Research

Section A: Instructions

1. *For research/academic institutions or PHD students attach 14 copies of the following.*

   i. *Research Application form*
   ii. *Study proposal.*
   iii. *Consent/authorization form or a request for waiver of consent/authorization- Setswana, English and back translation where applicable.*
   iv. *Questionnaires to be used. Setswana, English and back translation where applicable.*
   v. *Curriculum vitae/ resume of the Principal investigator(s)*
   vi. *Approval letter from other IRBs*
   vii. *Grant approval letter*

   viii. *Any other supporting materials i.e. recruitment scripts, brochures, flyers etc*

2. For undergraduates and graduates attach one copy of the above listed items/ documents.

Section B: Application Details

1. Study Title: (Include Version number and date) **Immunologic and virologic response to Highly Active antiretroviral therapy in treatment naïve patients with exposure to Isoniazid preventative therapy**
2. Date of submission: 10th May 2007
3. Type of Research: **i. Basic Science ( )** **ii. Public Health ( )** **iii. Clinical Research (X)** **iv. Human Biology ( )** **v. Other**_____

5. Principal Investigator*(Name & Qualifications)*:	**5(i). Local Contact Details** ***Name:***

Dr Robert Manda **Bsc. MB.ChB**	
Postal **Address: P/Bag 28 Lobatse**	**Postal** **Address:**
Phone **Number: 5330392**	**Phone** **Number:**
E mail **Address: robertmanda@yahoo.com**	**E mail** **Address:**
Name of affiliate **Institution/Organization: Lobatse Town Council**	**Name of** **Institution/Organization:**
Department *(If Government)*: Clinics	**Department *(If Government)*:**

6. Other Investigators /Co-Principal Investigators			
Name:	**Organization:**	**Email:**	**Telephone Number:**

7. Key Personnel working with data that may be linked to human subjects:			
Name:	**Organization:**	**Email:**	**Telephone Number:**

Section C: Description of Research

1. Brief Description of Study

Isoniazid Preventative Therapy (IPT) is one of the strategies used for stopping or preventing latent tuberculosis infection progressing to active TB in people living with HIV. Some studies have been conducted to explore the clinical, immunologic and virologic responses in patients with HIV and TB who received antiretroviral therapy. However, research is lacking to determine the immunologic and virologic responses in antiretroviral treatment naïve patients exposed to IPT. This will be a retrospective cohort study in a setting with a high TB/HIV co-infection rate. This study will provide evidence-based practice for health care workers taking care of HIV infected patients on HAART and exposed to Isoniazid preventative therapy.

2. Rationale/Justification *(Why the need to carry out this study in Botswana)*:

The TB/HIV co-infection rate in the country of 60 - 80% is one of the highest in the world. Highly active antiretroviral therapy is provided for free in all government health facilities and self-administered IPT is also widely spread in the country. Data on the primary resistance to both antiretroviral drugs and Isoniazid shows that the rates are still very low making the country an ideal place to determine the true immunologic and virologic responses in patients on HAART who are exposed to IPT.

3. Study Objectives *(Both General and Specific)*:

1) To identify the relationship between prior exposure to IPT and immunologic and virologic responses in antiretroviral treatment naïve patients currently on HAART.
2) To identify the relationship between concurrently taking IPT and HAART to immunologic and virologic responses.
3) To determine which HAART regimen is superior in the prior or currently IPT exposed patients

4. Expected Results *(Both Primary and Secondary endpoints)*:

1. Immunologic and virologic responses (CD4 & VL) will be superior in patients concurrently taking HAART and IPT than in the prior IPT exposed group.
2. The HAART regimens in the two categorize of patients will comparable.

Section D. Methodology

1. Study Design
Retrospective cohort study

2. Study sites *(Districts, Towns, Villages, Health facilities, Schools etc* :

Francistown and Lobatse DHT IDCC

3. Subject Population(s) *(Clinical condition, Gender, age, and other relevant Characteristics)*:

Antiretroviral treatment naïve patients of both gender and ≥12 years on HAART and exposed to Isoniazid preventative therapy

4. Sample size*(The number of subjects to be involved in the study and how these subjects will be selected from the population)*:
A total of 200 medical records will be reviewed and divided between the two sites. Francistown and Lobatse will have 150 and 50 medical records respectively.
Sample size determination was by the following criteria**:**

- A CD4 cell count of ≥50 and a ≤400 virological response is an appreciable change that the study will detect
- An assumption of equivalence in the immunologic and virologic responses in the two categorizes
- The required power and significance level of the study is 95% and 5% respectively.

The sample size has been deliberately increased to control for confounding variables in the analysis**.**

5. Subject Recruitment/Sampling Methods ***(Explain all procedures in detail)*****:**

Retrospective data collection on consecutive, unselected medical records of patients' with exposure to IPT and started on HAART between January 2003 and December 2006 will be identified and used in the study. A total of 200 eligible medical records will be reviewed between Francistown IDCC and Lobatse IDCC.

6. Data Collection Methods (*Explain all procedures in detail)*

Retrospective data collection of antiretroviral treatment naïve persons on HAART and with

prior exposure to Isoniazid therapy and persons concurrently on HAART and Isoniazid

therapy will be identified and reviewed. Only medical records of participants with

complete clinical, adherence assessments and CD4 and VL laboratory results

recorded at prescribed intervals for treatment and toxicity monitoring will be selected for the study. Additional data for these patients will be extracted from the IPT registers and

10. Approximate Date Study Recruitment will begin: June 2007

11. Estimated Duration of entire study: approximately 3 months

Section E: Subject Information

1. Inclusion Criteria

- ✓ Both gender of ≥12 years of age
- ✓ Non pregnant women
- ✓ Treatment naïve to ARV
- ✓ CD4 ≤ 200 or AIDS defining illness before HAART enrolment
- ✓ Prior exposure to IPT and on HAART
- ✓ Concurrently on IPT and HAART
- ✓ Adherence to IPT and ARVs of ≥85%
- ✓ Complete Immunologic and virologic assessments results at baseline, 12, 24 and 36 weeks of H

Taking Botswana recommended first line ARVs (CBV/EFV or CBV/NVP)

2. Exclusion Criteria:

- ✓ Exclusion of participants treated for TB and later offered INH therapy
- ✓ Exclude participants switched from first line to other HAART regimens

3. Does the study involve Vulnerable Groups? *(Tick all that Apply)*?

Elderly ( )
Children ( )
Pregnant women, fetuses, or neonates of uncertain viability or nonviable ( )
Prisoners ( )
Decisionally impaired Persons ( )
Minority and indigenous groups ( )
Low Literacy ( )
Economically Disadvantaged ( )
Other_____
N/A **(X)**

4. Does this study involve any use of a drug? No (X) Yes ( ). If yes, is the drug registered or given exemption status (IND studies) by the Drug Regulatory Unit in Botswana? *If yes attach proof)*_____

5. Reasonably foreseeable risk or discomforts to the subjects (*list in detail)*:

Not Applicable

6. Who will cover Subject Injury-Related Costs?

i. Sponsor ( )
ii. Third-Party Payers ( )
iii. Subjects ( )
iv. N/A (X)

v. Other
_____

Section G. Study Details

1. Capacity Building (*how will the study build capacity in the country*)
The study will provide evidence-based clinical practice for health care workers taking care of HIV patients on HAART and exposed to IPT.

2. Dissemination (*How will the study findings be disseminated*)

Dissertation and Medical Journal

3. Other Ethical Body(ies) Involved in the review of the study

University of South Africa
Department of Health Sciences

Section H: Sponsor Information

1. Name of Sponsor:_____

2. Type of Sponsor:

i. Government ( )
ii. Private Foundation ( )
iii. Industry ( )
iv. Internal ( )
v. Other ( )

3. Sponsor Contact Person:_____

4. Sponsor Contact Telephone: _____

Section I: Contact Information:

PI or other researchers for answers to questions about the study or research-related injuries*(You must offer at least two contacts)*:	**The HRDC representative who can answer questions about their rights as research subjects**
i).	Name_____ Head of Health Research Unit Ministry of Health

ii).	Private Bag 0038 Botswana Tel: (+267) 3914467 Fax: (+267) 3914697

Section J: Investigator's Statement

INVESTIGATOR'S STATEMENT OF ASSURANCE

I promise to abide with existing relevant International Declarations and National procedures and guidelines when undertaking research involving human subjects within the Republic of Botswana and agree to:
1. Ensure that all studies conducted on human participants are designed and conducted according to sound scientific and ethical standards within the framework of good clinical practice.
2. Report to the Health Research and Development Committee any information requested, serious or unexpected adverse events and any information related to national programs.
3. Unless if an emergency treatment for patient care, obtain prior approval from the HRDC before amending or altering the scope of the project or implementing changes in the approved consent form(s).
4. Submit progress reports as required by the HRDC.
5. Maintain all documentation including consent forms and progress reports.

Principal Investigator's Name: **Dr Robert Manda**

Principal investigator's Signature: **R. Manda** Date: **10th May, 2007**

Principal Investigator's Position: **Principal Medical Officer**

Local Investigator's Name:

Local investigator's Signature: Date:

Local Investigator's Position:

After Completion

1. **An electronic and hard copy of the report should be submitted to the Health Research Unit, Ministry of Health as well as other relevant Botswana Government Institutions/Organizations within 3 months of producing a bound report.**

2. **All continuing renewals should be submitted at least 6 weeks before the expiration.**

Section K. For Health Research Unit use ONLY

<table>
<tr><td>1. Date Received</td><td rowspan="3">6. Review Body [ ] Health Research Unit
[ ] HRDC</td></tr>
<tr><td>2. Final Outcome</td></tr>
<tr><td>3. Ref No:</td></tr>
<tr><td>4. Expiration Date:</td><td></td></tr>
<tr><td colspan="2">7. Continuing renewals extension

Date 1 _____

Date 2 _____

Date 3 _____</td></tr>
<tr><td colspan="2">8. Final Report Submission

( ) Yes Date _____
( ) No</td></tr>
</table>

ANNEXURE E

TELEPHONE: 3632000
FAX: 3914467
DEPT. OF PPM&E
HEALTH RESEARCH UNIT

MINISTRY OF HEALTH
PRIVATE BAG 0038
GABORONE
BOTSWANA

MINISTRY OF HEALTH

REFERENCE: PPM&E 13/18 US Vol I (29) May 18, 2007

Dr. Robert Manda
Lobatse Town Council
Private Bag 28
Lobatse

Research Permit: Immunologic and virologic response to Highly Active Antiretroviral Therapy in treatment naïve patients with exposure to Isoniazid Preventive Therapy.

Your application for a research permit for the above stated research protocol refers. We note that you have satisfactorily revised the protocol as per our suggestions. **Permission is therefore granted to conduct the above-mentioned study**. This approval is valid for a period of 1 year, effective May 18, 2007.

The permit does not however give you authority to collect data from the selected health facilities without prior approval from the management of the facilities.

The research should be conducted as outlined in the approved proposal. Any changes to the approved proposal will need to be resubmitted to the Health Research Unit in the Ministry of Health.

Furthermore, you are requested to submit at least one hardcopy and an electronic copy of the report to the Health Research Unit, Ministry of Health within 3 months of completion of the study. Copies should also be sent to relevant authorities.

Approval is for academic fulfillment only.

Thank you,

S. El – Halabi
For Permanent Secretary, Ministry of Health

PERMANENT SECRETARY
MINISTRY OF HEALTH
RESEARCH UNIT
18 MAY 2007
P/BAG 0038
GABORONE
REPUBLIC OF BOTSWANA

ANNEXURE F

Tel: 012-429-6770
Fax: 012-429-6688
E-MAIL: dvilll@unisa.ac.za

Department of Health Studies
P. O. Box 392
UNISA
0003
10 July 2007

Student number: 3513 – 825-4

Manda R
P.O.Box 353
Labotse
BOTSWANA

Dear Student

MASTER'S DISSERTATION: Immunological and vilorogical response in highly active antiretroviral therapy in patients exposed to Isoniazid preventive therapy

This letter serves an advance notification of your registered research title and appointed supervisor(s). You will receive official documentation in due course.

The appointed supervisor(s) is/are as follows:

Supervisor:	**Dr S Knight**			
	Tel:	**+27 (0)83 762 3123**	**E-mail:**	**knights@ukzn.ac.za**
Joint-supervisor:	**Prof L de Villiers**			
	Tel:	**+27 (0)12 429 6770**	**E-mail:**	**dvilll@unisa.ac.za**

In future, direct all correspondence relating to your research project to your **supervisor**. If you cannot get hold of your supervisor you may consult the joint supervisor. The most convenient means of communication is by e-mail.

The first step is to submit a research proposal. With this at their disposal, your supervisor(s) will guide you further through the process of writing and revising the proposal until it is accepted by the Research and Ethics Committee of the Department of Health Studies. Contact your supervisor for guidance on how to write a research proposal. Consult the tutorial letter MNUALL-L/301/2007. Study this tutorial letter.

After the research proposal has been accepted, you will commence with the dissertation. The research proposal will be dispersed into the dissertation. Your supervisor will guide you through the process of planning and executing your research and writing the dissertation. Under no circumstances are you allowed to enter the field and collect data without the permission of your supervisor. Permission will only be granted once the relevant chapters of the dissertation, the research methods and the data collection instruments have been approved.

All documentation concerning your proposal and dissertation must be in accordance with the technical and academic stipulations of the MNUALL-L/301/2007 tutorial letter. Electronic submission via e-mail is preferable. If you send your documents in printed format, you must include an electronic version saved on a memory stick or disk (stiffy). This will enable the supervisor to make some changes to your document and insert electronic comments. **NEVER** send your documents in an assignment envelope because it will be delivered to the assignment section and not to your supervisor. Remember to make backup copies of all your documents to prevent loss of important data and information.

You need to register annually and pay the full registration fee until your research has been completed and the dissertation has been submitted for examination.

The supervisor will grant permission to submit the dissertation for examination once he or she is convinced that the necessary scientific and technical standards have been met. You will not be allowed to submit without the supervisor's permission. Once all the chapters, introductory pages, annexures and the bibliography have been approved, the dissertation must be submitted for professional editing. This can be very costly and you are advised to make provision for this expense in your budget.

Wishing you success with your studies.

Yours sincerely

dde Villiers

Prof L de Villiers
RESEARCH COORDINATOR