OPTIMIZATION OF HIV AND TUBERCULOSIS CO-TREATMENT IN TANZANIA: DRUG-DRUG INTERACTIONS AND CLINICAL OUTCOMES

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ABSTRACT

Background: Sub-Saharan Africa has been greatly affected by the HIV epidemic, with an estimated 23.5 million people living with HIV/AIDS (PLWHA) residing within this region by the end of 2011, being the leading cause of morbidity and mortality. Tanzania is one of the countries in this region with an HIV prevalence of 5.7% i.e approximately 2.7 million PLWHA. The most common opportunistic infection in sub-Saharan Africa is tuberculosis (TB). Currently HIV and TB are the leading cause of morbidity and mortality in Tanzania. The management of these two infections in individuals with the dual infection is challenging due to drug-drug interactions that could potentially lead to toxicities or ineffective treatment outcomes for one or both diseases. This thesis aims to describe the socio-demographic and clinical characteristics as well as the clinical outcomes of treatment.

Methods: We first performed a baseline study of a clinical HIV infected population enrolled at the HIV care and treatment centre (CTC) at Muhimbili National Hospital between June 2004 and September 2008. Based on this clinical experience, a cohort of HIV infected patients, with or without TB who were HAART naïve with CD4 cell counts <200cells/µL were recruited and followed up for 48 weeks after HAART initiation. Demographic, clinical and laboratory data were collected at baseline and up to 48 weeks of HAART therapy. Plasma efavirenz concentrations and CYP2B6*6, CYP3A5*3,*6 and *7, ABCB1 and SLCO1B1 genotypes were determined. A 29-item questionnaire on neuropsychiatric manifestations was collected up to week 16 of follow up.

Results: Most patients presenting to the CTC had advanced immune deficiency. Significantly higher proportions were female patients. With the free access to HAART in the later years, patients presented earlier to the CTC in the course of HIV disease. For the co-infection cohort study a total of 255 HIV only patients and 231 HIV-TB patients were recruited. The HIV-TB patients had significantly lower body mass index, Karnofsky scores and haemoglobin compared to those with HIV only, despite similar baseline CD4 cell counts. Mortality was similar in both the HIV only and those with HIV-TB, being 10.9% (16 deaths/100person years) and 11.3% (17 deaths/100py) respectively with the predictors for mortality being advanced disease such as low CD4 counts, low baseline WBC, oral candidiasis and Kaposi sarcoma. HIV only patients had significantly higher plasma efavirenz concentrations compared to the HIV-TB patients 4 weeks after HAART initiation indicating an interaction with rifampicin. Female gender and those with CYP2B6*6/*6 genotype also had significantly higher plasma efavirenz concentrations. Pharmacogenetic variants play a role in plasma efavirenz concentrations and long-term efavirenz autoinduction. The proportion of patients with efavirenz concentrations below the therapeutic range (<1µg/ml) at week 16 was higher compared to the concentrations at week 4 predominantly affecting extensive metabolizers showing that efavirenz autoinduction continues up to week 16. The incidence of drug induced liver injury (DILI) was 7.8% being non-significantly higher in the HIV-TB patients compared to those with HIV only. The median time to DILI was 2 weeks and the predictors for DILI included CYP2B6*6/*6 genotype and a positive antibody result to hepatitis C infection, but not efavirenz concentrations. The overall incidence of neuropsychiatric manifestations was 57% and these were higher in the HIV only compared to those with HIV-TB (66.7% vs 47.4%). The HIV only patients were more symptomatic, with proportionately higher grades of manifestations compared to those with HIV-TB. The risk of neuropsychiatric manifestations was 3 times higher in HIV only compared to those with HIV-TB. There were comparable increases in the median body weight and median CD4 cell counts towards the end of the study between the HIV only and those with HIV-TB. A total of 11.7% (11 HIV only and 8 HIV-TB) of the patients were defined to have treatment failure.

Conclusion: Patients enrolled at the CTCs are predominantly females, and present with advanced immune deficiency that ultimately puts them at a higher risk of dying. Pharmacogenetic variants influence efavirenz concentrations where slow metabolizers are at a higher risk of presenting with higher efavirenz concentrations, DILI and neuropsychiatric manifestations. The DILI seen in our setting is mild, transient and does not require treatment interruption. Patients using efavirenz alone are at a higher risk of developing neuropsychiatric manifestations compared to those who concomitantly use rifampicin. The WHO recommended efavirenz dosage of 600mg daily can be used with rifampicin among Tanzanian patients without compromise to their treatment outcomes.

Key Words: HIV, TB, Efavirenz, Rifampicin, CYP2B6

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LIST OF ABBREVIATIONS

ABCB1  ATP binding cassette transporter
AFB   Acid-Fast Bacillus
AIDS  Acquired Immune Deficiency Syndrome
ALT   Alanine aminotransferase
ART   Antiretroviral therapy
AST   Aspartate aminotransferase
ATV/r Atazanavir boosted with ritonavir
AUC   Area Under the Curve
AZT   Zidovudine
BCG   Bacillus Calmette-Guerin
BMI   Body Mass Index
CCR5  Cysteine-cysteine chemokine receptor 5
CDC   Center for Disease Control
CNS   Central Nervous System
CPT   Cotrimoxazole Preventive Therapy
CRF   Case Report Forms
CSF   Cerebro-spinal fluid
CTC   Care and treatment centre
CYP450 Cytochrome P450
CYP2B6 Cytochrome P450 2B6
CYP3A5 Cytochrome P450 3A5
d4T    Stavudine
DILI  Drug Induced Liver Injury
DNA   Deoxyribonucleic Acid
DOTS  Direct Observed Therapy Short Course
EFV   Efavirenz
EIA   Enzyme immune assay
ELISA Enzyme Linked Immunosorbent Assay
EPTB  Extra pulmonary Tuberculosis
FDA   Food and drug administration
FDC   Fixed Dose combination
FTC   Emtricitabine
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>HAART</td>
<td>Highly Active Antiretroviral Therapy</td>
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<td>HBC</td>
<td>Home Based Care</td>
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<tr>
<td>HBsAg</td>
<td>Hepatitis B surface antigen</td>
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<td>HCV</td>
<td>Hepatitis C virus</td>
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<td>HIV</td>
<td>Human Immunodeficiency Virus</td>
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<td>IgG</td>
<td>Immunoglobulin G</td>
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<tr>
<td>IPT</td>
<td>Isoniazid Preventive Therapy</td>
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<tr>
<td>IQR</td>
<td>Interquartile range</td>
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<tr>
<td>IRIS</td>
<td>Immune reconstruction inflammatory syndrome</td>
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<tr>
<td>LPV/r</td>
<td>Lopinavir boosted by ritonavir</td>
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<tr>
<td>MDR-TB</td>
<td>Multidrug resistant Tuberculosis</td>
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<tr>
<td>MNH</td>
<td>Muhimbili National Hospital</td>
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<tr>
<td>MoHSW</td>
<td>Ministry of Health and Social Welfare</td>
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<tr>
<td>MR</td>
<td>Metabolic ratio</td>
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<tr>
<td>MUCHS</td>
<td>Muhimbili University College of Health Sciences</td>
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<tr>
<td>MUHAS</td>
<td>Muhimbili University of Health and Allied Sciences</td>
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<tr>
<td>NACP</td>
<td>National AIDS control Program</td>
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<tr>
<td>NNRTI</td>
<td>Non-nucleoside reverse transcriptase inhibitors</td>
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<tr>
<td>NRTI</td>
<td>Nucleoside reverse transcriptase inhibitors</td>
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<tr>
<td>NTLP</td>
<td>National Tuberculosis and Leprosy Program</td>
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<tr>
<td>OI</td>
<td>Opportunistic infections</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase Chain Reaction</td>
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<tr>
<td>PI</td>
<td>Protease Inhibitors</td>
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<tr>
<td>PLWHHA</td>
<td>People Living with HIV/AIDS</td>
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<tr>
<td>PPE</td>
<td>Pruritic papular eruptions</td>
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<tr>
<td>PTB</td>
<td>Pulmonary tuberculosis</td>
</tr>
<tr>
<td>RNA</td>
<td>Ribonucleic acid</td>
</tr>
<tr>
<td>SNP</td>
<td>Single Nucleotide Polymorphism</td>
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<tr>
<td>STI</td>
<td>Sexually transmitted Infections</td>
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<tr>
<td>TB</td>
<td>Tuberculosis</td>
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<tr>
<td>TDF</td>
<td>Tenofovir</td>
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<tr>
<td>VCT</td>
<td>Voluntary Counselling and Testing</td>
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<tr>
<td>VDRL</td>
<td>Venereal Disease Research Laboratory</td>
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<tr>
<td>WBC</td>
<td>White Blood Count</td>
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<tr>
<td>Acronym</td>
<td>Description</td>
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<tr>
<td>WHO</td>
<td>World Health Organization</td>
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<tr>
<td>XDR-TB</td>
<td>Extensively Drug resistant Tuberculosis</td>
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1 INTRODUCTION

1.1 HIV

1.1.1 History and Epidemiology of HIV

“31 years after the first reported cases of the acquired immunodeficiency syndrome (AIDS) and 29 years after the discovery of the etiologic agent, effective control of the HIV and AIDS pandemic remains elusive”.

In June 1981, a new syndrome later coined “Acquired Immune Deficiency syndrome” (AIDS) was first announced by the Centre for Disease Control (CDC) [1, 2]. By 1983, the etiological agent for AIDS was identified and later named as human immunodeficiency virus (HIV) [3]. HIV disease pathogenesis is complex and multifactorial [4, 5]. Following primary infection with HIV, a burst of viral replication disseminates the virus into the lymphoid organs [6, 7]. A robust cellular and humoral immune response usually inhibits viral replication within weeks, but the virus invariably escapes from the immune containment, producing a chronic persistent infection leading to advanced clinical disease [8, 9]. A subspecies of chimpanzees native to west equatorial Africa had been identified as the original source of the virus. Scientists believe that the chimpanzee version of the immunodeficiency virus (called simian immunodeficiency virus or SIV) was transmitted to humans and mutated into HIV when humans hunted these chimpanzees for meat and came into contact with their infected blood [10-12].

There are two types of HIV: HIV-1 and HIV-2. Both types are transmitted by sexual contact, through blood, and from mother to child (more so for HIV-1 than HIV-2), and they appear to cause clinically indistinguishable AIDS. However, it seems that HIV-2 is less easily transmitted, and the period between initial infection and illness is longer in the case of HIV-2. Worldwide, the predominant virus is HIV-1, and the relatively uncommon HIV-2 type is concentrated in West Africa and is rarely found elsewhere. The strains of HIV-1 can be classified into four groups: the "major" group M, the "outlier" group O and two new groups, N and P. These four groups may represent four separate introductions of simian immunodeficiency virus (SIV) into humans. More than 90 percent of HIV-1 infections belong to HIV-1 group M. Within group M there are known to be at least nine genetically distinct subtypes (or clades) of HIV-1. These are
subtypes A, B, C, D, F, G, H, J and K [13-15]. Occasionally, two virus subtypes can meet and mix their genetic material in the cell of an infected person to create a new hybrid virus [16]. Those that infect more than one person are known as "circulating recombinant forms" [17]. Several studies have demonstrated that HIV-1 subtypes are not randomly distributed around the globe but show distinct geographical distributions [18-23]. The resultant viral diversity could have potential implications for possible differential rates of transmission, disease progression, responses to antiretroviral therapy (ART) and vaccine development [24, 25]. Worldwide it has been shown that 48% of infections are caused by subtype C, 12% by subtype A, 11% by subtype B, 5% by subtype G, 2% by subtype D and 22% recombinants [26].

At the end of 2010, an estimated 34 million people were living with HIV/AIDS (PLWHA) globally, including 3.4 million children less than 15 years. There were 2.7 million new HIV infections in 2010, including 390,000 among children less than 15 years [27]. Globally, the annual number of people newly infected with HIV continues to decline, although there is stark regional variation. Between 2001 and 2009, the incidence of HIV infection has declined in 33 countries, 22 of them in sub-Saharan Africa. These trends reflect a combination of factors: the natural course of HIV epidemics, behavioural changes associated with greater awareness about the effects of the epidemics and with intensified prevention efforts and increasing coverage of antiretroviral therapy [28].

Sub-Saharan Africa remains the region most heavily affected by HIV. In 2010, about 68% of all PLWHA resided in sub-Saharan Africa, a region with only 12% of the global population. Sub-Saharan Africa also accounted for 70% of new HIV infections in 2010 [28]. The total number of new HIV infections in sub-Saharan Africa has dropped by more than 26% per year, down to 1.9 million from the estimated 2.6 million at the height of the epidemic in 1997.

1.1.2 Transmission of HIV, the symptoms and signs

The HIV/AIDS pandemic consists of many separate epidemics. Each epidemic has its own distinct origin, in terms of geography and specific populations affected, and involve different types and frequencies of risk behaviours and practices. HIV is transmitted through unprotected sexual intercourse (anal or vaginal), transfusion of
contaminated blood, sharing of contaminated needles, and from a mother to her infant during pregnancy, childbirth and breastfeeding [29]. Physiologically, women are up to four times more vulnerable to HIV infection than men. There are several reasons. Infected semen remains in the cervix for some time, there is a large surface area in the vagina and cervix exposed to the virus, and the vagina is more susceptible to small tears during sex. Young women’s cervixes are even more vulnerable, particularly when they first start having sex.

After entry of HIV into host cells using the reverse transcriptase enzyme of the host, the viral genome copies itself from RNA (ribonucleic acid) to DNA (deoxyribonucleic acid) genetic material. The viral DNA copy enters the host cell nucleus and incorporates itself into the cells own DNA using integrase enzyme, making the virus a part of the host cell nuclear protein. It thus establishes a latent infection for the rest of the life of the infected individual. HIV is then activated to produce new virions whenever the infected CD4 cell is activated. An HIV coded protease is active in the maturation of the virions at the cell surface. This viral production disturbs CD4 cell function and eventually exhausts the CD4 cells leading to increased immunodeficiency and increased vulnerability to opportunistic infections and death.

The symptoms of HIV vary depending on the stage of infection. Though people living with HIV tend to be most infectious in the first few months, many are unaware of their status until later stages. The first few weeks after initial infection, individuals may experience no symptoms or a flu-like illness including fever, headache, rash or sore throat.

As the infection progressively weakens the person’s immune system, the person becomes more susceptible to infections and the individual can develop other signs and symptoms such as swollen lymph nodes, weight loss, fever, diarrhoea and cough. They could also develop severe illnesses such as tuberculosis (TB), cryptococcal meningitis, and cancers such as lymphomas and Kaposi’s sarcoma. The most advanced stage of HIV infection is AIDS. It can take 10-15 years for an HIV-infected person to develop AIDS.
1.1.3 Diagnosis of HIV

The World Health Organization (WHO) recommends the use of immunological assays for the diagnosis of HIV [30, 31]. Immunological assays include positive results from an HIV antibody screening test (e.g., reactive enzyme immunoassay [EIA]) confirmed by a positive result from a supplemental HIV antibody test (e.g., Western blot or indirect immunofluorescence assay test). Rapid tests are EIAs that do not have to be repeated but require a confirmatory test if reactive. In children less than 18 months of age virological assays can be used for screening to determine HIV infection against the background of antibodies transmitted from the mother [32].

1.1.4 Treatment of HIV

The most advances in HIV treatment have come from inhibiting the activity of enzymes used by the HIV in its life cycle. Antiretroviral drugs are broadly classified by the phase of the HIV life cycle that the drug inhibits. Entry or fusion inhibitors interfere with the binding, fusion and entry of HIV into the host cell. The CCR5 receptor antagonists do not target the virus directly; rather they bind to the CCR5 receptor on the surface of the CD4 T-cell thereby blocking viral attachment to the cell. Nucleoside reverse transcriptase inhibitors (NRTI) inhibit reverse transcription by incorporating themselves into the newly synthesized viral DNA strand as faulty nucleotides. Unlike NRTI which act as competitive substrate inhibitors, the non- nucleoside reverse transcriptase inhibitors (NNRTI) are non-competitive inhibitors of reverse transcriptase enzyme. The protease inhibitors (PI) act by targeting the viral assembly thereby inhibiting the activity of the protease enzyme which is essential in the final assembly of new virions. Integrase inhibitors inhibit the integrase enzyme which is responsible for integration of the viral DNA into the DNA of the infected host cell. The maturation inhibitors inhibit the last step in gag processing in which the viral capsid polyprotein is cleaved, thereby blocking the conversion of the polyprotein into the mature capsid protein (p24).

In 1987, the U.S. Food and Drug Administration (FDA) approved the first antiviral drug zidovudine (ZDV; AZT) for use in preventing HIV replication by inhibiting the activity of the reverse transcriptase enzyme. This was used as a monotherapy for several years with very limited efficacy and later it was successfully paired with lamivudine (3TC) and used as combination therapy. The discovery of other classes of
antiretroviral drugs and potential development of resistance and cross-resistance to monotherapy warranted a switch from monotherapy to combination therapy. The introduction of viral load determination was crucial to prove this concept. This switch to combination antiretroviral therapy – highly active antiretroviral therapy (HAART) has had dramatic effects because the use combination therapy prevents mutated forms of HIV from evolving.

Currently there are six classes of antiretrovirals with over 26 different drugs used for treatment of HIV. These drugs are aimed at stopping HIV in its tracks by stopping the various stages of viral replication. These classes include nucleoside reverse transcriptase inhibitors (NRTI’s), non-nucleoside reverse transcriptase inhibitors (NNRTI’s), protease inhibitors (PI’s), fusion inhibitors, CCR5 antagonists and integrase inhibitors [33-35]. By the end of 2010, WHO reported that 6,650,000 people were receiving antiretroviral therapy in low- and middle-income countries, accounting for 47% coverage of the estimated 14.2 million people eligible for treatment [27].

Guidelines have been set forth by the WHO and each individual country on how to use and manage patients on HAART [34]. WHO currently recommends HAART initiation for all PLWHA with a CD4 count of \( \leq 350 \) cells/mm\(^3\) and for those with WHO clinical stage 3 or 4 if CD4 testing is not available. The Tanzanian guidelines based on WHO recommended first-line therapy consist of an NNRTI + two NRTIs, one of which should be zidovudine (AZT) or tenofovir (TDF). Countries have been advised to reduce the use of stavudine (d4T) in first-line regimens because of its well-recognized toxicities. Second-line HAART consists of a ritonavir-boosted protease inhibitor (PI) plus two NRTIs, one of which should be AZT or TDF, based on what was used in first-line therapy. Ritonavir-boosted atazanavir (ATV/r) or lopinavir/ritonavir (LPV/r) are the preferred PIs [34].

1.1.5 Preventive measures for HIV

The rate of new HIV infections and AIDS-related deaths has fallen globally resulting in a decline of the epidemic. Declines in new HIV infections across the world have been spurred by access to antiretroviral therapy and the changes in behaviour via several intervention programs. The most dramatic increases in antiretroviral therapy coverage have occurred in sub-Saharan Africa, with a 20% increase between 2009 and 2010 alone. The best success seen is in programs to prevent the transmission of HIV from
mothers to children, which can reduce rates of transmission by 92-99% [36, 37]. This primarily involves the use of a combination of antiretrovirals during pregnancy and after birth in the infant but also potentially include bottle-feeding rather than breastfeeding. As of 2012 there is no effective vaccine for HIV or AIDS. However further research is on-going in search of a truly effective vaccine [38, 39]. Social strategies have proved effective in changing people’s behaviour including sex education, provision of condoms both male and female, needle exchange programs, HIV testing centres, treatment of sexually transmitted infections (STI’s) and the use of social media to educate people. These strategies have widely differing levels of efficacy and social acceptance.

Other interventions include advocating male circumcision and the potential use of an antiretroviral based vaginal gel. Circumcision in men has been shown to reduce the risk of HIV infection in heterosexual men by between 38-66% [40]. More than 550,000 males were circumcised for HIV prevention in the priority countries of sub-Saharan Africa by the end of 2010. Based on these studies, the WHO and UNAIDS both recommended male circumcision as a method of preventing female-to-male HIV transmission in 2007 [41]. Provision of HAART as pre-exposure prophylaxis has been shown to protect 96% of partners of HIV infected individuals [37, 42, 43]. Post exposure prophylaxis with HAART is also recommended following needle-stick injuries or exposure to body fluids within the health care environment and to sexual assault victims. A vaginal gel containing tenofovir, a reverse transcriptase inhibitor, when used immediately before sex, reduces infection rates by approximately 40% among Africa women [44].

1.2 HIV IN TANZANIA

The first 3 patients with AIDS were reported from a hospital in the north-western region of Kagera in November 1983 [45, 46]. The patients’ clinical features were similar to those reported in neighbouring countries with extreme wasting (Slim disease). The AIDS cases subsequently reported were among young adults, both males and females who were involved in cross-border trade, commercial sex workers and truck drivers. Due to constant movement this group accelerated HIV transmission to other parts of the country. By the end of 1985, these cases were tested by the newly developed Enzyme-Linked Immunosorbent Assay (ELISA) and Western Blot assay and confirmed to be infected with HIV. By 1986 many regions in Tanzania had
reported AIDS cases to the Ministry of Health [47, 48].

The epidemic of AIDS and HIV infection in Tanzania is associated solely with HIV-1. In Tanzania HIV-1 subtypes A, C and D have been documented as major circulating strains [49-54]. The proportion of HIV subtype circulating recombinant forms in the country has also been increasing with studies reporting up to 27% recombinants [55-57]. More than 60% of detected recombinants are those of the subtype C that differs from neighbouring Kenya and Uganda that commonly have recombinants of A and D [58, 59].

The estimated prevalence of HIV when it was first identified in the mid 1980’s was 1.3%. The prevalence gradually increased reaching its peak at 7.2% in 2003-2004, after which there has been a steady decline in the country’s HIV prevalence [60, 61]. The current prevalence of HIV in Tanzania is estimated at 5.7%, and it is higher among women (6.6%) compared to men (4.4%) [62]. Data suggest that the most important factor fuelling the HIV epidemic in the country is unprotected heterosexual intercourse, which constitutes about 80% of all new infections while approximately 18% of infections are accounted for by mother to child transmissions [63].

Provision of free HAART at Care and treatment Centres (CTC) was rolled out country wide in October 2004. By the end of 2005, there were a total of 96 CTCs providing HAART, increasing to 825 in December 2010 [64]. By the end of 2010, the cumulative number of clients enrolled in HIV care was 740,040. A total of 244,148 patients were on ART. Of those, 92.2% were adults (>15 years) with majority of the patients (71.6%) being in the age group 25 – 49 years, with 7.8% being in those below 15 years, while the age group 15 – 24 years contributed the least proportion (4.4%) [64].

Following recommendations from WHO for treatment guidelines, the Ministry of Health and Social Welfare (MoHSW) together with the National AIDS Control Program (NACP) have come up with treatment guidelines tailored for Tanzania [34, 65]. According to these guidelines the MoHSW recommends all patients to be given triple therapy comprising of 2 NRTI + 1 NNRTI or 2 NRTI + 1 PI or 3 NRTI’s. The default first line regimen is the use of 2 NRTI + 1 NNRTI and this is commonly zidovudine (300mg) + lamivudine (150mg) + efavirenz (600mg). Patients receive their HAART from the CTC on a monthly basis [65].
Tanzania, like many other countries around the world, is taking action to try and decrease the number of HIV infections. Voluntary Counselling and Testing (VCT) is the predominant approach in mass HIV testing in Tanzania and this accounts for approximately 86% of referral of patients to CTCs. Condom promotion and distribution along with sex education has been advocated in most parts of the country. Free HAART is given as part of Prevention of Mother to Child Transmission (PMTCT), along with pre- and post-exposure prophylaxis to persons at risk. Male circumcision in Tanzania has a prevalence of 67%. Following the 2007 WHO recommendations, campaigns to circumcise men are on-going. There are several HIV vaccine trials ongoing in the country, which are mainly phase I/II trials like the HIVIS 03 and the TaMoVac 01 [66].

1.3 TUBERCULOSIS

1.3.1 History and Epidemiology of Tuberculosis

“With 2 billion persons, a third of the world population, estimated to be infected with mycobacteria” [67].

The presence of tuberculosis (TB) can be traced back to centuries ago. In 2008, evidence for tuberculosis infection has been discovered in human remains from the Neolithic era dating from 9,000 years ago, in a settlement in the eastern Mediterranean [68]. Over time, tuberculosis was known by many names including Contagion, Phthisis and White Plague. Scientists such as Hippocrates and many other at the time believed death by tuberculosis was inevitable and that it was a contagious disease characterized by fever, colourless urine, cough resulting in a thick sputa, and loss of thirst and appetite [69]. It was not until 1882 when a physician Robert Koch utilized a new staining method and applied it to the sputum of tuberculosis patients, revealing for the first time the causal agent of the disease: *Mycobacterium tuberculosis*, or Koch's bacillus [70, 71]. He made his result public at the Physiological Society of Berlin on the 24th of March 1882, in a famous lecture entitled *Über Tuberculose*, which was published three weeks later, and since then 24 March has been known as World Tuberculosis Day [70, 72].
It was not until 1943, 60 years later, that the first effective anti-tuberculosis agent, streptomycin, was isolated and used for treatment of TB [73]. Although this monotherapy cured several people, a substantial number of people developed TB again (relapse) and later resistance to streptomycin was seen [74]. Streptomycin in combination with isonicotinic acid hydrazide (isoniazid) showed much better outcomes and after rifampicin was discovered in 1957 the TB treatment was revolutionized [75-77]. Ultimately, results of clinical trials led by the British Medical Research Council showed that a four-drug regimen was recommended for use in patients with newly diagnosed tuberculosis. The backbone of such empirical regimens was the combination of isoniazid and rifampicin, the most effective and reasonably well-tolerated oral agents [78].

One in three people in the world is infected with *Mycobacterium tuberculosis*, however a relatively small proportion of infected people will go on to develop TB disease [79]. Despite the availability of highly efficacious treatment for decades, TB remains a major global health problem. In 1993, WHO declared TB a global public health emergency, at a time when an estimated 7–8 million cases and 1.3–1.6 million deaths occurred each year. There are 22 countries in the world which constitute 82% of global tuberculosis cases, and these are termed as 22 High-TB Burden Countries. There were an estimated 8.8 million incident cases of TB (range, 8.5 million–9.2 million) globally in 2010 [80]. TB is the second leading cause of death from an infectious disease worldwide (after HIV), which caused an estimated 1.5 million deaths in 2010. Globally, the absolute number of incident TB cases per year has been falling at an approximate rate of 1.3% per year since 2002. Likewise TB mortality has also been falling globally [81]. In 2010, 6.2 million people were diagnosed with TB and notified to national TB control programmes. Of these, 5.4 million had TB for the first time and 0.3 million had a recurrent episode of TB after being cured of TB in the past [80]. Globally, the ratio of female to male tuberculosis cases notified is 1/1.7 and 70% more smear-positive male than female tuberculosis patients are diagnosed every year and notified to the WHO [80].

### 1.3.2 Transmission of TB, the symptoms and signs

*Mycobacterium tuberculosis* (rod-shaped, non-spore-forming, aerobic bacterium) is spread by small airborne droplets, called droplet nuclei, generated by the coughing, sneezing, talking, or singing of a person with pulmonary or laryngeal tuberculosis. The
number of bacilli in the droplets, the virulence of the bacilli, exposure of the bacilli to ultraviolet light, degree of ventilation, and occasions for aerosolization all influence transmission. Introduction of *M. tuberculosis* into the lungs leads to infection of the respiratory system; however, the organisms can spread to other organs, such as the lymphatics, pleura, bones/joints, or meninges, and cause extrapulmonary tuberculosis. For persons with intact cell-mediated immunity, the next defensive step is formation of granulomas around the *M. tuberculosis* organisms restricting growth and establishing latency, by undergoing fibrosis and calcification, successfully controlling the infection so that the bacilli are contained in the dormant, healed lesions [82]. Lesions in persons with less effective immune systems progress to primary progressive tuberculosis [83-85]. Epidemiological information shows that there are differences between men and women in prevalence of infection, rate of progression from infection to disease, incidence of clinical disease, and mortality due to tuberculosis [86, 87].

About 90% of those infected with *M. tuberculosis* have latent TB infections which are asymptomatic with only a 10% lifetime chance of progression to active tuberculous disease [88]. Tuberculosis may infect any part of the body, but most commonly occurs in the lungs (known as pulmonary tuberculosis (PTB)) and approximately 15-20% of cases are termed Extrapulmonary TB (EPTB) when tuberculosis develops outside of the lungs [89]. General signs and symptoms include fever, chills, night sweats, loss of appetite, weight loss, and fatigue, and significant finger clubbing may also occur [90]. In patients with pulmonary TB along with the general symptoms they may also present with chest pains and coughing out blood.

EPTB occurs more commonly in immunosuppressed persons, commonly HIV patients and in young children. Common sites for extrapulmonary infection include the pleura, central nervous system, lymphatic system, genitourinary system, and the bones and joints among others. A potentially more serious, widespread form of TB is called "Disseminated or Miliary TB” makes up about 10% of extrapulmonary cases. Other signs of EPTB depend on the affected organs.

### 1.3.3 Diagnosis of TB

Active TB may be considered as a possible diagnosis when patients present with physical signs and symptoms suggestive of TB plus abnormal findings on a chest radiograph. The radiographs may show the characteristic findings of infiltrates with
cavitation in the upper and middle lobes of the lungs; however atypical features may be seen in immunocompromised patients.

Traditionally, in spite of modern advances, the first laboratory test used to detect active tuberculosis is microscopic examination of a sputum smear or other diagnostic specimen for the presence of acid-fast bacilli (AFB) using the Ziehl-Neelson staining. Definitive diagnosis of tuberculosis requires the identification of *M. tuberculosis* in a culture of a diagnostic specimen. Traditionally, culture has been grown on solid and liquid media such as Löwenstein-Jensen (LJ), or Kirchner and the various Middlebrook formulations. New fully automated systems that rely on non-radiometric detection of growth have been developed such as the MB/BacT (Biomerieux), BACTEC 9000 (Becton Dickinson), and the Mycobacterial Growth Indicator Tube (MGIT; Becton Dickinson) [91]. Newer rapid automated DNA tests are being used for the diagnosis of TB along with drug susceptibility testing (Xpert MTB/RIF is a TB-specific, cartridge-based nucleic amplification assay based on the GeneXpert multi-disease platform).

Presumptive diagnosis of pulmonary TB can be made in patients with abnormal findings on a chest radiograph suggestive of TB, presenting with signs and symptoms of TB in spite of at least two sputum smear examinations negative for AFB. Diagnostic criteria should include: radiographic abnormalities consistent with active pulmonary TB; no response to a course of broad-spectrum antibiotics; and a decision by a clinician to treat with a full course of anti-TB chemotherapy. A patient with positive culture but negative AFB sputum examinations is also a smear-negative case of pulmonary TB. In patients with EPTB, diagnosis should be based on one culture-positive specimen or histological or strong clinical evidence consistent with active extrapulmonary disease, followed by a decision by a clinician to treat with a full course of anti-TB chemotherapy. Immunological diagnostic tests such as the Mantoux tuberculin skin test are of limited application due to cross reactivity and poor sensitivity.

### 1.3.4 Treatment of TB

Directly Observed Treatment, Short-course (DOTS) was adopted by WHO and implemented in several countries and has been the gold standard of treatment [92]. It is the most effective way to ensure rapid sputum conversion of infectious patients, thereby stopping further transmission of *M. tuberculosis* to the community. WHO advises that all TB patients should have at least the first two months of their therapy
observed (and preferably the whole of it observed): this means an independent observer watching patients swallow their anti-TB therapy. Treatment with properly implemented DOTS has a success rate exceeding 95%.

WHO has set up guidelines whose principal purpose is to help national TB control programs (NTPs) in setting TB treatment policies. The first line drugs in the treatment of TB are rifampicin, isoniazid, ethambutol and pyrazinamide. The second line consists of 6 classes of drugs including aminoglycosides, polypeptides, fluoroquinolones and thioamides. TB treatment is divided into a 2 months intensive phase that comprises all the 4 first line anti-TB drugs followed by 4 months continuation phase with rifampicin and isoniazid (2HRZE/4HR). WHO now recommends a full 6 months of rifampicin will reduce the number of relapses and failures [93].

However, despite all measures, the emergence multidrug-resistant tuberculosis (MDR-TB) which is defined as tuberculosis that is resistant at least to isoniazid and rifampicin, the two most powerful first-line anti-TB drugs has been on the increase [94]. Worldwide, there were an estimated 650,000 MDR-TB cases in 2010. MDR-TB can develop in the course of the treatment of fully sensitive TB and this is always the result of patients missing doses or failing to complete a course of treatment, however, MDR-TB strains appear to be less fit and less transmissible. For patients diagnosed with MDR-TB, WHO recommends treatment of at least 20 months with a regimen that includes second-line anti-TB drugs [93].

In 2006, WHO announced a new epidemic of Extensively Drug Resistant Tuberculosism (XDR-TB) from South Africa [95, 96]. XDR-TB is defined as TB that has developed resistance to at least rifampicin and isoniazid as well as to any member of the quinolone family and at least one of the following second-line anti-TB injectable drugs: kanamycin, capreomycin, or amikacin [95]. It is clear that the spread of this strain of TB is closely associated with a high prevalence of HIV and poor infection control.

Currently the only available and most widely used vaccine is the Bacillus Calmette–Guérin (BCG) which, while it is effective against disseminated disease in childhood, confers inconsistent protection against contracting pulmonary TB [97].
1.4 TUBERCULOSIS IN TANZANIA

Tanzania is one among the 22 high burden countries in the world for TB ranking 14th overall [80]. The National Tuberculosis and Leprosy Programme (NTLP) was launched by the Ministry of Health and Social Welfare in 1977 to provide high quality and effective interventions to control TB and leprosy as a single combined programme [98]. The NTLP uses the gold standard DOTS strategy in the management of TB and to prevent development of anti-TB drug resistance. The first short-course regimen of 8 months was introduced in Tanzania in 1987 for sputum smear positive patients only. A six months short-course regimen for new smear positive, smear negative and extrapulmonary TB was introduced in 2006 in line with WHO recommendations [93, 99]. NTLP is endeavouring to achieve the WHO targets for TB control of detecting 70% of the infectious cases and treating successfully 85% of them based on Stop TB strategy of global TB control [81].

Tuberculosis continues to be among the major public health problems in the country, more than 20 years after launching of the programme. The number of tuberculosis cases has steadily increased from 11,753 in 1983 to about 65,665 in the year 2004, almost six-fold [99]. This rapid increase of tuberculosis in Tanzania is mainly attributed to the HIV epidemic affecting the young adult population aged 15-45 years, but factors like population growth and urban overcrowding have also contributed. Dar es Salaam, the country’s commercial capital contributes about 24% of all cases of TB in terms of absolute numbers [98].

Reports from 2009 show that there were 64,267 cases notified of all forms of tuberculosis (new and re-treatment). Among those, 93% were new tuberculosis cases and 7% were re-treatment cases. Of the new cases 41.5% were smear positive, 36.2% smear negative and 22.3% had extra-pulmonary TB [98]. The ratio of male to female among new smear positive TB cases notified in 2009 was 1.7. Countrywide, the rate of treatment success for new smear positive TB cases was 88.3% making the country able to achieve the WHO global target of 85% success [98]. The mortality rate among TB patients on treatment was reported to be 3.6%. The prevalence of MTB strains resistant to any of the four first-line drugs in new patients was 8.3%, while the prevalence of MDR-TB was 1.1%. In retreatment patients, the crude prevalence for any resistance and for MDR-TB was respectively 20.6% and 3.9%. The low levels of drug resistance
in Tanzania are likely due to a well performing TB control programme and the absence of noticeable involvement of the private sector in TB treatment [100].

Starting from 2006 Tanzania introduced in a phased manner 4-drug fixed dose combinations (FDCs) for all TB patients in line with the WHO/IUATLD recommendations in order to improve overall results and reduce the risk of multi-drug resistance. New patients with pulmonary TB should receive a regimen containing 6 months of rifampicin: 2HRZE/4HR [93, 99]. Three times weekly dosing throughout therapy [2(HRZE)x3/4(HR)x3] is another alternative provided that every dose is directly observed and the patient is not living with HIV or living in an HIV-prevalent setting. Retreatment patients will be provided with first line drugs plus streptomycin {2HRZES/1HRZE/5HRE}.

1.5 HIV AND TB CO-INFECTION

1.5.1 Epidemiology of HIV and TB co-infection

The HIV/AIDS epidemic is reviving the old TB problem in well-resourced countries and greatly worsening an existing problem in resource poor countries [28, 80]. HIV is the most important cause of the rapid increase of the current TB epidemic and both TB and HIV are fatally synergistic. TB is the leading infectious killer of PLWHA and the most common opportunistic infection (OI) [101, 102]. Almost one in four deaths that occurs among PLWHA is due to TB. HIV promotes both the progression of latent TB infection to active disease and a relapse of the disease in previously treated patients: individuals infected with M. tuberculosis who get infected with HIV have a 20-30 times higher risk of developing tuberculosis disease than those who are HIV negative [103-106]. HIV affects the immune system and increases the likelihood of people acquiring new TB infections. TB may accelerate the progression of HIV from asymptomatic to symptomatic disease and even AIDS, increases both mortality and the incidence of other opportunistic infections among PLWHA [107].

Globally the number of TB patients who had been diagnosed with HIV status reached 2.1 million in 2010, equivalent to 34% of notified cases of TB. Of the 8.8 million incident cases globally an estimated 1.1 million (13%) were found to be co-infected with HIV [80]. Overall, the African region accounted for a staggering 82% of all new TB cases co-infected with TB. Among TB patients known to be living with HIV, 46%
globally and 42% in the African region were on in 2010 [80]. The number of PLWHA who were screened for TB was approximately 58%, among those who were enrolled in HIV care worldwide in 2010. The treatment success and death rates reported for HIV-positive TB cases in 2009 were 72% and 20% respectively [80].

Like other countries, Tanzania also bears the brunt of TB patients who are co-infected with HIV. Of the 64,267 notified TB cases, 87.5% were counselled and tested for HIV of whom 37.2% were found to be co-infected with HIV [98, 108]. Results from 19,940 co-infected patients show that 86.6% were successfully treated, and 8.1% died during TB treatment while only a small proportion (0.2%) failed treatment. Approximately 41.5% of the new TB cases had smear positive PTB, 36.2% were smear negative for PTB and 22.3% presented with extra-pulmonary TB [98]. Research has shown that patients with disseminated TB presented with features of reactivation and newly acquired TB [109]. A low prevalence of TB drug resistance has been seen hence giving hope to better TB treatment outcomes and ultimately HIV management [100, 110].

TB occurs earlier in the course of HIV infection than other opportunistic infections. It progresses faster and harder to diagnose among HIV infected people, and is likely to be fatal if undiagnosed or left untreated. Even among HIV-infected patients, PTB is still the commonest presenting feature, however, EPTB or sputum smear-negative TB is common especially as immunosuppression advances [111, 112]. The commonest forms of extra-pulmonary TB are: pleural effusion, lymphoadenopathy, pericardial disease, miliary disease, meningitis, Spinal TB (Pott’s disease) and disseminated TB. Case-fatality is higher in people living with HIV with smear-negative pulmonary and extrapulmonary TB, as these patients are generally more immunosuppressed than those with smear-positive TB [113]. The case-fatality rate is reduced in patients who receive concurrent ART. High mortality rates have been reported among PLWHA who have drug-resistant TB [114], and rates can exceed 90% in patients co-infected with XDR-TB and HIV [115, 116]. Early detection and proper treatment of tuberculosis will therefore influence not only the life expectancy but also the quality of life of PLWHA.

1.5.2 Treatment of HIV-TB co-infection

Since the WHO declaration in 1993 that TB was a global emergency, the DOTS strategy has been the key public health intervention that has been widely used to affect global TB control [117]. Antiretroviral therapy improves survival in HIV-positive
patients and improves TB outcomes [118, 119]. In addition, antiretroviral therapy reduces TB rates by up to 90% at an individual level, by 60% at a population level and it reduces TB recurrence rates by 50% [120-123].

Unfortunately, drug-drug interactions between the current first-line TB regimen and certain commonly used antiretrovirals complicate treatment for co-infected patients. This potentially results in the loss of antiviral efficacy and the development of viral resistance [124]. Overlapping toxicities of anti-TB and antiretroviral agents occur frequently, necessitating discontinuation of therapy and increasing the risk of non-adherence further fuelling the development of drug resistance for both anti-TB and HAART [125]. Immuno-pathological reactions, termed "the immune reconstitution inflammatory syndrome" (IRIS), occur frequently when antiretroviral therapy is initiated in patients with tuberculosis (paradoxical TB-associated IRIS), or in which HAART therapy results in new presentation of previously undetected (likely subclinical) TB infection (unmasking TB-associated IRIS). Reports estimating the prevalence of TB-IRIS in patients with undergoing new ARVs are variable, ranging from as low as 7.6% in one to as high as 32% [126, 127].

WHO recommends that the first-line HAART regimen contain two nucleoside reverse transcriptase inhibitors (NRTIs) plus one non-nucleoside reverse transcriptase inhibitor (NNRTI) as in non-TB infected patients. The preferred NRTI backbone is zidovudine (AZT) or tenofovir disoproxil fumarate (TDF), combined with either lamivudine (3TC) or emtricitabine (FTC). For the NNRTI, WHO recommends efavirenz (EFV) due to fewer interactions compared to nevirapine [34, 93, 128]. The recommendations of WHO in 2009 are that TB treatment should be commenced first and ART subsequently commenced as soon as possible and within the first 8 weeks of starting TB treatment. When TB is diagnosed in patients already receiving HAART, TB treatment should be started immediately and HAART needs to be modified to prevent potential drug-drug interactions [93].

In all HIV-positive TB patients, co-trimoxazole preventive therapy (CPT) should be initiated as soon as possible and given throughout TB treatment [129]. Co-trimoxazole preventive therapy substantially reduces mortality in HIV-positive TB patients [117, 129].
NTLP in line with WHO, UNAIDS and the Stop TB Partnership have set a target of reducing TB mortality rates among PLWHA by 50% by the year 2015 compared with rates in 2004. The recommended interventions are collectively known as collaborative TB/HIV activities. These activities include HIV testing of TB patients, provision of ART and CPT to TB patients living with HIV, intensified TB case-finding among people living with HIV, isoniazid preventive therapy (IPT) for PLWHA who do not have active TB, and infection control in health care and congregate settings (the latter three activities are referred to as the “Three Is for HIV/TB”) [130].

1.6 PHARMACOKINETICS AND PHARMACOGENETICS OF RIFAMPICIN AND EFAVIRENZ

1.6.1 Drug-Drug Interactions

Drug-drug interactions can occur when two or more drugs are used concurrently and produce either a synergistic or antagonistic effect. Drug-drug interactions can lead to changed systemic exposure, resulting in variations in drug response of the co-administered drugs. There are a number of mechanisms by which drugs interact with each other, and most of them can be divided into two general categories: pharmacokinetic and pharmacodynamic interactions. With pharmacokinetic drug interactions, one drug affects the absorption, distribution, metabolism, or excretion of another. When pharmacodynamic drug interactions occur, two drugs have additive or antagonistic pharmacologic effects. Either type of drug interaction can result in adverse effects in some individuals.

The potential for clinically important drug-drug interactions can often be predicted based on the drug properties, method of drug administration, and patient-specific parameters [131]. Recent scientific developments particularly in the area of cytochrome P450 drug metabolizing enzymes and of late the role of drug transporters have revolutionized the study of drug interactions. The result has been a deluge of published drug interaction research including databases that have information on potential drug interactions, and this has overwhelmed most health care practitioners.

In the past decade, there have been numerous advances in HIV therapy which have turned it into a chronic and manageable disease. Patients often require treatment for co-
morbid conditions as well as HIV, and consequently, pharmacokinetic interactions between HAART and other drug classes are an increasing concern [132, 133]. As a result, this could lead to viral breakthrough and development of resistance or suboptimal disease management, or supra-therapeutic levels which may result in drug toxicity and possibly non-adherence and/or increased morbidity [133]. The efficacy and toxicity of the interacting drug(s) may also be similarly affected. Management options may vary depending upon a number of factors including the mechanism and clinical consequences of the interaction, availability of therapeutic alternatives, patient convenience and cost. Strategies include adjusting the dose and/or dosing frequency of one or both interacting drugs, or replacing one agent with another drug with lower interaction potential. Often close clinical, virological and therapeutic drug monitoring is warranted.

1.6.2 Pharmacogenetics

Pharmacogenetics refers to genetic differences in metabolic pathways that can affect individual responses to drugs, both in terms of therapeutic effect as well as adverse effects [134]. Pharmacogenetics is a rapidly growing field of interest encompassing genetic variation in genes encoding drug transporters, drug-metabolizing enzymes and drug targets, as well as genes related to the action of drugs. Pharmacogenetics can be used to find genetic polymorphisms in the genes encoding proteins and enzymes involved in drug transport, metabolism and action that can predict the usefulness of a particular drug, increasing the number of responders and decreasing the number of subjects affected by adverse drug reactions. It is evident that the polymorphism of drug-metabolizing genes has a high impact on inter-individual differences in drug response such as inhibition and induction of drug metabolism among a variety of other things [135]. Applications in pharmacogenetics are substantial and provide clinicians with information that could facilitate an individualized therapy of patients, both with respect to the choice of drug and the dose of a specific drug. Pharmacogenetics is, however, still in the beginning; knowledge about genetic variation at the level of drug metabolism is extensive, whereas the knowledge about inter-individual differences in the function of drug transporters and drug targets is scarcer.

Valuable databases have been created that store information on the various drug receptors, drug transporters and drug-metabolizing enzymes that can be used to find
characterized haplotypes, specific single nucleotide polymorphisms (SNPs) and allele frequencies in various ethnic groups [136, 137]. There are 57 known active CYP450 genes in the human genome which are responsible for 70–80% of all phase I-dependent metabolism of clinically used drugs [138, 139]. The functional importance of the variant alleles varies and the frequency of their distribution in different ethnic groups is different. The polymorphic forms of CYP450s are often responsible for the development of adverse drug reactions [140]. Four major phenotypes are known: poor metabolizers lacking functional enzyme, intermediate metabolizers being heterozygous for a defect allele, efficient metabolizers carrying two functional gene copies and ultrarapid metabolizers carrying more than two functional gene copies [141].

1.6.3 Rifampicin pharmacokinetics and pharmacogenetics

Rifampicin is a semisynthetic antibiotic derivative of rifamycin. Absorption of rifampicin is reduced by about 30% when the drug is ingested with food. Rifampicin is widely distributed throughout the body and is present in effective concentrations in many organs and body fluids, including the cerebro-spinal fluid (CSF). Rifampicin is about 80% protein bound. Most of the unbound fraction is not ionized and, therefore, diffuses freely into tissues. After an oral administration of 600mg of rifampicin peak concentration in plasma is reached in 2 to 4 hours [142-144]. Up to 30% of a dose of the drug is excreted in the urine and 60% to 65% in the faeces [145]. Rifampicin at therapeutic levels has demonstrated bactericidal activity and potent sterilizing effect against both intracellular and extracellular Mycobacterium tuberculosis organisms. Moderate rises in serum concentrations of bilirubin and transaminases, which are common at the outset of treatment, are often transient and without clinical significance, however, dose-related hepatitis can occur and could be potentially fatal.

Rifampicin is a known inducer of the CYP450 enzymes such as CYP2B6, CYP3A, CYP2C19, CYP2C8, CYP2C9 and CYP1A2 [146-152]. Administration of rifampicin with drugs that undergo biotransformation through these metabolic pathways may accelerate elimination of co-administered drugs.

1.6.4 Efavirenz pharmacokinetics and pharmacogenetics

Efavirenz is a NNRTI and is used as part of HAART for the treatment of HIV-1 [34, 146]. The oral bioavailability of efavirenz is affected by food as seen by increases in
peak plasma concentrations and area under the curve (AUC) of the drug after a normal to high calorie diet compared to a fasting state. It is primarily excreted in faeces, though a small proportion is also excreted via urine. Efavirenz is about 99.5% - 99.75% bound to plasma proteins, principally albumin. Its long half-life (40–55 h after repeated dosing) allows durable, long-lasting reduction in HIV RNA after once-a-day dosing (600 mg), presenting an advantage for treatment compliance and efficacy [151, 153]. Efavirenz is widely distributed in body compartments and is likely to be effective in protected tissues such as the central nervous system and testes [152, 154].

Efavirenz is extensively metabolized in the liver by oxidation via the CYP450 system. It has been shown to exhibit multiple interactions with CYP450 system such as being a substrate [153, 155], an inhibitor [156], and an inducer of CYP450 [157]. Further research has shown that efavirenz enhances its own metabolism after repeated dosing [153].

In vitro data together with in vivo evidence from literature strongly suggest that in the cytochrome P450 system the CYP2B6 is the main catalyst of efavirenz metabolism [158-160]. Efavirenz is primarily metabolized by hepatic CYP2B6, with some contributions from CYP2A6, CYP3A4/5 and UGT2B7 enzymes [160-162]. It is metabolized to inactive hydroxylated metabolites that include 7- and 8-hydroxyefavirenz [155]. Based on in vitro data from human liver microsomes, the 7- and 8- hydroxyefavirenz on average account for 23% and 77% of overall efavirenz metabolism respectively. The 7-hydroxylation is mainly that accounts for 23% of efavirenz metabolism that is mediated by CYP2A6 while the 8-hydroxyefavirenz mediated by CYP2B6 [163-166].

Inter-individual variability in efavirenz pharmacokinetics have been reported and linked to increased risk of central nervous system (CNS) toxicity or virological failure [167-169]. This variability has been linked to the CYP2B6 genetic variants. The CYP2B6 gene is highly polymorphic as reflected by 29 associated alleles, many suballeles, and SNPs [136, 170]. Of the variants identified so far, the CYP2B6*6 haplotype defined by two non-synonymous SNPs, 516G>T (Q172H) and 785A>G (K262R), is clinically important because this allele or the SNP tagging it (G516T) occurs at high frequency in all ethnic populations (14–62%) and has been associated with functional consequences in expressed systems [170-176]. Subsequent to the
demonstration that CYP2B6 is the principal clearance mechanism of efavirenz in vitro, several studies have documented that the CYP2B6*6 allele or its tagging SNP results in increased risk for higher efavirenz exposure and/or adverse effects [170, 177, 178].

The frequency of CYP2B6-G516T among Japanese (3.3%) [178], Caucasian (6%) [179], Thai 8.9% [180], African-American (20%) [177] or African population (23%) [181, 182] further shows and confirms the differences among the ethnicities. Unlike the associations between CYP2B6 516G>T (SNP) and efavirenz exposure, other biological factors such as gender and body weight may play varying roles in the influence of efavirenz plasma concentrations. Some authors found higher efavirenz concentrations in women, while no such gender association was reported by other authors [167, 183, 184] and likewise, with the association of body weight to plasma efavirenz concentrations [163, 183, 185].

Clinical studies with HIV-infected patients have repeatedly shown that CYP2B6 polymorphisms such as CYP2B6*6/*6 decrease efavirenz clearance and may be associated with CNS adverse effects [170]. The CYP2B6*6/*6 genotype is associated with an approximate 3-fold increase in efavirenz exposure compared with CYP2B6*1/*1 genotype [186]. In vitro studies showed that Vmax values for the formation of 8-hydroxyefavirenz were substantially decreased (by approximately 70%) in human liver microsomes (HLMs) with CYP2B6*6/*6 genotype versus HLMs with CYP2B6*1/*6 and CYP2B6*1/*1 genotypes [187]. The low clearance of efavirenz in CYP2B6*6/*6 haplotype has been associated with a reduced CYP2B6 protein expression in the liver [158].

### 1.6.5 Efavirenz and rifampicin pharmacokinetics and pharmacogenetics

Despite its well-known interaction potential via induction of the CYP450, rifampicin is commonly used in the treatment of HIV/AIDS patients co-infected with TB. Efavirenz-based antiretroviral regimen is preferred during rifampicin-containing antituberculous therapy. The adult fixed dose of 600 mg per day is associated with wide inter-individual variability in plasma concentrations, as well as clinical outcome and this variability is even greater during co-administration with rifampicin or rifampicin-containing antituberculous therapy suggesting a variable degree of drug–drug interaction [167-169, 188-190].

The available pharmacokinetic data showed that rifampicin reduced the area under the
concentration–time curve (AUC) of efavirenz by 13 – 25% [191-193]. While some authors reported a 7 – 13-fold induction, others found only 2.5-fold increase in activity [194-196]. The proposed mechanism of the rifampicin–efavirenz interaction is induction of \( CYP3A \) and \( CYP2B6 \). Rifampicin enhances efavirenz metabolism probably via induction of \( CYP2B6 \)-mediated 8-hydroxylation, and 8-hydroxyefavirenz exists primarily as a conjugate in plasma and urine samples.

The reduction of efavirenz during rifampicin co-administration led some experts to recommend an increased efavirenz dose when co-administered with rifampicin [197, 198]. It was recommended that the approximate 20% decrease in efavirenz in the presence of rifampicin would be overcome by increasing the dose to 800 mg/day from the usual 600mg/day [191, 198]. Clinically, efavirenz 800 mg/day was used in patients with HIV-TB co-infection on rifampicin-containing therapy but the increased dose did not show superior virologic suppression rates [199-201]. Rather, the increased dose was associated with higher frequencies of CNS and hepatic toxicities associated with high efavirenz plasma concentrations. Recent studies have shown that individuals with the \( CYP2B6 \) 516 TT genotype are at risk of high efavirenz plasma exposures even in the presence of rifampicin-containing therapy [181, 202]. Thus, increase in efavirenz dose during rifampicin-containing therapy may not be necessary in individuals with slow metabolizing phenotype.
2 RATIONALE

HIV is now considered a chronic disease and the widespread use of HAART is destined to improve the quality of life of HIV infected individuals. As seen over the years, comorbidity with TB is abundant. With the presence of effective therapy for both HIV and TB, concurrent treatment is complicated due to drug-drug interactions. Due to these interactions, some scientists suggested an increase in efavirenz dose from the standard 600mg/day to 800mg/day to counter the effects of reduced efavirenz concentrations when co-administered with rifampicin during TB treatment [191, 198, 203, 204]. Alternatively, other scientists suggest that the standard efavirenz dose of 600mg/day is adequate in HIV positive patients receiving concomitant HAART and anti-TB therapy [192, 205, 206]. Thus an increased dose of EFV when used with RIF may be optimal in some populations whereas in other populations such general recommendation may result in more adverse event without better treatment outcome.

Currently, there is little data available on the optimal regimens for concomitant treatment of TB and HIV in Africans due to wide genetic heterogeneity. It is known that there is a higher frequency of CYP2B6 defective alleles in African populations, and the degree of interaction between EFV and RIF varies between populations based on these polymorphisms. Co-treatment with efavirenz and rifampicin could affect the clinical, immunological and virological outcomes of HIV disease as well as TB treatment outcomes. Knowledge how to co-treat HIV and TB effectively while minimizing risk of drug-drug interaction is crucial in Africa in general and Tanzania in particular.
3 OBJECTIVES

3.1 BROAD OBJECTIVE

To assess the clinical outcomes as a result of drug-drug interactions during HIV/TB co-treatment based on pharmacokinetic and pharmacogenetic aspects of interactions between rifampicin and efavirenz.

3.2 SPECIFIC OBJECTIVES

Paper I – to describe the socio-demographic and clinical features of patients enrolled at the care and treatment clinic at Muhimbili National Hospital following the free roll out of anti-retroviral drugs that was started in Tanzania in 2004 and assess the impact this free roll out has had on HIV.

Paper II – Here we describe the socio-demographic characteristics, clinical presentation and associated mortality of two cohorts of patients, those who are HIV infected only and those who are HIV positive and co-infected with TB: to describe the socio-demographic characteristics, clinical profile and associated mortality of patients with HIV and HIV-TB co-infection.

Paper III – to study factors influencing efavirenz auto-induction and the resulting effects on long-term efavirenz exposure, by comparing the changes in plasma efavirenz concentrations and metabolic ratio (MR) between week 4 and week 16 of therapy in HIV only patients in Tanzania based on gender and pharmacogenetic influences.

Paper IV – To investigate the timing, incidence, clinical presentation, pharmacokinetics and pharmacogenetic predictors for antiretroviral and antituberculosis drug induced liver injury (DILI) in HIV patients with or without TB co-infection.

Paper V – To determine the incidence, clinical presentations and predictors for neuropsychiatric manifestations of efavirenz based antiretroviral therapy (HAART) with or without concomitant rifampicin based anti-tuberculosis treatment.
4 METHODOLOGY

4.1 PAPER I

The HIV CTC of Muhimbili National Hospital (MNH), in Dar es Salaam, Tanzania was set up in June 2004. The clinic was part of the National HIV care program that was started countrywide to provide care and treatment including provision of free ARV's. Patients' clinical information at the clinic was monitored using a clinic visit form. This information was and is still being regularly entered into an HIV database.

For the purposes of our study, we used the data of patients enrolled between June 2004 and September 2008. Eligible patients for this analysis were those aged 18 years and above with complete data. Incomplete data for this analysis included all individuals whose identification was in doubt, i.e. names, age and sex were not indicated or were actually in doubt. Also, incomplete data included all those who at baseline had no clinical information in their records and had no baseline CD4 counts nor clinical HIV staging. Individuals missing any one of these important variables were excluded.

The clinic enrolled HIV positive patients referred from voluntary testing and counselling centres as well as hospitals in and around Dar es Salaam. At enrolment a structured first-visit form was used to collect patients' social demographic and clinical information, physical findings and anthropometric measurements. Opportunistic infections were diagnosed on the basis of standard clinical definitions and individual laboratory investigations. CD4 T-lymphocyte counts were determined using FACS Count System (Becton Dickinson, San Jose, CA, USA) at the Central Pathology Laboratory in MNH. HIV disease severity was categorized using the WHO HIV disease staging whereby stage I and II was categorized as mild disease, stage III as moderate disease and stage IV was categorized as severe disease. Immune suppression as indicated by CD4 T-cell counts was divided into < 100 cells/µL, 100-200 cells/µL and > 200 cells/µL.

4.2 PAPERS II-V

4.2.1 Study setting and population

This was a prospective study conducted at 3 different sites all within Dar es Salaam, namely MNH, Infectious Disease Centre (IDC) and Mwananyamala District Hospital.
Patients enrolled in the study included: Newly diagnosed HIV-infected patients who were naïve to antiretroviral therapy, consenting adults $\geq 18$ years of age, CD4 cell count $< 200 \text{cells}/\mu\text{L}$ with excluded TB disease at recruitment. These patients were referred to as HIV-only. Another group of patients were those who were newly diagnosed to have TB disease (pulmonary or extrapulmonary), with concomitant HIV infection and naïve to antiretroviral therapy, with a CD4 cell count $< 200 \text{cells}/\mu\text{L}$. These patients with HIV and TB co-infection were referred to as HIV-TB. We excluded pregnant women, prisoners, haemoglobin $< 8 \text{gms}/\text{dL}$, a history of treated TB infection within the last 5 years and severely ill patients.

HIV was diagnosed using the algorithm set forth by the Ministry of Health and Social Welfare (MoHSW) based on the serial testing strategy recommended by WHO (two rapid tests, ELISA, and Western Blot). TB was defined as a definitive or presumed clinical illness consistent with TB symptoms and signs. The definitive diagnosis was made if sputum smear was AFB positive or if histology was consistent with the presence of TB granulomas. A presumptive diagnosis of TB was established if the patient presented with symptoms suggestive of TB (fever, cough, weight loss, night sweats over 2 weeks duration) even if sputum smears were negative for AFB. This was done following the NTLP algorithm, whereby patients presenting with such symptoms initially receive broad spectrum antibiotics for at least two weeks. In case of poor response to antibiotics a sputum smear was repeated and chest radiograph done. An abnormal chest radiograph with persistent symptoms despite treatment and in the absence of other causes of such symptoms, patients were started on anti TB with the presumptive diagnosis of AFB smear negative TB.

### 4.2.2 Recruitment and follow up

After informed consent and appropriate pre-test counselling patients were seen at predetermined intervals where clinical and laboratory assessments were done. All information was recorded into a case report form (CRF) and later entered into a Microsoft Access Database. The HIV only patients were seen at weeks 0, 1, 2, 4, 8, 12, 16, 24, 36 and 48. At week 0 the demographic characteristics, a detailed history of present and past illnesses was taken along with a general physical examination. HAART was initiated at week 0 for the HIV only, whereas for those with HIV-TB co-infection, after 4 weeks of anti-TB treatment HAART was initiated. For those co-
infected with HIV and TB scheduled visits were on weeks 0, 2, 4, 6, 8, 12, 20, 28, 40 and 52. Education on the treatment regimens being provided and potential side effects and toxicities of the drugs was given along with adherence counselling by the study nurse. Assessment of drug adherence (to either HAART alone or HAART plus anti-TB) was done at each visit and patients were encouraged to have treatment assistants or use alarms as methods of enhancing adherence.

The study physicians did clinical evaluations for any adverse event and patient progress at every clinical visit. New TB diagnosis in HIV-only patients or worsening of TB symptoms in HIV-TB patients was noted and recorded in the CRFs. A verbal autopsy questionnaire was administered by the clinician to the relatives of those reported to have died during the study period. This questionnaire was administered in order to ascertain the probable cause of death in a situation where conduct of a real autopsy was not possible. For patients who missed their scheduled clinic visits telephone calls were placed to these patients to determine their whereabouts and appointments were rescheduled and they were encouraged to visit the clinic. For those who were lost to follow up, a home based care (HBC) worker was sent to follow this patient up at their given residence addresses.

Patient follow up and treatment chart

Key:
- Anti TB – Time point (week) when anti TB in initiated
- HAART – Time point when HAART is initiated
- EFV – Time points when blood is collected for plasma efavirenz measurement
4.2.3 **Laboratory investigations**

The laboratory investigations were done according to the same clinical schedule in both groups. The routine laboratory testing was performed at the Central Pathology Laboratory in MNH, including complete blood count, CD4 cell count, viral load assessment, and serum biochemistry. In addition hepatitis B surface antigen, hepatitis C serology and venereal disease research laboratory (VDRL) was done. The serum biochemistry was determined using a COBAS MIRA chemistry analyzer (GMI, MI, USA) after it was calibrated. The determination of hepatitis B surface antigen (HBsAg) and anti-hepatitis C virus IgG antibody (anti HCV) was done using the antibody capture ELISA (Adaltis – EIAgen kit).

Genomic DNA was isolated from peripheral blood leukocytes using QIAamp DNA Maxi Kit (QIAGEN GmbH. Hilden. Germany). Genotyping was carried out at the Division of Clinical Pharmacology, Department of Laboratory Medicine, Karolinska University Hospital-Huddinge, Karolinska Institutet, Stockholm, Sweden. Genotyping for SNPs were done by real time PCR using pre-developed Taqman assay reagents for allelic discrimination (Applied Biosystems Genotyping Assays) according to the manufacturer’s instructions. Allelic discrimination reactions were performed using TaqMan® (Applied Biosystems, CA, USA) genotyping assays with the following ID number for each SNP: (C__7586657_20 for ABCBI 3435C>T rs1045642, C__11711730_20 for CYP2B6 c.516G>T rs3745274 [CYP2B6*6], C__26201809_30 for CYP3A5 6986A>G rs776746 [CYP3A5*3], C__30203950_10 for CYP3A5 g.14690G>A rs10264272 [CYP3A5*6], C__32287188_10 for CYP3A5 g.27131_27132insT rs41303343 [CYP3A5*7] on ABI 7500 FAST (Applied Biosystems, Foster City, CA). The final volume for each reaction was 10µl, consisting of 2x TaqMan Universal PCR Master Mix (Applied Biosystems), 20 X drug metabolising genotype assay mix and 10 ng genomic DNA. The PCR profile consisted of an initial step at 50°C for 2 min and 50 cycles with 95°C for 10 minutes and 92°C for 15 sec. Genotyping for SLC01B1 388A>G (rs2306283) and 521T>C (rs4149056) was done using LightCycler® based method as described previously [207]. Haplotype analysis was done using Haplovie v.4.1 software.

On the 4\textsuperscript{th} week of efavirenz-based HAART, 8 ml of blood were collected 16 hrs post efavirenz dosing, centrifuged, and 2 mL plasma aliquot was taken and stored at -80°C
for determination of efavirenz and its metabolite concentration. Plasma samples were sent in dry ice to the Department of Clinical Pharmacology and Pharmacoepidemiology, University of Heidelberg, Germany. The determination of plasma Efavirenz and 8-hydroxyefavirenz concentrations by LC/MS/MS was performed as described previously [208, 209]. The lower limits of quantification in plasma were 10.0 ng/mL for efavirenz and 0.4 ng/mL for 8-hydroxyefavirenz.

4.2.4 Treatment

All HIV patients in the present study received efavirenz based HAART together with two NRTIs. Efavirenz was available as a 600 mg tablet for oral administration to be used once daily at bedtime. The nucleoside back bone was either zidovudine (300mg) plus lamivudine (150mg) twice daily or stavudine (30mg) plus lamivudine (150mg) also twice daily. The choice of which NRTI backbone to use was left to the decision of the attending clinician.

HIV patients co-infected with TB, they were initiated on rifampicin containing anti-TB treatment for the first 4 weeks, after which HAART will be added on to their treatment. The TB treatment followed the WHO and NTLP guidelines with DOTS divided in two phases. The Intensive Phase of anti-TB therapy consisted of 2 months treatment with 4 drugs namely rifampicin (150mg), isoniazid (75mg), pyrazinamide (400mg) and ethambutol (275mg) in fixed dose combinations (FDC), where dosage depended on the patient’s body weight. The Continuation Phase consisted of 4 months with 2 drugs, namely rifampicin and isoniazid also in a fixed dose combination. Cotrimoxazole (960mg once daily) prophylaxis was provided to the patients. Any other treatment that was given in addition to the study treatment during the study was documented in the CRF’s.

4.3 ETHICAL CONSIDERATIONS

Ethical permit to conduct the study for paper I was obtained from Muhimbili University College of Health Sciences (MUCHS) under the title “The study of enhancing adherence to antiretroviral drugs in the management of HIV/AIDS patients in Tanzania”.

Ethical approvals for papers II-V were covered under the ethical permit titled “Optimization of Tuberculosis and HIV co-treatment in Tanzania and Ethiopia: Pharmacokinetic and pharmacogenetic aspects of drug-drug interactions between
rifampicin and efavirenz” obtained from Muhimbili University of Health and Allied Sciences (MUHAS), Tanzania and the Karolinska Institutet, Sweden.

Prior to recruitment of the study participants, we obtained a written informed consent from each participant.
5 RESULTS AND DISCUSSION

5.1 PAPER I

Since the CTC clinic at MNH was set up in June 2004 up to September 2008, there were a total of 4108 HIV patients enrolled into the clinic. A total of 2542 patients were enrolled between 2004-2005 and a further 1566 were enrolled between 2006-2008. We excluded patients who had incomplete data. After exclusion, a total of 2408 patients were used for analysis. The excluded patients presented with similar characteristics to those included in the analysis like mean ages, gender distribution and WHO clinical staging.

The overall median age at enrolment was 37 years, with males being significantly older (p = 0.003) and with most patients (62.7%) being below 40 years of age. This indicates that the young people below the age of 40 years accounted for the majority of clinic attendees in keeping with earlier reported national and global findings on the age group of patients most affected with HIV. There were twice as many females compared to males who were enrolled into the clinic (ratio 2.2:1). Significant differences were seen in the distribution of marital status between those enrolled 2004-2005 and 2006-2008 with most patients (47.1%) being married, and this was true among both male and female patients. The mean body mass index (BMI) of the studied patients was 22.8 ± 4.6 kg/m2 though females presented with slightly higher BMI compared to the males (23.3 ± 4.8 vs 21.8 ± 4.0).

At presentation to the clinic patients who were enrolled earlier were sicker than those enrolled later (2004-2005); 62.4 % were WHO stages I and II, 26.2% stage III and 11.3% stage IV compared to (2006-2008) 78.6% were WHO stages I & II, 16.5% stage III and 4.9% stage IV respectively (p ≤ 0.001). Two thirds (65.7%) of all patients had CD4 cell counts below 200 cells/µL, of whom more than half had CD4+ T lymphocyte counts below 100 cells. Although patients who were enrolled to the clinic had severe immunosuppression, those enrolled during the first years of the program were found to be even more immunocompromised as compared to those enrolled later (127 cells /µL [IQR 174] versus 167 cells/µL [IQR 256] (p<0.001). This was also true with clinical presentation, where more patients were symptomatic at the beginning of the program (2004-2005) compared to the period 2006-2008. The number of symptoms at enrolment...
was linearly inversely related to the level of CD4 cell counts. This difference in immune status and clinical characteristics could be explained by the easy access of ARVs which could have increased awareness and reduced stigma of the disease thereby leading to early presentation to HIV clinics.

Women had significantly higher baseline CD4 cell counts and BMI compared to their male counterparts. The reason why males presented with lower CD4 cell counts was not clear from this study, however we can only speculate that males presented late due to stigma which was probably higher among men as compared to females. The other reason could be the difference in health seeking behaviour of men compared to women as seen by higher numbers of women enrolled at clinics. Approximately a fifth of the patients reported to have suffered from TB over the past five years. This is in keeping with the fact that TB is the most common infectious disease among HIV infected patients and that it is highly prevalent in sub Saharan Africa.

Prior to free access to ARVs, some of the patients who came to the clinic were not naïve to HAART as they had acquired them through private pharmacies. The absence and difficulty to access appropriate treatment early in the program sometimes led to some patients (2.7%) seeking alternative forms of treatment such as traditional medicines and spiritual healing. With increased access to HAART, there was a reduction of patients who sought out alternative treatment options as seen in patients who were enrolled later in the program. However, despite the improved access to ARVs, the pattern of clinical presentation has not changed much with time because patients still present late with severe immunosuppression to the HIV clinics.
5.2 PAPERS II-V

Flow Chart showing patient recruitment and follow up

Key:  
LTFU – Lost to follow up  
ETB – Patients were withdrawn due to new TB diagnosis  
Withdrew – Patients voluntarily withdrew themselves from the study  
Died – Patients that died within the study period
General baseline results for the cohort

A total of 486 HIV-1 infected individuals were recruited of whom 255 (53%) had HIV only while 231 (47%) have HIV and TB co-infection. There were significantly more females among the HIV only patients compared to the HIV-TB patients (p<0.001). Patients with HIV-TB co-infection had a lower median BMI of 19.4 kg/m² compared to 21.3 kg/m² among patients with HIV alone (p<0.001). Past history of opportunistic infections revealed that 13% had suffered from TB and 12% had a history of herpes zoster infection presenting with post herpetic scars.

More (40%) of the HIV-TB co-infected patients presented with Karnofsky scores of ≤80% compared to those with HIV alone (13%). The most common presenting symptoms were weight loss (44.2%), cough (39.9%), fever (35.4%), loss of appetite (29.6%), headache (25.7%) and skin rash (25.3%). The most common clinical findings were pruritic papulard eruption (PPE) (18.9%), lymphadenopathy (18.7%) and oral candidiasis (15.0%). Patients with HIV-TB co-infection had a significantly lower mean haemoglobin level (9.97 ± 1.55) compared to those infected with HIV alone (10.62 ± 1.73). The median CD4 cell counts and log transformed viral loads were similar in both HIV only patients and those co-infected with TB. Among the HIV-TB co-infected patients 67% were sputum positive for AFB with the remaining 33% being AFB negative or having EPTB.

A combination of zidovudine, lamivudine and efavirenz was initiated in 83.1% of the patients with HIV alone while only 49.3% of the HIV-TB co-infected patients were initiated with the same combination. This was due to the low baseline haemoglobin levels among those with HIV-TB co-infection.

General follow up results of the cohort.

At the end of the study (48 weeks after HAART initiation) we managed to successfully follow up a total of 161 HIV only patients and 126 HIV-TB patients. In the HIV only patients a total of 9.8% withdrew themselves from the study and 9.4% were lost to follow up whereas among the HIV-TB patients 18.6% withdrew from the study and a further 15.6% were lost to follow up. This proportion was higher, however not significantly so in the HIV-TB patients. We believe that most patients tend to go back to regions of origin after initiation of treatment and improvement of their health.
During the follow up period there was a steady increase in the weight of both HIV only and the HIV-TB patients. This shows a clear benefit from HAART initiation as seen by the overall well being and functional status of the patient. This was also evident from few reported opportunistic infections among these patients. CD4 cell count is the single most important parameter in monitoring ART in HIV-infected individuals especially in resource-constrained countries like Tanzania [7, 210, 211]. There was a gradual increase in the CD4 cell counts in both the HIV-only patients and those with HIV-TB. The median CD4 counts at 12, 24, 36 and 48 weeks after HAART initiation among HIV only patients was 182, 199, 212 and 250 cells/µL respectively while in the HIV-TB patients it was 200, 211, 251 and 274 cells/µL respectively. The most dramatic changes were seen within the first 12 weeks where the HIV only patients had a median increase of 92 cells/µL and 106 cells/µL in those with HIV-TB from their baseline levels.

HIV viral load is a useful tool for monitoring of HAART [212]. It is not used routinely for monitoring HAART in resource-limited countries. In our study we were able to obtain results from 163 patients. A total of 32.5% (33.3% HIV only and 31.2% HIV-TB) had detectable viral load (>50 copies/mL) at 48 weeks. Significant differences in mean log plasma efavirenz concentration at week 4 was seen between the patients who had virological failure (3.06 ± 0.63) and those who did not (3.28 ± 0.42) (p = 0.023). However, no significant differences were observed at week 16. The difference in efavirenz concentrations was not apparent when stratified by disease status (i.e. HIV only vs HIV-TB) at both weeks 4 and 16. Similarly, patients with virological failure had significantly lower median CD4 cell counts compared to those without failure (218cells/µL [IQR=224] vs 275cells/µL [IQR=203]) (p = 0.042), however when stratified by disease status, this difference was not significant. There were proportionately more patients with *CYP2B6*1/*6 genotypes (48.8%) with treatment failure compared to those with *1/*6 and *6/*6 (39.0% and 12.2% respectively).

5.3 PAPER II

From the 486 recruited patients a total of 54 (11.1%) patients died during the 48 week follow up period after HAART initiation. This compares well with other studies that showed similar rates of mortality [213, 214]. Proportionately there were more males
(13.1%) [20 deaths/100 person years (py)] who died compared to females (9.6%) [14 deaths/100py]. Of the 54 patients who died, 28 [16 deaths/100py] had HIV only and 26 [17/100py] had HIV-TB co-infection. This is of interest since the mortality rate in the HIV only patients and those with HIV-TB were similar. Studies have shown that with good anti-TB therapy, mortality among HIV-TB patients need not be higher than those with HIV only [215, 216].

Of the patients who died, 54% were WHO clinical stage III and 11% were WHO clinical stage IV. There were statistically significant differences in BMI among those patients who died compared to those who didn’t (19.3±3.6 versus 21.1±4.2) (p=0.003). Likewise, highly significant differences were seen in the median baseline CD4 cell counts between patients who died and those who didn’t. Patients who died had lower CD4 counts that those who didn’t (48 [IQR=101] versus 98 [IQR=116]) (p<0.001). Viral load levels were significantly higher among patients who died, compared to those who didn’t (p=0.015).

The median time to death among these patients was 13 weeks [17 deaths/100py]. In the HIV only patients 16 (57.2%) died in the first 16 weeks of HAART initiation. A large proportion (21%) of the patients died towards the last third of the follow up period. Among the 26 HIV-TB patients who died, 6 (23%) died while still on anti-TB therapy before HAART was initiated at the 4th week of treatment. Following HAART initiation, 46.2% died while on anti-TB treatment and HAART, and a further 8 (30.8%) died after the completion of TB treatment while continuing with HAART. However, in both the HIV only and those with HIV-TB over 50% died within the first 4 months of HAART initiation. Improvement of immune status associated with HAART could explain the lower mortality rates during the later periods of the study.

After analysing the verbal autopsy reports from the relatives of the deceased and hospital records, there was no specific pattern seen in either the HIV only or the HIV-TB patients and the symptoms at death showed a wide variation. Multivariable analysis showed that clinical predictors of mortality were the presence of oral candidiasis, Kaposi’s sarcoma and low Karnofsky scores. Laboratory parameters that were predictors of mortality include low baseline white blood count (WBC) and CD4 cell counts. The risk factors for mortality are those of severe immunosuppression due to late presentation to the CTC.
Self-reported adherence to HAART was 98% over the past week for HIV only patients and 97% for those with HIV-TB co-infection on HAART and anti-TB therapy. The good adherence rates may be due to persistent counselling given to patients on adherence during each clinic visit and the encouragement to have treatment assistants [217]. In this study 4.7% of the HIV only developed active TB after HAART initiation. These were classified as HAART-related TB with similar prevalence’s to those reported in other studies [218, 219]. Among HIV-TB patients on anti-TB who were started on HAART only, 3% of the patients developed exaggerated features of TB, which we defined as TB IRIS. These features were in the form of increased fever episodes and enlargement of lymph nodes.

5.4 PAPER III

A total of 255 HIV only patients were recruited into the study. Of these only 129 patients were used for analysis due to the available data on efavirenz plasma concentrations at both time points (i.e. 4th week and 16th week after HAART initiation). The mean age of these patients was 39.6±9.1 years, with the male to female ratio of 1:2.1. The median BMI and baseline CD4 cell counts among these patients was 23(20-26) and 103 (44-160) cells/µL respectively. The allele frequencies from 120 patients for CYP2B6*6, CYP3A5*3, CYP3A5*6, CYP3A5*7 and ABCB1 were 39.6%, 21.7%, 18.8%, 11.3% and 15.4% respectively. For the CYP2B6 the genotype frequencies for *1/*1, *1/*6 and *6/*6 were 37.5%, 45.8% and 16.7% respectively.

The proportion of subjects with sub-therapeutic efavirenz plasma levels (<1 µg/ml) increased by 57% at week 16 as compared with the value at week 4, whereas the proportion of those with risk for toxicity (>4 µg/ml) decreased by 44%. The magnitude of change was influenced mainly by CYP2B6 genotype. The most affected group was patients with CYP2B6*1/*1 followed by those with CYP2B6*1/*6 genotype whereas among CYP2B6 slow metabolizers (2B6*6/*6), there was no significant change in the plasma efavirenz concentrations between weeks 4 and 16. During HAART the levels of efavirenz decrease and those of this metabolite (8-hydroxyefavirenz) increase; this led to a lowered efavirenz concentrations at week 16 of therapy.

Our results indicate that the enzymatic activity of CYP2B6 at week 16 of therapy is
higher than at week 4 thereby suggesting that the effect of efavirenz auto-induction in reducing efavirenz plasma exposure continues up to week 16 of therapy. The extent of this change in plasma concentrations of efavirenz, its metabolite (8-hydroxyefavirenz) and the metabolic ratio between weeks 4 and 16, were higher among CYP2B6*1 carriers than in those with the CYP2B6*6/*6 genotype. This implies that patients who are carriers of the functional enzyme are more likely to experience autoinduction of efavirenz to an extent that may have consequences for the long term HAART outcome especially in the CYP2B6 extensive metabolisers (CYP2B6*1/*1).

The relevance of the CYP3A5 genotype in affecting the extent of enzyme inducibility was reflected among CYP2B6 slow metabolizers. In the CYP2B6*6/*6 group, carriers of one or two CYP3A5*1 alleles had higher 8-hydroxyefavirenz values at week 16 compared to those lacking the allele. No changes were seen in patients lacking both CYP2B6*1 and CYP3A5*1 alleles, showing that CYP3A5 is an alternative pathway in efavirenz metabolism. Gender had no impact on the changes in the proportion of patients within the different efavirenz therapeutic ranges, plasma efavirenz levels or the metabolic ratios at week 4 and 16.

5.5 PAPER IV

A total of 473 patients with complete laboratory data were used for analysis, of whom 253 were HIV only and 220 were those with HIV-TB co-infections. As per our case definition of DILI based on one or more AST/ALT levels ≥ 2 X UNL, 37 patients (7.8%) developed drug induced liver injury (DILI). Fifteen (5.9%, 6.3 per 1000 person-week) patients developed DILI among HIV only patients while twenty-two (10.0%, 10.7 per 1000 person-week) HIV-TB patients developed DILI, an almost 2-fold incidence of DILI among the HIV-TB patients compared to those with HIV only. These results are similar to those reported elsewhere describing that concomitant HAART and anti-TB therapy exacerbates the incidence of DILI [220, 221]. The DILI was transient, with AST and/or ALT levels coming back to within normal limits by 12 weeks of HAART with no treatment modification required for patients with DILI. The low incidence and tolerability of DILI among Tanzanian HIV patients is comparable to other reports from Africa [220, 222].

Proportionally more males developed DILI compared to females. Patients who
developed DILI had lower baseline CD4 cell counts compared to those without DILI (p=0.056). No association with DILI was seen among patients who used fluconazole or consumed alcohol. In a subgroup of HIV-TB patients, pronounced DILI was observed before the introduction of HAART indicating that rifampicin may contribute to the hepatotoxicity, however in the majority of cases the DILI appeared later in conjunction with the introduction of efavirenz.

The prevalence of HBsAg and anti-HCV antibodies was 10.4 % and 3.1% respectively in the cohort, however, a significant 25% of the patients with positive HCV antibody developed DILI (p=0.028). We observed that hepatitis C infection was a significant risk factor for the development of DILI rather than hepatitis B. All our study subjects received lamivudine as part of HAART and this might lead to alleviating HBV co-infection as a risk factor for DILI. Significant differences in the distribution of CYP2B6 genotype were seen between patients with and without DILI. The frequency of CYP2B6*6 (c.516TT) and CYP2B6*6/*6 genotype was overrepresented among patients with DILI. The variant CYP2B6*6 allele was associated with higher efavirenz plasma concentration and in our study it was found to be a risk factor for DILI, however, plasma efavirenz concentration on its own was not a risk factor. Interestingly, efavirenz concentrations were higher in patients with low BMI in the HIV only patients receiving efavirenz-based HAART whereas no such difference in BMI was seen in HIV-TB patients receiving rifampicin and efavirenz.

Of the 54 DILI patients a total of 7 (13%) died during the study period with median time to death being 4 weeks. Causes of death among these patients were not associated with DILI as shown in verbal autopsy reports. Gradual and comparable increases in CD4 cell counts and body weight were seen among patients with and without DILI from baseline to 12 weeks after HAART initiation. Despite the high pill burden especially in those on both anti-TB and HAART and potential toxicity, patients’ self-reported adherence was high (>95%).

5.6 PAPER V

Data from 458 patients (243 HIV only and 215 HIV-TB) was used for analysis as these had documentation on the neuropsychiatric manifestations. Of the 458 patients, 264 (57.6%) had some form of neuropsychiatric manifestations during the 16-week study period comparable to incidences reported from other studies [151, 223, 224].
Significant differences in the presence of neuropsychiatry manifestations were seen between the HIV only compared to those with HIV-TB (66.7% and 47.4% respectively) (p<0.001). HIV only patients were more symptomatic compared to the HIV-TB patients where just over a third (33.4%) of the HIV only compared to over half (52.6%) of those with HIV-TB did not present with any neuropsychiatric manifestations. However, for symptomatic patients, higher severity was reported in general in those with HIV only compared to those with HIV-TB. This difference in severity of symptoms between the HIV only and HIV-TB was statistically significant (p<0.001).

The median time to neuropsychiatric manifestations was 2 weeks during the 16 week follow up. However, in the HIV only patients, the median time to manifestations was 1 week after HAART initiation while in patients with HIV-TB this was seen at 6 weeks (i.e. 2 weeks after HAART was initiated). No differences were seen in median efavirenz concentrations and the metabolite 8-hydroxyefavirenz among patients with and without neuropsychiatric manifestations at weeks 4 and at 16 weeks respectively. There were no gender differences between patients with and without neuropsychiatric manifestations. There were no differences in plasma efavirenz concentrations between those with and without neuropsychiatric manifestations when stratified by disease status (HIV only and HIV-TB). The single most significant predictor of neuropsychiatric manifestations was the use of efavirenz only, compared to use of efavirenz and rifampicin. Baseline age, BMI and CD4 did not differ among patients with and without neuropsychiatric manifestations

The group 1 symptoms of depression and anxiety seem to occur in both HIV only and HIV-TB patients despite the absence of efavirenz in the beginning of the evaluation in the HIV-TB patients when they are on anti-TB alone. These symptoms were mild, and not specific to the known efavirenz neurotoxic side effects. The higher proportion of symptoms in the HIV only could also be due to the stress brought on by psychological adjustment to recent HIV diagnosis. HIV disease status on its own is a risk factor for neuropsychological impairments despite the HIV stage [225, 226]. Patients with low CD4 nadir are also more likely to have these impairments as seen in our study where most of the patients had lower CD4 cell counts with a larger proportion having counts below 100 cells/µL. Addition of efavirenz based HAART caused an increase in these symptoms in the first week of therapy. However, the waning off of these symptoms in
subsequent weeks could also be explained by the immune reconstitution as seen by increase in CD4 counts at 12 weeks, efavirenz auto-induction and the use of concomitant rifampicin in the HIV-TB patients. Despite presence of the neuropsychiatric manifestations, patients’ self-reported adherence to HAART in both the HIV only using HAART alone and the HIV-TB patients using both anti-TB therapy and HAART was over 95%.
6 CONCLUSIONS

The results of the study indicate that patients enrolled at HIV clinics around Dar es Salaam are predominantly women, and they present to the clinics fairly late with severe immunosuppression. The results also show that persons below the age of 40 were those most affected by the disease and that the time at presentation to the HIV clinics influences the clinical spectrum of symptoms and signs seen at the initial evaluation. Improved access to HIV care and treatment services has been associated with early presentation to the clinics in the course of the HIV disease. HIV related TB is high among patients attending HIV clinics.

Mortality of HIV positive patients who are initiated on HAART is still considerable with mortality occurring early in the treatment duration. There was no significant difference in mortality between HIV only patients started on an efavirenz based HAART and the HIV-TB patients started on rifampicin-based anti TB therapy and efavirenz based HAART. Predictors of mortality were those of advanced disease such as low Karnofsky scores, low baseline CD4 cell count and low white blood cell count and the presence of opportunistic infections such as oral candidiasis and Kaposi sarcoma.

We investigated the influence of pharmacogenetic variations on efavirenz concentrations among our patients. A little over 40% (42.2%) of the patients had the extensive metabolizer genotype. Our results indicate that CYP2B6 genotype determines not only the plasma efavirenz concentration at a given time point but also the long-term effect of efavirenz auto induction on plasma exposure. Individuals with the extensive metabolizer phenotype experience a pronounced efavirenz autoinduction effect which may influence the long term therapeutic outcome. The effect of efavirenz autoinduction on reducing plasma exposure continues up to week 16. The magnitude of change was influenced mainly by the CYP2B6 genotype with the most affected group being those with *1/*1 (rapid metabolizers) followed by *1/*6 and lastly by those with *6/*6 (slow metabolizers) genotypes.

Efavirenz-based HAART and rifampicin-based anti-TB DILI does occur in Tanzanian HIV patients, presenting early after HAART initiation. Despite these patients having DILI, in the majority of these patients the elevations of the liver enzymes was relatively
mild, transient and did not require any modification of the treatment regimens. The risk for developing DILI was attributed to a positive anti-HCV IgG antibody and the presence of CYP2B6*6/*6 genotype. Despite the presence of DILI, there is good tolerance to HAART and anti-TB therapy with good clinical and immunological outcomes.

Incidence of neuropsychiatric manifestations due to efavirenz-based HAART is high in Tanzanian HIV patients. Despite a relatively high proportion of patients reporting neuropsychiatric manifestations attributed to efavirenz, these symptoms are transient and not severe. There is wide inter-patient variability in plasma EFV concentration as seen in differences in gender and CYP2B6 genotypes. The use of rifampicin-containing anti-TB therapy concomitantly with efavirenz decreases the risk of developing neuropsychiatric manifestations up to three times compared to those on efavirenz-based HAART. Plasma efavirenz concentrations at week 4 after HAART initiation, and the CYP2B6 genotype are not positively correlated with development of neuropsychiatric manifestations.

A significant proportion of patients (32.5%) developed treatment failure. However, no differences were noted between the HIV only and HIV-TB patients. Both the HIV only and the HIV-TB patients showed comparable clinical and immunological outcomes.

Overall, free access to HAART has greatly improved patients lives and clinical outcomes. The recommended efavirenz dose of 600mg when used with rifampicin shows similar clinical outcomes compared to those using efavirenz-based HAART alone in terms of toxicities (DILI and neuropsychiatric manifestations), immunological (CD4 cell counts) and virological (viral load) outcomes despite the pharmacokinetic and pharmacogenetic drug-drug interactions.
7 RECOMMENDATIONS

More efforts need to be put in place to educate the public on the need for regular HIV testing so that the problem is caught earlier, allowing for subsequent appropriate accessibility to preventive and curative services.

We recommend the reduction in national in-programme delays in initiating HAART for HIV patients with and without TB before the development of advanced HIV disease in order to reduce morbidity and mortality.

We would like to encourage clinicians to avoid reluctance in combining HAART and anti-TB therapy for fear of toxicity in Tanzanian patients. As we saw from our study, co-treatment is tolerable among patients, with good clinical and immunological outcomes as well as good reported adherence to both HAART and anti-TB.

Genotyping for CYP2B6 polymorphism in HIV clinics could help identify patients with poor metabolizer phenotype who are at risk of developing CNS toxicity, and the rapid metabolisers whose long-term therapeutic outcome might be compromised.

Measurement of plasma efavirenz concentrations could assist in therapeutic drug monitoring to ensure patients are within therapeutic ranges to avoid suboptimal efficacious response or even the development of acquired drug resistance.

The WHO recommended efavirenz dosage of 600mg daily can be used with rifampicin among Tanzanian patients without compromise to their treatment outcomes.
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9 REFERENCES


