RESPONSE TO ANTIRETROVIRAL THERAPY IN HIV-INFECTED PATIENTS ATTENDING CARE AND TREATMENT CENTRE IN DAR-ES-SALAAM, TANZANIA

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A DISSERTATION SUBMITED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FORTHE DEGREE OF MASTER OF SCIENCE IN ONE HEALTH MOLECULAR BIOLOGY OF SOKOINE UNIVERSITY OF AGRICULTURE. MOROGORO, TANZANIA

2013

ABSTRACT

Access to antiretroviral therapy and *Human immunodeficiency virus* (HIV) care is increasing in resource-limited settings like Tanzania. This study was conducted to evaluate prevalence and risk factors associated with treatment failure, in HIV patient on Anti Retroviral Therapy (ART) for more than six months in Dar es Salaam. A cross-sectional observational study that involved 70 conveniently sampled and consented HIV-infected patients who were on antiretroviral therapy for more than six month at PASADA Care and Treatment Centre (CTC) in Dar es Salaam. The findings of this study indicated that majority of Patient 78.5% (n=55) were experiencing ARVs benefits, they attained total viral suppression (<400 viral copies/ml. Ten percent (n=7) of patients were reported to have virological failure suppression (>10,000 viral copies/ml), and 10.4% (n=8) of them found to have partially viral suppression (400-9999 viral copies/ml). On the other hand, 27.1% (n=19) of participants were reported to have an immunological failure. Poor adherence to the treatment regime and drug toxicity were the main factors observed to associate with treatment failure in this study ($P<0.05$). Although many HIV-infected patients attending CTC in Dar es Salaam had advanced HIV infection, the majority of patients who were receiving antiretroviral therapy were experiencing viral suppression and clinical benefit. In order to optimize the likelihood of viral suppression and prolong benefits of improved quality of life to those HIV patients on ART attending CTC, early HIV testing, initiation of therapy with a potent, durable regimen, accompanied by stable drug supplies and proper counselling on drug adherence are important. Furthermore prospective studies on treatment failures, factors associating with treatment failures and studies that will aim at developing low-cost molecular methods to identify virological failure and ARV sensitivity were urgently needed.

DECLARATION

I, Peter Benard Mtesigwa do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work done within the period of registration and that it has neither been submitted nor being concurrently submitted in any other institution.

Signature……. Date……

Peter Benard Mtesigwa

(MSc. OHMB Candidate)

The above declaration is confirmed

Signature……. Date……

Dr. Christopher J Kasanga

(Supervisor)

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ACKNOWLEDGEMENTS

This work has been accomplished through the assistance and heart full co-operation of different people, agencies and organizations. I believe that all the people I had contacted helped me directly or indirectly by giving me moral, material support and using their time for this particular work. Although I cannot mention them all, I am obliged to express my profound gratitude to few of them.

First of all, I would like to thank God who kept me and has been keeping me healthy physically and mentally, it is through his mercy and grace that finally I came to finish this research work despite all the difficulties I have passed through in the preparation of this dissertation.

Secondly, I would like to thank my research supervisors Dr. Christopher J Kasanga and Prof Mercky Matee for their guidance in research, critical reading of the manuscript chapters, encouragement and valuable comments that helped to shape this work. Thirdly, I would be unfair if I leave out my fellow students who worked hand in hand with me especially through advice and minor consultations. These include Edward Mayila, Sydney Mwanza, Emmanuel Kifaro, Frank Banda, DR Matangila Jr and my beloved sisters Emma Njau and Dr Vivi Maketa.

Furthermore, I thank all patients and their families for their participation in the study and for their motivation and collaboration. I also thank the medical personnel of the PASADA Care and Treatment Centre and MUHAS Microbiology Laboratory staff for their support and technical advice in the implementation and carrying out of this study. The support and encouragement that they gave me was unique and very helpful. Thanks to Dr C Moshilo for her editorial support.

Financial support for this study was provided by the Welcome Trust through SACIDS.

TABLE OF CONTENTS

LIST OF TABLES

FIGURE

LIST OF ABBREVIATIONS AND SYMBOLS

AIDS	Acquired Immune Deficiency Syndrome
ALT	Alanine Aminotransferase
ART	Antiretroviral treatment
ARV	Antiretroviral
CD4	Cluster of Differentiation 4
CI	Confidence Interval
CPK	Creatine phosphokinase
CTC	Care and Treatment Centre
DNA	Deoxyribonucleic acid
EDTA	Ethyldiaminetetracetic acid
FBC	Full Blood Count
HAART	Highly active antiretroviral therapy
HBC	Home Based care
HBV	hepatitis B virus
HCV	hepatitis C virus
HIV	Human Immunodeficiency Virus
IQR	Inter Quartile Range,
LTRs	Long terminal repeats
MOHSW	Ministry of Health and Social Welfare
NACP	National AIDS Control Programme
NCTP	National HIV Care and Treatment Plan
NDHSA	National Department of Health South Africa.
NNRTI	Non-nucleoside reverse transcriptase inhibitors
NRTI	Nucleoside reverse transcriptase inhibitors

OHL	Oral Hairy Leukoplakia
OI	Opportunistic infection
PASADA	Pastoral Activities and Services for people with AIDS Dar-es-salaam Archdiocese
PCR	Polymerase Chain Reaction
PI	Protease inhibitors
RNA	Ribonucleic acid
S.D	Standard Deviation
SACIDS	Southern Africa Consortium for Infectious Diseases Surveillance
STI	Sexually Transmitted Infection
SUA	Sokoine University of Agriculture
TB	Tuberculosis
UNAIDS	Joint United National Program for HIV/AIDS
VCT	Voluntary counselling and testing
VL	Viral Load
WHO	World Health Organization

CHAPTER ONE

1.0 INTRODUCTION

1.1.1 HIV and AIDS

The current pandemic of acquired immune deficiency syndrome (AIDS) is caused by a member of family known as human immunodeficiency virus (HIV) (UNAIDS, 2011). A patient who is infected with HIV has, at first, a normal immune response to the presence of the virus. However, as time passes, things begin to change, as the virus continues to replicate over the years after the initial infection the patient begins to lose CD4+ cells (Michele *et al.*, 2011). The depletion of CD4+ T cells is correlated with high turnover of the human immunodeficiency virus HIV-1 and associated with apoptosis (Michele *et al.*, 2011). After the level of these cells drops below 200 cells per mm^3, the patient is said, according to the Centres for Disease Control (CDC) and the World Health Organization (WHO), to have clinical or "full-blown" AIDS. With depleted CD4+ T cells, immune system rendered under functional, the immune system of the AIDS patient is no longer able to fend for infections that normally are not threatening to health individuals.

1.1.2 Antiretroviral (ARV)

The purpose of anti-retroviral therapy is to reduce the HIV viral load as much as possible preferably to undetectable levels. This assures less damage on the immune system following HIV pathogenesis with ultimate improvement in the immune functioning and delayed onset of AIDS. This actually enhances quality of life, reduce opportunistic infections and reduce the impact of HIV transmission in the community (NACP, 2005).

Recommended ARV Drugs are triple therapy in any case, consisting of either 2 (two) nucleotide reverse transcriptase inhibitors NRTI and 1 (one) non-nucleoside reverse transcriptase inhibitors NNRTI or 2 (two) nucleotide reverse transcriptase inhibitors

NRTI and 1(one) protease inhibitors PI or 3 nucleotide reverse transcriptase inhibitors NRTI's (NACP, 2009).

Changing therapy may be necessary due to treatment failure, toxicity, and patient intolerance to the combination or inability of the patient to adhere to the treatment regimen (NACP, 2009). Clinical disease progression is a marker of treatment failure and necessitates a review of the patient's therapy (Geretti, 2006).

1.1.3 Monitoring HIV Patients

Monitoring HIV patients begins before initiation of antiretroviral therapy (ARV), with the clinical status of the patient and laboratory markers guiding when to recommend commencement of therapy (WHO, 2006). Conventionally, this decision is based on the predictive values for disease progression and decline of CD4 lymphocyte count and HIV-RNA (Hammer *et al.*, 2006).

The presence or absence of HIV-related signs and symptoms also significantly influences the decision to initiate therapy (WHO, 2007). Increasingly, concerns related to drug toxicities, pill burden and the ability of patients to adhere to strict and complicated treatment regimens have complicated the decision-making process for physicians and patients toward drugs (Asim, 2004).

Once treatment begins, the clinical progress of the patient needs to be reviewed regularly. Laboratory monitoring is focused on markers of efficacy of the regimen and drug toxicities (NACP, 2009).

Since laboratory monitoring cannot always predict the development of complications regular clinical evaluation to patient taking ARV is required to complement monitoring of patients on ARV (NDHSA, 2004).

1.1.4 Monitoring Patients on ARV Therapy in Tanzania

According to (NACP, 2009) in Tanzania, Patients on ARV have been monitored both clinically and through a number of Laboratory tests. Clinical Monitoring is associated with weight gain and reduced morbidity from opportunistic infections and improvement in the quality of life. Appearance or persisting opportunistic infections, or lack of weight gain, may indicate treatment failure hence the need to consider changing regimens. Laboratories have been used to monitor disease progress, drug efficacy and antiretroviral treatment safety.

Depend on the fact that, as HIV disease advances, patient's immune status deteriorates. Measurements of CD4 counts play part as important immunological marker of disease progression and assist in decision making on when to start antiretroviral treatment. Since successful antiretroviral therapy results in decrease of viral load, immune recovery and therefore increase in number of CD4 cells, instead of using HIV drug resistance tests, or HIV-RNA Viral load for testing drug's efficacy, Periodically every 6 months the CD4 count have been used to monitor the efficacy to antiretroviral therapy.

1.2 Problem Statement and Justification

Antiretroviral drugs are known to produce short and long term side effects in some patients which may pose challenge to treatment adherence. However, successful suppression of HIV replication is influenced by the inherent strength of the prescribed regimen, patient adherence to treatment and pre-existing or emerging resistance to antiretroviral (ARV) agents. On the other hand, due to the presence of drug pressure, invention of drug-resistant mutants is an inherent aspect of HIV-1 replication. Under conditions that allow ongoing viral replication, drug-resistant mutants might get hold of a selective advantage over wild-type virus and become dominant within the quasispecies

(Jourdain *et al.*, 2004) a condition which may result into treatment failure and leads to change into more expensive treatment regime.

In Tanzania, since 2004 patients examined and counselled to starts medication without knowing their viral load and drug resistance status. Once they are enrolled, clinical and immunological parameters are used for monitoring patient's prognosis and identifying treatment failure. Therefore, treatment regime has been changed depending on immunological and clinical parameters without knowing to which particular ARV patient's virus are sensitive to.

As a part of disease monitoring to avoid the emergence of drug-resistance viral strains in the near future, there is a need to continuously understanding on how currently administered ARV responds in HIV-infected patients while detecting the emergence of ARV drug resistance if any, as well as factors associating with them.

1.3 Objectives

1.3.1 Main Objective

The main objective of this study was to determine prevalence, patterns and factors associated with virological/immunological failure to Antiretroviral Therapy (ART) among HIV-Infected patients attending a Care and Treatment Centre in Dar-es-salaam, Tanzania

1.3.2 Specific Objectives

The specific objectives of this study were

i. To determine the prevalence of virological/immunological failure for HIV-infected patients receiving antiretroviral therapy (ART) in Dar-es-salaam, Tanzania

ii. To identify factors associated with virological/immunological failure for patients receiving antiretroviral therapy (ART) in Dar-es-salaam, Tanzania

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 HIV and AIDS

The current pandemic of acquired immune deficiency syndrome (AIDS) is caused by a member of family known as human immunodeficiency virus (HIV) (UNAIDS, 2011). HIV genome is similar to and yet different from other retroviruses, one very striking difference is the presence of a number of small proteins, all of which play a role in the infectious process (Frank, 2004). After the viral RNA genome enters the cytoplasm of the host cell, it is immediately converted into double stranded DNA by reverse transcriptase, an enzyme carried as a part of the virus structure (Nathan *et al.,* 2003). This DNA enters the nucleus where it is integrated into the host cell genome by a site-specific recombination event, catalyzed by the viral integrase (Nathan *et al.,* 2003).

The integrated form of HIV-1, also known as the provirus, is approximately 9.8 kilo bases in length (Muesing *et al.,* 1985). Both ends of the provirus are flanked by a repeated sequence known as the long terminal repeats (LTRs). The genes of HIV are located in the central region of the provirus DNA and encode at least nine proteins (Gallo *et al.,* 1988). These proteins are divided into three classes, major structural proteins, Gag, Pol, and Env, regulatory proteins, Tat and Rev and accessory proteins, Vpu, Vpr, Vif, and Nef (Gallo *et al.,* 1988)

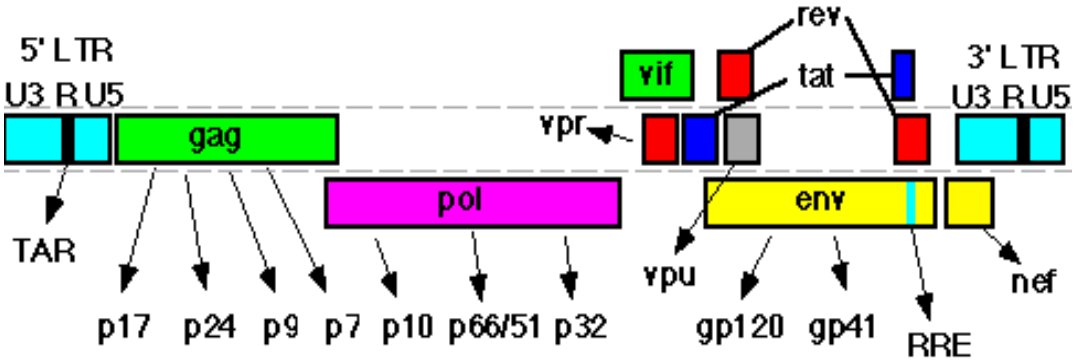

Figure 1: Orientation of HIV - 1 Genome

A patient who is infected with HIV has, at first, a normal immune response to the presence of the virus. The patient produces antibody and within 4 to 10 weeks can be found to be "HIV positive" meaning that these antibodies are present in the circulation (NACP, 2009). However, as time passes, things begin to change, as the virus continues to replicate over the years after the initial infection the patient begins to lose CD4+ cells. The depletion of CD4+ T cells is correlated with high turnover of the human immunodeficiency virus HIV-1 and associated with apoptosis (Michele *et al.,* 2011). The molecular mechanism of apoptosis in HIV infection, however, is largely unknown. T-cell apoptosis might be affected by viral proteins such as HIV-1 Tat and gp120 (Michele *et al.,* 2011). T-cell-receptor (TCR) induced apoptosis also shown to involve the CD95 (APO-1/Fas) receptor (Michele *et al.,* 2011). After the level of these cells drops below 200 cells per $mm^{3,}$ the patient is said, according to the Centres for Disease Control and the World Health Organization, to have clinical or "full-blown" AIDS.

With the deficiency of CD4+ T cells, immune system of AIDS patient is no longer able to fend infections that are not normally threatening to health individuals. Opportunistic infections such as *Cytomegalovirus, Pneumocystis pneumonia* and *Cryptosporidium* become severe illnesses. In addition to this, HIV invades the central nervous system,

causing AIDS related dementia. If left untreated AIDS has a greater than 95% case fatality rate (McDonald *et al.*, 1998).

2.2 Epidemiology/Impact of HIV and AIDS

By the end of 2007, it was estimated that a total of 33.2 (30.6 – 36.1) million people worldwide were living with HIV/AIDS (Oladipo, 2010). Sub-Saharan Africa is the world's most severely affected region, with only 10% of the world's population, sheltering about two thirds of the global total number of people living with HIV/AIDS (NACP, 2009).

According to the WHO an estimated 6.6 million people in low- and middle-income countries were receiving antiretroviral therapy for HIV/AIDS at the end of 2010 (WHO Media centre, 2011).

As well elaborated by National Guidelines for the Management of HIV/AIDS, since 1983 when the first AIDS cases were reported in Tanzania, the HIV epidemic has spread rapidly to all districts and communities and has affected all sectors of the society (NACP, 2009).

During the year 2003 a total of 18,929 AIDS cases were reported to the National AIDS Control Programme (NACP) from the 21 regions, bringing the cumulative total of reported cases since the epidemic broke to 176,102 (NACP, 2009).

In 2007, about 2 million persons were estimated to be living with HIV and AIDS, with approximately 600,000 (30%) in need of ART (UNAIDS, 2007). The HIV pandemic has had a profound impact on the health care system of all countries worldwide but mostly

those in Sub-Saharan Africa. It has reduced resources available for other health problems which has an unfavourable effect on the quality of health care services being provided (UNAIDS, 2007). In Tanzania for example, most of the urban district and regional hospitals report a bed occupancy rate of up to 50-60% for HIV-related conditions (Mwita *et al.*, 2007).

2.3 Tanzania's National Response for Prevention and Interventions of HIV/ AIDS

During all this period of the HIV epidemic in Tanzania, the country has responded in several ways (NACP, 2005), one of them being National HIV Care and Treatment Plan (NCTP) which was launched in October 2004. It had the main focus on care and treatment services, targeting more than 400,000 patients on care and treatment by the end of 2008. Simultaneously, it focused on follow up on disease progression in 1.2 million HIV+ persons who are not eligible for antiretroviral therapy (ART) (National AIDS Control Programme, 2009). This plan came up with, Care and Treatment Clinics (CTC) set up, in both public and private health providers throughout Tanzania (NACP, 2009). For the delivery of care and treatment to ensure that effective monitoring and evaluation programme takes place

2.4 Monitoring HIV Patients

Monitoring HIV patients begins before initiation of antiretroviral therapy (ARV), with the clinical status of the patient and laboratory markers guiding when to recommend commencement of therapy (WHO, 2006). Conventionally, this decision is based on the predictive values for disease progression and decline of CD4 lymphocyte count and HIV-RNA (Hammer *et al.*, 2006)

The presence or absence of HIV-related signs and symptoms also significantly influences the decision to initiate therapy (WHO, 2007). Increasingly, concerns related to drug toxicities, pills burden and the ability of patients to adhere to strict and complicated treatment regimens have complicated the decision-making process for physicians and patients toward drugs (Asim, 2004). Despite promised price-reductions and increased availability of generic drugs in some countries, cost remains a major factor in deciding when to start therapy in many parts of the world (WHO, 2006).

Guidelines vary from country to country. Early intervention in an asymptomatic patient is commencement of ARV if the CD4 lymphocyte count is less than 500 Cells/ml. A less aggressive approach is to recommend therapy when the count is below 350 Cells/ml. Depending on the financial resources of the patient, treatment typically may be delayed until the CD4 count is 200 Cells/ml (Panel on Antiretroviral Therapy and Medical Management of HIV, 2011), while a declining CD4 count and/or rising viral load over time may be considered as one of the reasons to initiate therapy. Most physicians would recommend therapy for patients with symptomatic HIV conditions, such as the presence of recurrent oral candidiasis, oral hairy leukoplakia (OHL) or unexplained weight loss (World Health Organization, 2006). Also factors like, commitment of the patient to commencing therapy, understanding of the lifelong nature of such treatment and understanding the importance of adherence to drug regimens on a daily basis all affect the timing of the recommendation. Once treatment begins, the clinical progress of the patient needs to be reviewed regularly. Laboratory monitoring is focused on markers of efficacy of the regimen and drug toxicities (NACP, 2009). The frequency of review is dictated by the drugs selected, the development of adverse events and the available resources.

2.5 Laboratory Monitoring of HIV Patients on ARV Treatment

2.5.1 Efficacy Monitoring

Quantification of HIV-1 RNA in plasma is the basis of ARV efficacy monitoring (Murray *et al.,* 1999). The ultimate goal of combination ARV is undetectable plasma HIV-RNA (HIV/AIDS Antiretroviral Newsletter: 2002). This should be achievable in most treatment-naive patients receiving highly active ARV therapy (HAART). The "gold standard" remains a triple drug combination of two nucleoside reverse transcriptase inhibitors (NRTI) plus protease inhibitor toxicities (National Guidelines for the Management of HIV/AIDS: 2009).

2.5.2 Immunological Markers

Following successful initiation of HAART, a rise in CD4 lymphocyte count of 90-150 cells would be expected in six months (HIV/AIDS Antiretroviral Newsletter 2002). To some extent, this rise is dependent on the CD4 count at the time of commencement of therapy, with a lesser response expected with a lower initial count (HIV/AIDS Antiretroviral Newsletter 2002). There is often a biphasic response with an initial rise in CD4 count after 1-2 months of therapy as cells are redistributed from bone marrow (Julie *et al.,* 2012). This may be followed by a decline in CD4 cell numbers followed by a second, slower rise with continued suppression of viral replication and the second-phase rise in CD4 cell numbers may continue for more than 12 months (Julie *et al.,* 2012).

As HIV disease progression is unlikely in a patient with a CD4 count above 350 Cells/ml, this should be the minimum immunological goal of therapy. However, risk of disease progression is significantly reduced if the CD4 cell count can be maintained above 200 Cells/ml (NACP, 2009).

2.5.3 Laboratory Toxicity Monitoring

Prior to the commencement of ARV in a resource-unlimited clinic, it is recommended that the following set of laboratory tests at baseline and each follow-up visit be performed: full blood count (FBC) and differential, liver enzymes, serum creatinine and serum amylase, fasting serum glucose, fasting serum cholesterol/ triglycerides, electrolytes, CPK, T-cell subsets and HIV RNA.

Where resources are limited, regular FBC, serum ALT and Createnine are the minimum requirements for safety monitoring (NACP, 2009). Toxicities associated with ARV may appear soon after the commencement of therapy (Kebba *et al.*, 2002). An NNTRI-induced rash or an abacavir hypersensitivity reaction may present within a few days (Kebba *et al.*, 2002). A clinically significant decline in haemoglobin, sufficient to require interruption of therapy, may occur within the first month of zidovudine therapy. Some toxicity appears in the medium term. Lip dystrophy and significant hyper-lipidaemia associated with PI therapy typically present following 6-18 months of therapy (Geretti, 2006). Adverse reactions to ARV are not always typical and can be unpredictable. Renal colic associated with indinavir and pancreatitis caused by ddI can occur at any time (Geretti, 2006).

2.6 Clinical Monitoring

Since laboratory monitoring cannot always predict the development of complications regular clinical evaluation to patient taking ARV is required to complement (NDHSA, 2004).

Probably the most common long-term side effects of combination ARV are NRTI-associated lipo-atrophy and PI-associated lipodystrophy and the related abnormalities in

serum lipids and glucose (Lichtenstein *et al.,* 2001). The exact aetiology of these metabolic complications remains uncertain. However, it is clear that NRTIs cause mitochondrial toxicity resulting in multiple end-organ damage (William *et al.,* 2003). The clinical picture includes peripheral fat loss, hepatic and pancreatic toxicity and peripheral neuropathy (Lichtenstein *et al.,* 2001). Treatment with protease inhibitors can result in a similar, but quite distinct syndrome, of fat redistribution and metabolic abnormalities (Lichtenstein *et al.,* 2001).

It is critical that patients be clinically assessed for the early development of these side effects, particularly the body composition changes (Steven *et al.,* 2003). In fact, the changes may be permanent in many patients, even if the drugs are stopped. While serum lipids and liver enzymes may help predict those patients at risk, the best method of monitoring these newly emerged toxicities is regular clinical review (WHO, 2006).

2 .7 Indications for Changing Therapy

Recommended ARV Drugs are triple therapy in any case, consisting of either two nucleotide reverse transcriptase inhibitors NRTI, and one non-nucleoside reverse transcriptase inhibitors NNRTI or two nucleotide reverse transcriptase inhibitors NRTI and one protease inhibitors PI or three nucleotide reverse transcriptase inhibitors NRTI's (NACP, 2009). Changing therapy may be necessary due to treatment failure, toxicity, and patient intolerance to the combination or inability of the patient to adhere to the treatment regimen (NACP, 2009). Clinical disease progression is a marker of treatment failure and necessitates a review of the patient's therapy (Geretti, 2006).

Virological treatment failure can be defined as a failure to achieve undetectable HIV-RNA or at least a 2 log10 decline in viral load from baseline, after a reasonable time on

therapy, typically 1-2 months (Steven *et al.,* 2000). A viral load rebound to detectable levels or a rebound of 0.5 log10 from the nadir also indicates failing therapy, or for adults, a viral load greater than 10,000 copies is proposed to reflect viral replication suggestive of treatment failure (NACP, 2009).

Immunological failure is less easily defined, as individual CD4 count responses to ARV are less predictable (NACP, 2009). The response is dependent on such variables as disease stage, prior ARV and the drugs taken. As stated above, a patient with a CD4 count of less than 200 Cells/ml is at significant risk of HIV disease progression and a failure to achieve this level indicates the need to review the therapy. A declining CD4 count over time is also a marker of treatment failure (NACP, 2009).

Decisions to change therapy can be straightforward or, require a thorough review of the patient's clinical status, immunological and virological markers, treatment history and available, useful drugs. The role of resistance assays in guiding therapy changes is still being developed (NACP, 2009).

2.8 Studies Done on Response to Antiretroviral Therapy

Studies done on different parts of the world have shown remarkable progression in the survival of HIV-infected patients treated with antiretroviral therapy (ART) in Senegal (Laurent *et al.,*2002), Nigeria (Idoko *et al.,* 2002), South Africa (Orrell *et al.,* 2003), and Uganda (Weidle *et al.,* 2002, Kebba *et al.,* 2002).

As free access to ART strategies is being scaling up in resource-limited settings, a primary concern is that the benefit of therapy should be optimized. Emerging resistance to non-nucleoside reverse-transcriptase inhibitors (NNRTIs) has been documented not

only in the United States (Grant *et al.,* 2002) and Europe (Porter *et al.,* 2001) but also it has been associated with single-dose nevirapine for prevention of vertical transmission of HIV in Uganda (Eshleman *et al.,* 2001) and Thailand (Jourdain *et al.,* 2004). Resistance to NNRTIs poses a serious threat to the sustained success of ART where nevirapine-based combination pills are commonly used.

Evaluation of small cohorts in Ivory Coast (Adje *et al.,* 2001), Zimbabwe (Kantor *et al.,* 2002), and Uganda (Weidle *et al.,* 2001) has demonstrated ARV treatment failure and drug resistance (Richard *et al.,* 2004) found a high prevalence of resistance 52% (n=50) in cross-sectional cohort study conducted in Uganda.

Few studies have determined risk factors for virological/immunological failure in persons receiving HAART, during routine visits to clinics in resource-limited settings to be poor adherence, tuberculosis diagnosed after ART initiation, sub therapeutic NNRTI concentrations, general clinical symptoms, and lower weight than at baseline and low ARV plasma concentrations (Weidle *et al.,* 2002, and Laurent *et al.,* 2005).

Previous study reported to be conducted in Tanzania, on ARV drug resistance, participants were treatment naive pregnant women, the study found HIV Drug Resistant prevalence to be very low < 5% (Somi *et al., 2008).* Dissimilar to the previous study, this study aimed at examining clinical and behavioural factors associated with failure of virological suppression to HIV-infected patients on ART in Dar-es-salaam Tanzania, by conducting a cross-section, observational study.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Site

The time of the study was November 2011–September 2012, the study was carried out at Pastoral Activities and Services for people with AIDS Dar-es-salaam Archdiocese (PASADA) Upendano CTC. An organization which serves more than 800,000 people every year through its Upendano Clinic and 15 diocesan health facilities located throughout Dar es Salaam capital city. Operating under the Roman Catholic Archdiocese of Dar es Salaam, PASADA targets the poorest of the poor, offering comprehensive care and support to all people living with AIDS, regardless of religious affiliation. The services offered includes voluntary counselling and testing; home-based care; educational psychological, social, and economic support to orphans and vulnerable children; diagnosis and treatment of AIDS and opportunistic infections and prevention of mother to child transmission (PMTCT) in its 15 sites.

3.2 Study Design

A one year cross-sectional, observational study was conducted, which included 75 patients conveniently identified and consented prior to be enrolled in the study. Out of all 75 identified participants, 3 dined to consent and 2 excluded due to exclusion criteria of the study. Each patient participated in a clinical evaluation and laboratory assessment.

3.3 Study Participants

Patients on ARV treatment for six months and above from different areas of Dar es salaam attending PASADA Care and Treatment Centre for people living with HIV.

3.4 Clinical Evaluation

For each participant a clinical evaluation report, including gender, age, WHO clinical staging, medical history data, diagnosis, prophylaxis, and treatment for opportunistic infections and an up-to-date antiretroviral history were obtained through patient CTC form number 2.

3.5 Laboratory Assessment

Blood samples for laboratory tests were collected from each patient, to include, Full blood cell count (FBC), CD4 lymphocyte count, alanine aminotransaminase (ALT), aspartate aminotransaminase level (AST), Serum Creatnine, Hepatitis B Virus (HBV), Hepatitis C Virus (HCV) and quantitative measurement of HIV-RNA load.

Baseline and at 6 months CD4 count values for each participant were obtained from CTC form number 2 and during the study period, the current CD4 count were done using BD FACCS COUNT Flow Cytometer Machine. Tri level Quality control samples were performed on daily basis prior to run batch of patient's samples. All patients found to have 50% drop in CD4 count from peak value within 6 months, or return to pre-ART baseline CD4 count or lower were regarded to have immunological failure.

Whole blood Samples for viral load were collected in EDTA vacutainer and transferred to MUHAS HAVARD Laboratory where plasma were separated from cells and stored at -80^0 C prior being tested using Amplicor Monitor standard assay, version 1.5 (Roche

Molecular Systems), with a detection limit of 400 copies/ml. Trilevel quality controls sample were included in every batch of 12 samples. In this study all patient tested and found having viral load greater than 10,000 copies were regarded as having viral replication reflecting of virological treatment failure.

For monitoring antiretroviral treatment safety Full blood cells count (FBC), serum creatinine, and Alanine amino-transifarase (ALT) were monitored. FBC were analyzed using EDTA Blood in Abbort Celldyn 3700 Haematological analyzer while serum creatinine and ALT were analyzed using Bio systems A15 Random Access Chemistry Analyzer. For FBC Trilevel Quality control samples were performed prior running patients' specimen, and for chemistry two level quality control samples were included in every batch of patients' specimens. HBV and HCV serology was qualitatively measured using SD Bioline immunochromatographic rapid test, known positive and negative samples tested using ELISA were used as quality control samples and included in every batch of patient specimen.

3.6 Sample Storage and Transportation Procedures

All samples needed to be stored were stored in tightly closed and labelled tubes and kept in an upright position in racks. Temperature requirement were monitored during specimen storage and records of all samples were kept. EDTA blood samples for HIV Viral Load were processed in 4hrs from collection time to obtain 2 aliquots of 1ml plasma. Then were transported to MUHAS/HAVARD Laboratory to be store at (-80^{o} C) and processed Adherence to safety precautions in the laboratory at all steps starting from specimen collection, storage, transportation and disposal of biohazard wastes were done to minimize risk of transmission of HIV, hepatitis B virus (HBV) and other blood-borne diseases agents to laboratory workers.

3.7 Ethical Consideration – Ethical Clearance

The ethical clearance and permission to conduct this study obtained from managements of SUA and further permission obtained from the management of PASADA CTC.

Informed written consent statement from patients was a mandatory to all willing participants. There were no Patients with mental problems or children under the legal age of consenting. Confidentiality to all observations and participant details were highly assured during and after research conducting period.

3.8 Statistical Data Analysis

Statistical Analyses were conducted using STATA version 12.0 (StataCorp, Texas, USA). Pearson's Chi-square test assessed associations between Virological failure or Immunological failure and other categorical variables such as CD4 Count, Haemoglobin Concentration Level and WHO staging. CD4 counts were divided into two groups, those started ART treatment with CD4 count, less than100 Cells/ml and the other group was those stated ART with CD4 count equal or greater than100 cells/mm^3. As an indicator for adverse effects and drugs toxicity, haemoglobin concentration levels were divided into four groups >10g/dL (Normal), 9.0-9.9 g/dL (Mild toxicity), 7.0-8.9 g/dL (Moderate toxicity) and <7.0 g/dL (Severe toxicity). Univariable and multivariable logistic regression analyses were performed with a stepwise-descending selection procedure to identify possible risk factors associated with virological failure or Immunological failure. All variables with P value less than 0.30 in univariable analysis were included in multivariable analysis. Adjusted Odds Ratios (AORs) and 95% confidence intervals were reported and statistical significance was determined at $P<0.05$

CHAPTER FOUR

4.O RESULTS AND DISCUSSION

4.1 Results

4.1.1 Demographic Characteristics

Overall, 70 HIV- infected patients were involved in this cross sectional study, out of them 76.8% (53/70) were female patients. The mean age was 44.1 years with the mean haemoglobin concentration level of 11.2 g/dL. Among 70 HIV- infected patients, 63.8% (44), 20.3% (14) and 15.9% (11) patients were reported to live in Temeke, Ilala and Kinondoni districts respectively (table 1). CD4 count was reported to increase over time from 142 Cells/ml mean CD4 at baseline to 262.5 Cells/ml mean CD4 after 6 months of ART initiation and 323.5 Cells/ml mean CD4 measured during the study (table 1). Most of the HIV- infected patients 48.6% (34) were reported to be in stage IV compared to 31.4% (22) patients who were in stage II, 18.6% (13) patients who were in stage III and 1.4% (1) patient who was in stage I (Table 1).

Of all study participants 92.9% (65/70) were reported to adhere to the ART treatment, while 22.9% (16/70) patients were reported to be on TB treatment, 52.9% (37/70) were reported to be on OIS prophylaxis and 2.9% (2/70) were reported to be infected with Hepatitis B Virus (Table 1).

Table 1: Baseline Characteristics of the Study Population (N=70)

Characteristics	**Measure**
Age (Year), Mean ± S.D	44.12 ± 10.39
Sex, Female % (n/N)	76.8% (53/70)
District, % (n/N)	
1. Temeke	63.8% (44/70)
2. Kinondoni	15.9% (11/70)
3. Ilala	20.3% (14/70)
WHO Stage, % (n/N)	
I	1.4% (1/70)
II	31.4% (22/70)
III	18.6% (13/70)
IV	48.6% (34/70)
Baseline CD4 Count (Cells/mm^3), Median [IQR]	142 [79-219]
After 6 months CD4 Count (Cells/mm^3), Median [IQR]	262.5 [171-366]
Recent CD4 Count (Cells/mm^3), Median [IQR]	323.5 [190-486]
Treatment Adherence, % (n/N)	92.9% (65/70)
Immunological Failure, % (n/N)	27.1% (19/70)
Virological Failure, % (n/N)	10% (7/70)
Viral Load, Median [IQR]	<400 [0-400]
Total suppressed viral load	78.57(55/70)
Partially suppressed viral load	10.43(8/70)
TB Treatment, % (n/N)	22.9% (16/70)
Haemoglobin Concentration Level (g/dL), Mean ± S.D	11.18 ± 1.98
OIS Prophylaxis, % (n/N)	52.9% (37/70)
Hepatitis B Virus, % (n/N)	2.9% (2/70)
Lymphocytes, Median [IQR]	2 [1.5-2.4]
ALT, Median [IQR]	15 [10-19]
Createnine, Median [IQR]	77.5 [70.3-85.1]

IQR=Inter Quartile Range; S.D=Standard Deviation

4.1.2 Prevalence of Virological and Immunological Failure for HIV- Infected Patients on ART Treatment

This study found 10% (7/70) HIV- infected patients on ART have virological failure, 57.1% (4/7) were male and 42.9% (3/7) female. Virological failure was observed to be higher, 57.1% (4/7) in HIV- infected patients at stage IV compared to 42.9% (3/7) on other stages.

On the other hand 27.1% (19/70) of HIV- infected patients were reported to have an immunological failure. Of whom 73.7% (14/19) were female and 26.3% (5/19) were male. In relation to WHO stage, 57.9% (11/19) HIV- infected patients with immunological failure were reported to be in stage IV, 31.6% (6/19) patients in stage II and 10.5% (2/19) be in stage III.

4.1.3 Risk Factors Associated with Virological Failure

In the univariate logistic regression analysis, HIV- infected patients with < 100 Cells/ml CD4 counts had 3.61 times the odds of having virological failure compared to patients with =/ > 100 Cells/ml CD4 counts P=0.11, CI 0.7-17.8. HIV- infected patients on ARV treatment for more than six months with mild drug toxicity symptoms (haemoglobin concentration level 9.0-9.9 g/dL) had significantly 13.0 times the odds of having virological failure compared to patients who did not show drugs adverse effects and toxicity symptoms (haemoglobin concentration level =/>10.0 g/dL) P<0.05 CI 1.4-118.2 (Table 2). By comparison, WHO stage did not significantly influence virological failure. HIV- infected patients in stage IV had 1.18 times the odds of having virological failure compared to patients in stage II P=0.83, CI 0.2-5.8. HIV- infected patients who adhered to the ART treatment were less significantly likely to have virological failure, they had

0.01 times the odds of having virological failure compared to those who did not adhere to the treatment P<0.05, CI 0.0-0.1 (Table 2).

In the multivariate logistic regression analysis, after adjusting for HIV Co infections like patients on ART treatment while taking ant-TB and those taking OIS prophylaxis, none of the possible risk factors was significantly associated with virological failure. HIV-infected patients with <100 Cells/ml CD4 counts had 7.23 times the odds of having virological failure compared to the patients with =/ > 100 Cells/ml CD4 counts p=0.1, CI 0.4-107 (Table 2). HIV- infected patients with haemoglobin concentration level between 9.0-9.9 g/dL had 5.77 times the odds of having virological failure compared to patients with >10.0 g/dL P=0.3, CI 0.1-206.5. HIV- infected patients who were in stage II had 7.36 times the odds of having virological failure compared to the those in stage IV P=0.17, CI 0.4-127.1 (Table 2).

HIV- infected patients who adhered to the ART treatment were less likely to have virological failure, they had 0.05 times the odds of having virological failure compared to those who did not adhere to the treatment P=0.1, CI 0.0-1.8 (Table 2).

Table 2: Risk factors Associated with Virological Failure

Variable	Prevalence %(n/N)	Univariate Analysis			Multivariate analysis		
		Odds Ratio	95% CI	P Value	Odds Ratio	95% CI	P Value
Baseline CD4 Count, Cells/mm^3							
< 100	19.1% (4/21)	3.61	0.73-17.82	0.115	7.23	0.4-107.0	0.15
>/= 100	6.1% (3/49)	1.00	-	-	1.00	-	-
Haemoglobin Categories, g/dL							
9.0-9.9	33.3% (2/6)	13.00	1.43-118.28	0.023	5.77	0.16-206.54	0.33
7.0-8.9	12.5% (1/8)	3.71	0.29-46.48	0.309	5.13	0.22-119.81	0.30
<7.0	100% (2/2)	-	-	-	-	-	-
WHO Stage							
I	0% (0/1)	-	-	-	-	-	-
II	13.6% (3/22)	1.18	0.24-5.89	0.836	7.36	0.43-127.15	0.17
III	0% (0/13)	-	-	-	-	-	-
IV	11.8% (4/34)	1.00	-	-	1.00	-	-
TB Treatment							
No	11.1% (6/54)	1.00	-	-	1.00	-	-
Yes	6.3% (1/16)	0.53	0.06-4.79	0.575	-	-	-
Treatment Adherence							
	80% (4/5)	1.00	-	-	1.00	-	-
Yes	4.6% (3/65)	0.01	0.001-0.14	<0.0001	0.05	0.002-1.82	0.104
OIS Prophylaxis							
No	6.1% (2/33)	1.00	-	-	1.00	-	-
Yes	13.5% (5/37)	2.42	0.44-13.43	0.311	-	-	-

4.1.3 Risk Factors Associated with Immunological Failure

HIV- infected patients with haemoglobin concentration level 9.0-9.9 g/dL had significantly 8.8 times the odds of having immunological failure to patients with haemoglobin concentration level =/>10.0 g/dL, P<0.05, CI 1.4-54.9. HIV- infected patients who adhered to the ART treatment were less significantly likely to have

immunological failure, they had 0.08 times the odds of having immunological failure compared to those who did not adhere to the treatment P <0.05, CI 0.0-0.7 (Table 3).

In the multivariate analysis, after adjusting for TB treatment, none of the possible risk factors was significantly related with immunological failure. HIV- infected patients <100 Cells/ml CD4 count had 2.59 times the odds of having immunological failure compared to those with >100 Cells/ml CD4 count P=0.22, CI 0.5-11.9. HIV- infected patients in stage II and III were less likely to have immunological failure, they respectively had 0.89 and 0.57 times the odds of having immunological failure compared to those patients in stage P=0.87, CI 0.1-4.0 and P=0.54, CI 0.0-3.5 respectively (Table 3).

Table 3: Risk Factors Associated with Immunological Failure

Variable	Prevalence %(n/N)	Univariate Analysis			Multivariate analysis		
		Odds Ratio	95% CI	P Value	Odds Ratio	95% CI	P Value
Baseline CD4 Count, Cells/mm^3							
=/< 100	23.8% (5/21)	0.78	0.24-2.54	0682	2.59	0.5-11.9	0.224
> 100	28.6% (14/49)	1.00	-	-	1.00	-	-
Haemoglobin Categories, g/dL							
>10.0	18.5% (10/54)	1.00	-	-	1.00	-	-
9.0-9.9	66.7% (4/6)	8.8	1.41-54.91	0.02	5.39	0.5-53.9	0.152
7.0-8.9	50% (4/8)	4.4	0.94-20.66	0.06	5.09	0.8-30.3	0.074
<7.0	50% (1/2)	4.4	0.25-76.49	0.30	$1.84e^{-7}$	-	0.994
WHO Stage							
I	0% (0/1)	-	-	-	-	-	-
II	27.3% (6/22)	0.78	0.24-2.56	0.68	0.89	0.19-4.05	0.877
III	15.4% (2/13)	0.38	0.07-2.02	0.25	0.57	0.09-3.5	0.544
IV	32.4% (11/34)	1.00	-	-	1.00	-	-
TB Treatment							
No	25.9% (14/54)	1.00	-	-	1.00	-	-
Yes	31.3% (5/16)	1.29	0.38-4.39	0.67	-	-	-
Treatment Adherence							
No	80% (4/5)	1.00	-	-	1.00	-	-
Yes	23.1% (15/65)	0.08	0.008-0.72	0.02	$4.12e^{-8}$	-	0.99
OIS Prophylaxis							
No	21.2% (7/33)		1.00	-	1.00	-	-
Yes	32.4% (12/37)	1.78	0.604-5.26	0.29	1.46	0.38-5.59	0.58

4.2 Discussion

Study found that, most of HIV patients on ART treatments experienced biological and clinical benefit. This mean that, the current treatment regime is working properly, and contributes on improving the quality of life for people living with HIV since majority of participants (78.57%) attained total viral suppression (<400 viral copies/ml). This situation also revealed on the study previously conducted in Tanzania which reported

very low prevalence < 5% of HIV Drug Resistant among treatment naive pregnant women (Somi *et al.,* 2008), and the prevalence of viral suppression seen in industrialized countries where HAART was first introduced (Deeks *et al.,* 1999).

Together with this convincing picture, the study observed a total of 20.43% patients having either virological failure (>10000 viral copies/ml) or partially suppressed viral road (>400 & <10000 viral copies/ml). This tells us that, one out of five of HIV patients under ART due to some reasons, their virus continue to replicate regardless of being on medication. Under the conditions that allow ongoing viral replication like this, drug-resistant mutants might get hold of a selective advantage over wild-type virus and become dominant within the quasispcies. These may become source of infecting others with the treatment resistant viral strains. Despite of the power and limitation of this study, these alarming result, tells us, if ART regime continue to be managed the same way, in a near future the clinical and biological benefits, that majority of HIV- infected patients are experiencing might wane out due to domination of resistant viral strains as revealed in Uganda where (Richard *et al.,* 2004) found a high prevalence of HIV drug resistance of 52% (n=50) among Ugandan.

A study also experienced that virological failure does not necessarily occurs in conjunction with immunological failure, since out of 19 patients found to have immunological failure (Table 1)., only 23.32% (5/19) had virological failure. This means that, there are some patients having immunological failure but they do experience biological and epidemiological benefits of ARV by having suppressed viral reproduction. This condition leaves an answered questions on accuracy and reliability of using immunological marker like CD4 count to monitoring treatment efficacy and define treatment failure, In view of the fact that it is not clear indicated for a patient present with

immunologic failure in the setting of virological suppression should prompt change in the ARV regimen or not. Study results demonstrate that poor treatment adherence, drug adverse effects and toxicity were associated with virological/immunological failure, same as other studies conducted in resource-limited settings (Laurent *et al.,* 2005, Macharia *et al.,* 2003, and Lively *et al.,* 2003) which observed treatment interruption caused by unreliable drug supply was a significant challenge, this study signify adherence to therapy as a necessary factor for viral suppression. In relation to WHO stages. Study observed that most of patients with immunological failure 57.9% were those started ARV at WHO stage IV, although the risk differences between WHO stages were not statistically proven to be significant. This became differently to other studies (Weidle *et al.,* 2002, and Laurent *et al.,* 2005) that signified risk factors for virological/immunological failure in persons receiving HAART, during routine visits to clinics in resource-limited settings to be tuberculosis diagnosed after ART initiation, sub therapeutic NNRTI concentrations, general clinical symptoms and lower weight than at baseline. This study took in consideration factors like TB, HBV and HCV co- infection (Table 2, 3) as well as other factors like time interval in ARVs, and gender difference but they were all not statistically significant.

On the other hand, the power of this study is not sufficient to generalize the study result to general population due to little number of participants, the assessment of adherence by this study was limited to information obtained from CTC form number 2, Haemoglobin levels were not measured longitudinally and study did not include clinic-based pill counts to assess adherence to treatment, therefore results may reflect reporting bias and underestimation of adherence in both suppressed and unsuppressed group.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

These Study findings observed most of HIV patients on ART treatments experienced biological and clinical benefit. The percentage of patient found to have either partially viral suppression or virological failure revealed in this study 20.4% is alarming. If this conditions that allow ongoing viral replication will be left un-intervened, in a near future, drug-resistant mutants may get hold of a selective advantage over wild-type virus and become dominant within the quasispcies. Study demonstrated that poor treatment adherence, and mild anaemia as indicator of drug adverse effects and toxicity being associated with both virological/immunological failures, also study experienced that virological failure does not necessarily occurs in conjunction with immunological failure.

5.2 Recommendations

- Early HIV Testing which can be achieved through strengthen VCT and PITC services and close monitoring for drug adverse effects and toxicity are needed on proper management of HIV infected/AIDS patients.
- Despite the additional cost, Human immunideficient viral load measurement, and HIV 1 drug resistance testing are of most important on monitoring ARV efficacy, having them available at affordable price to most of Tanzanians may benefit patients at risk for virological failure.
- Consequences of continuing providing regimen without reliable monitoring tool or prior knowing if the regimen is sensitive or not sensitive to particular patient must be considered, because with this alarming results over time, the clinical benefit will wane if drug resistance increases. Therefore prospective studies that

will develop low-cost methods to identify virological failure and ARV sensitivity are urgently needed.

- There is a need for further studying on treatment failures, factors associating with treatment failures and explore more on causes of poor treatment adherent while put all limitations of this study in consideration in order to make use of those result in intervention.

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