

**ASSESSMENT OF ADHERENCE TO ANTIRETROVIRAL
TREATMENT IN ORPHANED CHILDREN IN DAR ES SALAAM
TANZANIA**

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TREATMENT IN ORPHANED CHILDREN IN DAR ES SALAAM
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By

Dr. Nassoro A. Mopei, BVM

**A Dissertation Submitted in (Partial) Fulfillment of the Requirements for the
Degree of Master of Science (Clinical Pharmacology) of the
Muhimbili University of Health and Allied Sciences**

**Muhimbili University of Health and Allied Sciences
November, 2014**

CERTIFICATION

The undersigned certify that they have read and hereby recommend for acceptance by Muhimbili University of Health and Allied Sciences, a dissertation titled “*Assessment of Adherence to Antiretroviral Treatment in Orphaned Children in Dar es Salaam-Tanzania*”, presented in (partial) fulfillment of the requirements for the Degree of Master of Science (Clinical Pharmacology) of the Muhimbili University of Health and Allied Sciences.

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DECLARATION AND COPYRIGHT

I, **Dr. Nassoro Athumani Mopei** declare that this **dissertation** is my own original work and that it has not been presented to any other university for similar or any other degree award.

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DEDICATION

This work is dedicated to my father Mr. Athumani S. Mopei, my mother Hadija S. Mbullu and my friends; Dr. Sarah Hoyle and Eng. James Hoyle for their immense contribution to my education.

ABSTRACT

The study was conducted at pediatric HIV clinics of Dar es Salaam Municipal Hospitals between June, 2013 and September, 2013 to determine ART adherence levels and factors influencing adherence to ART among HIV-infected orphan children.

A total of 216 HIV-infected orphan children aged between 2 and 14 years receiving ART and their caretakers were recruited. Data were collected using questionnaire and review of patient record forms (CTC2). On the other hand blood samples were collected for determination of nevirapine plasma levels by High Performance Liquid Chromatography (HPLC). Data analysis was performed using SPSS version 20 software.

The ART adherent proportions established were 79.6% by caretaker self report, 82.9% by clinic attendance consistency and 72.2% by nevirapine plasma levels determination. It was observed that 72% of HIV-infected patients on ART had increased CD4 counts whereas 28% of patients had blunted CD4 count responses. It was revealed that 25.5% of interviewed research subjects are not familiar with major means of HIV transmission. Forgetfulness was cited by 90.7% of respondents to be one of challenges facing caretakers in taking orphan child for drug refill. An estimated 75.5% of study population reported to spend more than two hours from arriving at clinic to getting ARVs.

It was concluded that significant proportions of HIV-infected orphans on ART attending CTCs in Dar es Salaam have inadequate adherence and inappropriate CD4 response. Inadequate HIV/AIDS knowledge, unnecessary long waiting time and forgetfulness were identified to impair ART adherence of orphans. The study recommended more attention should be paid on ART adherence in HIV-infected orphan children including initiation of interventions to promote adherence in this pediatric category.

TABLE OF CONTENTS

CERTIFICATION	ii
DECLARATION AND COPYRIGHT	iii
ACKNOWLEDGEMENT	iv
ABSTRACT	vi
TABLE OF CONTENTS	vii
LIST OF TABLES	x
LIST OF FIGURES	xii
LIST OF ACRONYMS	xiii
DEFINITION OF TERMS	xiv
1. INTRODUCTION	1
2. LITERATURE REVIEW	4
2.1 Antiretroviral Therapy (ART)	4
2.2 ART Adherence	12
2.3 ART Adherence Measurements	16
2.4 Core Indicators for Assessment of Adherence at Health Facilities	23
3. STATEMENT OF THE RESEARCH PROBLEM.....	26
4. STUDY JUSTIFICATION.....	28
5. RESEARCH QUESTIONS	29
6. RESEARCH OBJECTIVES.....	29
6.1 Broad Objective	29
6.2 Specific Objectives	29
7. HYPOTHESIS.....	30

7.1 Null hypothesis	30
7.2 Alternative hypothesis	30
8. RESEARCH METHODOLOGY	31
8.1 Description of the study area	31
8.2 Study population	31
8.3 Sampling procedure and sample size	31
8.4 Study design.....	33
8.5 Study setting	33
8.6 Ethical Considerations	41
8.7 Pre-testing of the data collection methods	41
8.8 Data Collection	41
8.9 Statistical Data analysis	42
9. RESULTS	43
9.1 Description of study population.....	43
9.2 ART adherence in orphans.....	48
9.3 Findings related to the challenges on ART in orphans	61
9.4 Investigation into knowledge regarding HIV/AIDS among caregivers of orphans.....	62
10. DISCUSSION.....	64
11. LIMITATIONS OF THE STUDY	75
12. CONCLUSION	76
13. RECOMMENDATIONS	77
REFERENCES	79
LIST OF APPENDICES	102
Appendix I: Approval of Ethical Clearance	102

Appendix IIa: Consent Form- English Version	103
Appendix IIb: Consent Form- Kiswahili Version.....	105
Appendix IIIa: Questionnaire to Caretaker for Assessment of ART Adherence in Orphans (English Version)	108
Appendix IIIb: Dodoso kwa Mzazi/Mlezi wa Mtoto Yatima kwa Ajili ya Kutathmini Matumizi Thabiti ya Dawa za Kukata Makali ya Virusi Vya Ukimwi (ARVs).....	111
Appendix IVa: Patient Record Review Form (English Version)	114
Appendix Va: CTC2 (Front Pages)	118
Appendix Vb: CTC2 (Back Pages).....	119

LIST OF TABLES

Table 1:	HIV-infected children receiving ARVs in Dar es Salaam Municipal Hospital CTCs as per 20 th February, 2013	31
Table 2:	The recruited research subjects	43
Table 3:	Orphan age grouping	44
Table 4:	Caretaker age grouping	44
Table 5:	Sociodemographic characteristics of the caretakers (N=216)	46
Table 6:	Characteristics of HIV-infected children (N=216)	47
Table 7:	Adherence measures by different approaches	48
Table 8:	The association between nevirapine plasma concentrations and other adherence measures	49
Table 9:	Univariate logistic regression for association between nevirapine plasma concentrations and other adherence measures	50
Table 10:	Multivariate logistic regression for association between nevirapine plasma concentrations and other adherence measures	50
Table 11:	Agreement between nevirapine plasma concentration versus caretaker report, clinic attendance consistency and clinician estimated adherence	51
Table 12:	Performance of caretaker report and clinic attendance consistency as compared with nevirapine plasma concentration	52
Table 13:	Trends in CD4 counts (N=164)	53
Table 14:	Association between Changes in CD4 counts and Initial CD4 counts (N=164).....	54

Table 15:	Association between changes in CD4 counts and duration of ART (N=164)	54
Table 16:	ART adherence measures and its association with change in CD4 counts (N=164).....	55
Table 17:	The association between caregivers' and patients' demographic/clinical characteristics and nevirapine plasma concentrations	57
Table 18:	Univariate logistic regression for caregivers' and patients' demographic/clinical characteristics associated with nevirapine plasma concentrations	58
Table 19:	Multivariate binary logistic regression for caregivers' and patients' demographic/clinical characteristics associated with nevirapine plasma concentrations.....	60
Table 20:	Proportions of respondents facing challenges in care of orphans (N=216)	61
Table 21:	Proportions of respondents facing challenges in care of orphans (N=216)	63

LIST OF FIGURES

Figure 1:	Structural formula of nevirapine	5
Figure 2:	A flow scheme for HPLC	10
Figure 3:	A Chromatogram of blank plasma obtained after analysis of plasma sample collected from a volunteer	36
Figure 4:	A Chromatogram of Internal Standard (IS)	36
Figure 5:	A Chromatogram obtained during validation in day 2 from C6.	37
Figure 6:	Chromatogram showing peaks of nevirapine and carbamazepine and their retention times from plasma of one HIV infected child (P1) undergoing ART	38
Figure 7:	ART levels by different adherence assessment methods.....	48
Figure 8:	Trends in CD4 counts	53

LIST OF ACRONYMS

AIDS	Acquired Immunodeficiency Syndrome
ART	Antiretroviral Therapy
ARVs	Antiretroviral drugs
CTC	Care and Treatment Centres
DMO	District Medical Officer
HIV	Human immunodeficiency virus
IAA	Initiative on Antiretroviral Adherence
INRUD	International Network for Rational Use of Drugs
IS	Internal Standard
MoHSW	Ministry of Health and Social Welfare
MUHAS	Muhimbili University of Health and Allied Sciences
NACP	National Control Aids Programme
NVP	Nevirapine
OR	Odds ratio
PI	Principal Investigator
PLH	People Living with HIV/AIDS
rpm	Rotations per minute
RAs	Research Assistants
SIDA	Swedish International Development Cooperation Agency
TACAIDS	Tanzania Commission for AIDS
TDM	Therapeutic Drug Monitoring
Tshs	Tanzanian shillings
UNICEF	United Nations Children's Fund
UNAIDS	United Nations Programme on HIV/AIDS
VVU	Virusi Vya Ukimwi
WHO	World Health Organization

DEFINITION OF TERMS

ADHERENCE	Adherence to a medication regimen is generally defined as the extent to which patients take medications as prescribed by their health care providers.
AIDS ORPHAN	AIDS orphan refers specifically to that category of children who has at least one parent dead from AIDS (UNAIDS, 2010).
AIDS STIGMA	AIDS stigma refers to socially shared perceptions about the devalued status of people living with HIV/AIDS. The stigmatized individual is seen to be a person who possesses “an undesirable difference” which then leads to social devaluation and discrimination. In this case, there is “spoiled identity” for the person concerned (Goffman, 1963).
AN ORPHAN	An orphan is defined by the United Nations as a child under the age of 18 years who has lost one or both parents; maternal and paternal orphans have respectively lost their mother and father only, and double orphans have lost both parents.
ANTIRETROVIRAL DRUGS	Drugs that inhibit replication of HIV.
CARETAKER	A person who lives with the child and participates in the child’s daily care and takes the responsibility in giving the child medication and bring the child to clinic.

NON-ADHERENCE

Intentional nonadherence is an active process whereby the patient chooses to deviate from the treatment regimen. This may be a rational decision process in which the individual weighs the risk and benefits of treatment against any adverse effects (Lowry *et al.*, 2005).

Unintentional nonadherence is a passive process in which the patient may be careless or forgetful about adhering to the treatment regimen. This is also referred to as the execution of the prescribed regimen (Vrijens *et al.*, 2008).

1. INTRODUCTION

Human immunodeficiency virus (HIV) infection and Acquired Immunodeficiency Syndrome (AIDS) continues to be a serious worldwide public health problem. HIV/AIDS epidemic affects entire population both adults and children resulting in unique social and economic consequences. One of the worst consequences of AIDS deaths is an increase in the number of orphans (UNAIDS, 2010). It is estimated that more than 16 million children under 18 have been orphaned by AIDS worldwide. Around 14.8 million of these children live in sub-Saharan Africa. In Tanzania, there are about 1 million children without parents due to HIV and AIDS; of that number about 40,000 are infected with HIV (Mmbando *et al.*, 2009; TACAIDS, 2009).

HIV infection among orphaned children constitutes an important issue in Africa. According to previous studies, orphans aged under 15 who have lost one or both parents constitute an especially vulnerable group of HIV positive children. In most resource-limited settings, the death of one or two parents has much greater effect on the caregiving situation (Mellins *et al.*, 2004). Due to lack of social services in most resource-limited settings, HIV-infected orphaned children are often taken care of by different adult relatives, making it difficult to provide consistent, ongoing support (Nyandiko *et al.*, 2006).

To reduce children's HIV/AIDS related mortality and progression, early ART initiation and high levels of ART adherence are particularly critical. Sustaining good adherence to ART in children is difficult as it is influenced by factors such as child behaviour, parental care, abuse, poverty, poor access to health services, HIV status of caregivers, their attitudes and beliefs (Paterson, 2000; Powell, 2007).

The orphan crisis due to HIV/AIDS sets in numerous challenges. The rising numbers of children who have lost one or both parents are threatening traditional systems of care. These challenges have an influence on orphans who are on ART to adhere to their treatment (UNICEF, 2006; Van Dky, 2005). Compared with non-orphans, orphans may be denied access to healthcare, more likely to be diagnosed as WHO Clinical Stage 4 (most severe HIV infection stage) prior to ART initiation than non-orphans (Strode and Grant, 2001; Vreeman *et*

al., 2009). Evidence shows that orphans living with extended families or in foster care are frequently subject to discrimination and often stigmatized by society through association with AIDS. HIV-infected orphans face particular adherence challenges related to stigma, family structure, access to treatment, and resources (Haberer and Mellins, 2009). They also face numerous stressors potentially impeding their cooperation with treatment which present significant barriers to maintaining 100% ART adherence (Mellins *et al.*, 2003; Vreeman *et al.*, 2008). This suggests that the orphaned children are at particular risk (Ntanda *et al.*, 2009).

ART adherence matters in HIV-infected orphans are more complicated and matter of great concern than in the case of other categories of children; periodic assessments and reinforcements are necessary. This group needs more care and attention to promote their adherence to ART than do patients who have less difficulty with adherence, a more forgiving medication regimen, or both (Farris *et al.*, 2004; Weingarten *et al.*, 2002).

Accurate and reliable measures of adherence and better understanding of both barriers and facilitators of adherence are needed to maintain the ART benefits, reduce the risks associated with poor adherence, to help clinicians identify patients who need assistance with their medication taking, to design and evaluate effective interventions to enhance adherence, and to interpret the role of adherence in evaluating clinical outcomes and making treatment decisions. Decisions to change recommendations, medications, and/or communication style to promote adherence depend on valid and reliable measurement of adherence (Farley *et al.*, 2003; Wagner, 2004; WHO, 2006).

The ability to fully understand ART adherence has been hampered by limitations in the measurement tools. Several methods allow adherence assessment to medication; however, nearly all methods are associated with known limitations and thus fail to capture the important dimensions of adherence (Berg and Arnsten, 2006; Gill *et al.*, 2005). Adherence measurements in HIV patients in recent research have suggested that use of multiple measures will be of greater benefit than continue search for single defining adherence measure (Chesney, 2000). This has led to the recommendation of a multi-method approach that combines several methods as the current state-of-the-art in measurement of adherence

behavior (WHO, 2003). The assessment of ART adherence by several adherence-measuring methods enables comparison of the information provided by these various methods which provides opportunity to fully understand ART adherence practices in HIV-infected orphans.

This study was designed to identify the levels of ART adherence among HIV-infected orphans attending CTC in Dar es Salaam by multi-method approach involving caregiver 3-day recall, consistency of clinic attendance and nevirapine plasma levels determination. Furthermore, the study investigated factors influencing adherence to antiretroviral therapy among orphans.

2. LITERATURE REVIEW

2.1 Antiretroviral Therapy (ART)

2.1.1 Overview of Antiretroviral Therapy

ART is treatment of people infected with human immunodeficiency virus (HIV) using anti-HIV drugs. The introduction of powerful new antiretroviral therapies in the late 1990s led to significant improvements in the health and longevity of patients with HIV infection. ART use in HIV-infected patients leads to reduced plasma HIV RNA levels, increased CD4 cell counts, decreased incidence of opportunistic infections, improved growth and development, and decreased morbidity and mortality (Burns and Mofenson, 1999; de Martino *et al.*, 2000).

In resource-rich countries, the prognosis of paediatric HIV infection has undergone a dramatic transformation since the availability of antiretroviral treatment particularly with the advent of highly active antiretroviral therapy (HAART) regimens (Gaur, 2008). Antiretroviral therapy for HIV infection in children has dramatically improved their survival and quality of life (Paterson, 2000).

The currently existing and commercially available antiretroviral drugs in Tanzania fall into the following four main categories:

- Nucleoside reverse transcriptase inhibitors (NRTIs); Zidovudine (AZT), Lamivudine (3TC), Abacavir (ABC), Emtricitabine (FTC), Stavudine (d4T).
- Non-nucleoside reverse transcriptase inhibitors (NNRTIs); Nevirapine (NVP), Efavirenz (EFV).
- Nucleotide reverse transcriptase inhibitors (Nucleotide analogues); Tenofovir (TDF).
- Protease inhibitors (PIs); Lopinavir (LPV), Ritonavir, Atazanavir (ATV).

Prior to the advent of combination antiretroviral regimens, many HIV-infected patients were on monotherapy, especially regimens of zidovudine (ZDV). The standard treatment consists of a combination of at least three drugs (often called highly active antiretroviral therapy or HAART) that suppress HIV replication (Muma *et al.*, 1995). The recommended triple therapy

consist of 2 NRTI + 1 NNRTI, 2 NRTI + 1 PI, 3 NRTI's or 2NRTIs + 2PIs if boosted regimen is available. Three drugs are used in order to reduce the likelihood of the virus developing resistance. The use of monotherapy in the treatment of HIV infection is prohibited. It is important to remember that there is no single combination that is best for every patient and/or that can be tolerated by all patients. Practitioners should recommend regimens on the basis of a patient's clinical condition, lifestyle and ability to tolerate the regimen.

Recommended First-Line ARV Regimens in Infants and Children: Zidovudine (AZT), Lamivudine (3TC), Nevirapine (NVP), Abacavir (ABC), Efavirenz (EFV), Stavudine (d4T), and Lopinavir/r. Available paediatric Fixed Dose Combination (FDC): AZT+3TC+NVP; NVP+d4T+3TC; 3TC+AZT

2.1.2 Pharmacological profile of nevirapine

Nevirapine is a dipyridodiazepinone and has two chemical names, 11-cyclopropyl-5,11-dihydro-4-methyl-6H-dipyrido-[3,2-b: 2',3'-e] [1,4]diazepin-6-one and 5,11-dihydro-6H-11-cyclopropyl-4-methyl-dipyrido-[3,2-b: 2',3'-e] [1,4] diazepin-6-one. The molecular formula is $C_{15}H_{14}N_4O$. Its structural formula is shown below in figure 1



Figure 1: Structural formula of nevirapine

Nevirapine is the prototypic member of a class of antiretroviral compounds referred to as nonnucleoside reverse transcriptase inhibitors. In sub-Saharan Africa, India, Southeast Asia, and other resource-poor regions of the world, the nevirapine -containing regimens are often preferable to others for HIV treatment and prevention because of high efficacy, its relative safety, long half-life, and low cost.

It is a potent and selective noncompetitive inhibitor of the reverse transcriptase enzyme, an important therapeutic target for the treatment of HIV-1 infection. Nevirapine inhibits replication of HIV-1 by interfering with viral RNA-directed DNA polymerase (reverse transcriptase). It binds directly to herodimeric HIV-1 reverse transcriptase and exerts a virustatic effect by acting as a specific, noncompetitive HIV-1 reverse transcriptase inhibitor; it appears to inhibit viral RNA and DNA-dependent DNA polymerase activities by disrupting the catalytic site of the enzyme

The therapeutic range for nevirapine plasma trough concentration is considered to be between 3000 µg/L (C_{\min}) and 8,000 µg/L (C_{\max}) (La Porte *et al.*, 2006). In completed clinical studies, nevirapine has demonstrated antiretroviral activity both as monotherapy and in combination with nucleoside analogues. When administered with zidovudine or the combination zidovudine/didanosine, the antiviral effect has been profound and sustained. Nevirapine does not appear to harm pregnant women therefore it is considered the safest NNRTI for use by pregnant women in the first three months of pregnancy. It is also used to prevent transmission of HIV from a pregnant woman to her new child (Stringer *et al.*, 2003).

Nevirapine is readily absorbed (> 90%) after oral administration in healthy volunteers and in adults with HIV-1 infection with a peak plasma concentration at 4 hr. It is widely distributed throughout body tissues including the central nervous system. The concentration of the drug in the CNS is 45% of that in plasma. It crosses the placenta and has been detected in breast milk, and is active in the adult at an oral dose of 200 mg administered twice daily after a two week lead-in dose of 200 mg per day. Nevirapine pharmacokinetics exhibit marked interpatient variability that can be explained in part by genetic differences, drug-drug interactions, pregnancy and ethnic factors (de Vries-Sluijs *et al.*, 2003; Veldkamp *et al.*, 2001).

Despite different chemical structures and pharmacokinetics, NNRTIs share a similar metabolism, and both efavirenz and nevirapine are converted to inactive metabolites by cytochrome P450 (CYP) enzyme systems, primarily by CYP2B6 with lesser involvement of CYP3A4. The metabolites 2- and 3-hydroxyNVP are primarily formed by CYP3A4 and

CYP2B6 respectively. Drug transporters expressed in key target tissues may have a role in drug intake and efflux, but little is known about the transporters that influence the disposition of nevirapine. However, the multidrug resistance protein 7, encoded by the adenosine triphosphate-binding cassette gene ABCC10, has been implicated in the efflux process of the metabolites. Greater than 80% of the nevirapine is excreted in urine is in the form of glucuronide conjugates of hydroxylated metabolites (Moffat *et al.*, 2005; Riska *et al.*, 1999; Ward *et al.*, 2003). There are no significant drug-drug interactions noted with the nucleoside reverse transcriptase inhibitors. Other commonly used drugs, such as ketoconazole, dapsone, rifampin, rifabutin and trimethoprim-sulfamethoxazole, appear not to be significantly affected. Resistance to nevirapine is rapid when administered as a monotherapy but this is altered and made less clinically relevant when nevirapine is given in combination with one or more of the nucleosides. Nevirapine has a safety profile that does not overlap with other antiretroviral therapies, the most common treatment-limiting reaction being rash.

The CYP2B6 and CYP3A4 genes are highly polymorphic and are subject to pronounced interindividual variability in expression and activity. The polymorphisms associated with an altered expression of these enzymes may theoretically have an influence on nevirapine pharmacokinetics and patient response (Lang *et al.*, 2001; Relling, 1989; Whirl-Carrillo *et al.*, 2012). The clinical impact of the CYP2B6 and CYP3A4 polymorphism on NVP pharmacokinetics has been described in various settings; the study in South Africa explored associations between CYP2B6, CYP3A, and ABCB1 polymorphisms and nevirapine hepatotoxicity, showed that the T allele in the ABCB1 gene at position 3435 was significantly related to decreased risk of liver toxicity from nevirapine (Haas *et al.* 2006). In Thailand, it has been reported that CYP2B6-G516T polymorphism significantly affected the drug metabolism of efavirenz in HIV-infected Thai children, while its impact on nevirapine concentrations was less pronounced after intra-partum single-dose nevirapine in HIV-infected mothers (Saitoh *et al.*, 2007). Rotger *et al.* (2005) found a significant association between the CYP2B6 516 T/T genotype and greater plasma exposure of nevirapine.

2.1.3 Therapeutic drug monitoring (TDM) of nevirapine

The therapeutic drug monitoring (TDM) approach for the optimization and individualization of drug administration is of huge interest in the treatment of HIV because it seems able to increase the efficiency of treatment and possibly, lower the adverse effects of antiretrovirals. TDM of ARVs aims to improve ART efficacy and safety by maintaining individual patients ARV plasma concentrations within a therapeutic range. It can potentially identify patients with sub-therapeutic, toxic or appropriate drug concentrations. ARV TDM is therefore potentially a rational tool to optimise efficacy and minimise toxicity of ARV therapy (Csajka *et al.*, 2003; de Vries-Sluijs *et al.*, 2003; Duong *et al.*, 2004).

Long-term virologic suppression of HIV has been associated with maintenance of efficacy plasma trough concentrations above 3000 ng/ mL for nevirapine (C_{12h}) in adult and pediatric patients with HIV infection. Higher nevirapine plasma trough concentrations (>4300 ng/ml) have been reported to be associated with reduced nevirapine resistance compared to the range of 3000-4300 ng/ml concentrations in adults, but the recommended target remains 3000 ng/ml for nevirapine due to the concern for potential toxicity and sustained virologic suppression at a lower target in other studies. A number of studies exploring the association of exposure to nevirapine with virologic response yielded a target trough concentration of 3.0 mg/L. For therapeutic drug monitoring (TDM) of nevirapine a trough plasma concentration >3.0 mg/L is suggested (Gonzalez de Requena *et al.*, 2005).

Due to plasma levels variation over a time within a patient and also due to high risks of developing drug resistance, there is a need of continuous monitoring the drug kinetics in patients undergoing NVP based HAART for the purpose of ensuring that the drug levels are maintained within therapeutic plasma concentration ranges. Resistance to NNRTIs develops very quickly especially when sub therapeutic drug levels are maintained at the site of action for a long time (Delaugerre *et al.*, 2001). The pharmacological characteristics of nevirapine make it an attractive candidate for therapeutic drug monitoring. Nevirapine is suitable for TDM for several reasons, including considerable interpatient variability in concentrations among patients who take the same dose and data indicating relationships between the

concentration of drug in the body, the anti-HIV effect and in some cases, toxicity (de Vries-Sluijs *et al.*, 2003; Duong *et al.*, 2005; Veldkamp *et al.*, 2001).

2.1.4 Plasma Drug Concentration Determination by High-Performance Liquid Chromatography (HPLC)

The development of HPLC methods for the determination of drugs has received considerable attention in recent years (Ahuja and Dong, 2005; McMaster, 2007). HPLC is a powerful tool in analysis; it is a chromatographic technique used to separate the components in a mixture, to identify and quantify each component. HPLC is basically a highly improved form of column chromatography. Instead of a solvent being allowed to drip through a column under gravity, it is forced through under high pressures of up to 400 atmospheres. That makes it much faster. It also allows you to use a very much smaller particle size for the column packing material which gives a much greater surface area for interactions between the stationary phase and the molecules flowing past it. This allows a much better separation of the components of the mixture.

The schematic of an HPLC instrument typically includes a sampler, pumps, and a detector. The sampler brings the sample mixture into the mobile phase stream which carries it into the column. The pumps deliver the desired flow and composition of the mobile phase through the column. The detector generates a signal proportional to the amount of sample component emerging from the column, hence allowing for quantitative analysis of the sample components (figure 2).

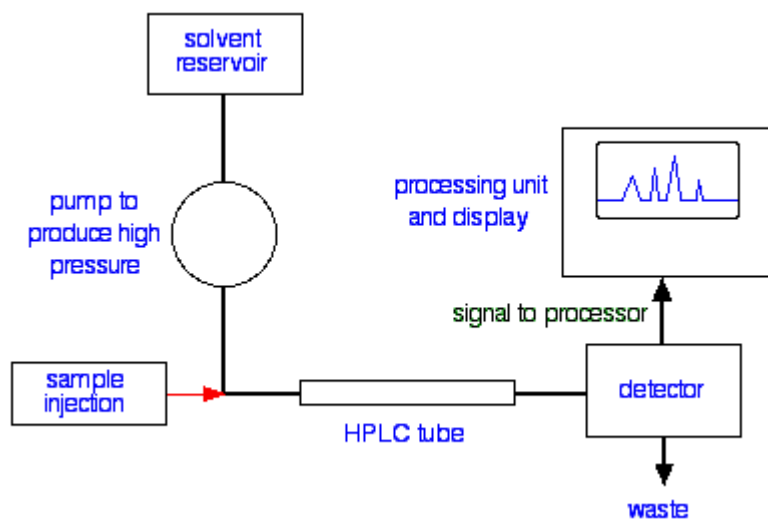


Figure 2: A flow scheme for HPLC

Normal-phase chromatography was one of the first kinds of HPLC that chemists developed. Also known as normal-phase HPLC (NP-HPLC) this method separates analytes based on their affinity for a polar stationary surface such as silica, hence it is based on analyte ability to engage in polar interactions (such as hydrogen-bonding or dipole-dipole type of interactions) with the sorbent surface. NP-HPLC uses a non-polar, non-aqueous mobile phase (e.g. Chloroform), and works effectively for separating analytes readily soluble in non-polar solvents.

The term reversed-phase describes the chromatography mode that is just the opposite of normal phase, namely the use of a polar mobile phase and a non-polar (hydrophobic) stationary phase. In this case, the column size is the same, but the silica is modified to make it non-polar by attaching long hydrocarbon chains to its surface typically with either 8 or 18 carbon atoms in them. A polar solvent is used - for example, a mixture of water and an alcohol such as methanol. RP-HPLC operates on the principle of hydrophobic interactions, which originates from the high symmetry in the dipolar water structure and plays the most important role in all processes in life science. RP-HPLC allows the measurement of these interactive forces. The binding of the analyte to the stationary phase is proportional to the contact surface area around the non-polar segment of the analyte molecule upon association with the ligand on

the stationary phase. This solvophobic effect is dominated by the force of water for "cavity-reduction" around the analyte and the C₁₈-chain versus the complex of both. Reversed phase HPLC is the most commonly used form of HPLC.

HPLC relies on pumps, a high-pressure pump (solvent delivery system or solvent manager) is used to generate and meter a specified flow rate of mobile phase, typically milliliters per minute. An injector (sample manager or autosampler) is able to introduce (inject) the sample mixture to be separated and analyzed is introduced, in a discrete small volume (typically microliters) into the continuously flowing mobile phase stream that carries the sample into the HPLC column. The column contains the chromatographic packing material needed to effect the separation. This packing material is called the stationary phase because it is held in place by the column hardware. The components of the sample move through the column at different velocities, which are function of specific physical interactions with the sorbent (also called stationary phase). The velocity of each component depends on its chemical nature, on the nature of the stationary phase (column) and on the composition of the mobile phase. A detector is needed to see the separated compound bands as they elute from the HPLC column (most compounds have no color, so we cannot see them with our eyes). The mobile phase exits the detector and can be sent to waste, or collected, as desired (Ahuja and Rasmussen, 2007; Snyder *et al.*, 2009)

2.2 ART Adherence

2.2.1 Background information on ART Adherence

Adherence is an old issue in health care but an emerging one in the light of the new therapy options in HIV management (Haynes *et al.*, 1996; Ley, 1997). Drug adherence is a key part of highly active antiretroviral therapy (HAART) (Chesney *et al.*, 2000).

Adherence to a medication regimen is generally defined as the extent to which patients take medications as prescribed by their health care providers. It refers to the whole process from choosing, starting, managing to maintaining a given therapeutic medication regimen to control HIV viral replication and improve function of the immune system. Non-adherence is the discontinuity or cessation of part or all of the treatment such as dose missing, underdosing, or overdosing, and drug holidays (Osterberg and Blaschke, 2005).

Rates of adherence for individual patients are usually reported as the percentage of the prescribed doses of the medication actually taken by the patient over a specified period.

Drug adherence level is calculated by the following formula:

$$\frac{\text{Total number of drugs taken}}{\text{Total number of drugs prescribed}} \times 100\%$$

There is no consensual standard for what constitutes adequate adherence. Some trials consider rates of greater than 80 percent to be acceptable, whereas others consider rates of greater than 95 percent to be mandatory for adequate adherence, particularly among patients with serious conditions such as infection with HIV (Pullar *et al.*, 1989; Spilker, 1991). In the treatment of patients with HIV infection or AIDS, it is essential to achieve more than 95 percent adherence to highly active antiretroviral therapy (HAART) in order to suppress viral replication and avoid the emergence of resistance (Osterberg and Blaschke, 2005). Numerous studies have shown that an even higher level of adherence (e.g. >95%) is necessary for durable suppression of HIV-1 virus load. However, the cutoff depends on the measure that was used (Arnsten *et al.*, 2001; Miller and Hay, 2000).

Adherence to ART regimen is a prerequisite for the efficacy and durability of any antiretroviral therapy regimens (Harries *et al.*, 2004; Munakata *et al.*, 2006). Studies from both resource-rich and resource-limited settings have repeatedly demonstrated that high levels of ART adherence are associated with better immunological and virological outcomes, decreased risk of developing an AIDS-defining illness, and improved survival (Abaasa *et al.*, 2000; Glass *et al.* 2006).

Treatment interruptions, even when structured and monitored clinically, lead to significant adverse events, including an increased risk of morbidity and mortality (El-Sadr *et al.*, 2006). Nonadherence to HAART is a major public health concern because it leads to virologic, immunologic, clinical and treatment failure, resistance and cross resistance and the transmission of resistant viruses to other patients, thus limiting future therapeutic options, both for the individual and the community (Williams and Friedland, 1997).

2.2.2 ART Adherence behavior in children

Pediatric ART adherence is complex and current levels are often suboptimal (Mellins *et al.*, 2004). Parental, familial, or social determinants of the child's context may play important roles in mediating ART adherence. Factors associated with adherence are age, sex, caregiver type, income, disclosure to child, caregiver-child communication, caregiver health belief, depression, stress, stigma and forgetfulness (Quitter *et al.*, 2000). Pediatric adherence to ART is a dynamic process involving many factors, which may be categorized as characteristics of the child, the caregiver(s) and family, the regimen and society and culture. Family support and the caregiver's perception and attitude towards HIV and AIDS can have an influence on the quality of care for pediatric patients who are on ART and consequently on their adherence to this treatment. These factors make ART adherence behavior in children complex (Giacomet *et al.*, 2003; Haberer and Mellins, 2009). Many HIV-infected children live in poverty and face food scarcity, both of which have been associated with poor adherence in developing settings (Cupsa *et al.*, 2000; Fassinou *et al.* 2004).

Developing countries are confronting different challenges for achieving and maintaining ART adherence as they scale-up pediatric ART programs. The obstacles facing pediatric antiretroviral therapy (ART) delivery in resource-limited countries include lack of health care infrastructure, limited availability of pediatric drug formulations, lack of early HIV diagnostic and monitoring techniques, limited manpower with expertise in pediatric HIV care, limited donor funding, and competing public health priorities with limited health care budget (Paintsil, 2011).

The stigma associated with AIDS and HIV infection has long been recognized as a significant barrier in the worldwide fight against HIV/AIDS. Gaps in knowledge and lack of in-depth information about HIV/AIDS fuel the fear of causal transmission, leading to stigmatizing action to avoid persons with HIV/AIDS (Mann *et al.*, 1992). Across cultures, AIDS-related stigma and discrimination have serious individual and public health ramifications; these include a reluctance to be tested for HIV, deter infected individuals from seeking medical treatment for HIV-related problems in local health care facilities or in a timely fashion and to disclose positive test results to partners, as well as poor treatment adherence (Bharat *et al.*, 2001; Greeff *et al.*, 2008). Children are more vulnerable to HIV/AIDS-related stigma and discrimination and this may reduce access to treatment (Brackis-Cott *et al.*, 2003). Stigma can affect children directly when it leads to active discrimination, or status loss. It can also affect children indirectly when caregivers suffer the effects of stigma and discrimination, or when children or parents take certain courses of action to avoid expected stigma or discrimination (Brackis-Cott *et al.*, 2003).

Adherence in children may even pose greater challenges especially if children do not have proper care. Given the influential role that caregivers play in linking People Living with HIV/AIDS (PLH) with medical care and support services it seems likely that they are also actively involved in facilitating ART treatment regimens. Maintaining high levels of adherence in children is particularly challenging due to reliance on adult caregivers (Eley and Nuttall, 2007; Watson and Farley, 1999). ARV treatment for children requires collaboration between the child and caregiver in terms of commitment of caregiver and cooperation of the

child (Quitter *et al.*, 2000). The child–caregiver relationship is an important feature related to adherence, children whose caregiver was strongly involved in the child’s matters were more likely to be ART adherent. The relationship between the caregiver and the child may have a profound influence on adherence. Giving medication is an interactive process that is shaped by the child's behavior and the caregiver's expectations. When communication is poor, adherence has been shown to suffer. Caregivers’ involvement may directly affect child care in monitoring ART adherence (Naar-King *et al.*, 2009; Bikaako-Kajura *et al.*, 2006). This result is consistent with a study from the US which showed that better medication adherence can be achieved by greater caregiver-child relationships. Caregivers’ involvement may directly affect child care in monitoring ART adherence (Naar-King *et al.*, 2009; Bikaako-Kajura *et al.*, 2006).

Disclosed HIV status has been identified as one of the factors associated with better adherence (Bikaako-Kajura *et al.*, 2006; Haberer *et al.*, 2011); however a few caregivers do not reveal the status to the child. This is because of the stigma attached to HIV infection and they may not be able to handle the effects that come with such disclosure (Stein, 2003). A study in the United States showed that such caregiver/family factors as poor parent–child communication, higher caregiver stress and less HIV disclosure to the child were most strongly associated with non-adherence (Mellins *et al.*, 2004). Unwillingness to disclose the child’s HIV status may also negatively impact adherence, since children are totally reliant on an adult to ensure that they take their medication (Nabukeera-Barungi *et al.*, 2007; Reddington *et al.*, 2000).

2.2.3 Challenges of maintaining ART adherence in orphaned children

Factors that can possibly influence orphans to adhere to antiretroviral treatment may vary depending on their circumstances ((Stine, 2005). For orphans who have a surviving parent, the parent takes over the responsibility to care for the children. As for orphans who are left in the care of extended family members owing to the death of both parents, it is a challenge for these caregivers to meet the needs of these children and their own children. In cases where the orphaned child is infected with HIV, the burden faced by the caregivers is exacerbated by the

increasing demands to meet the health care needs of the child and the provision of psychosocial support (Van Dky, 2005).

Children living with HIV or orphaned as a result of AIDS are often victims of discrimination (Campbell *et al.*, 2005). The burden faced by orphans and vulnerable children is often exacerbated by high levels of stigma and discrimination associated with HIV and AIDS which remains a significant problem. When stigma is manifested in the orphan-caregiver relationship, through hostility, violence, or differential resource allocation, it can have severe negative impacts on children under care (Strode and Grant, 2001). Evidence accumulated from studies on ART adherence in orphaned children revealed caregiving situation to be main determinant of adherence in this pediatric population (Vreeman *et al.*, 2008; Blè and Floridaia, 2007).

2.3 ART Adherence Measurements

2.3.1 Overview on ART Measurement Methods

ART adherence can be measured using multiple methods, which can be categorized as subjective or objective. Subjective measures include provider assessment and child and caregiver self-report. Objective measures consist of pharmacy refill data, announced and unannounced pill counts, and electronic monitoring. There is no gold standard for adherence measurement, and all measures have advantages and disadvantages, depending on individual preferences, available resources, and the goal of clinical care versus research (Alcoba *et al.*, 2003; Osterberg and Blaschke, 2005).

Directly observed therapy, measurement of concentrations of a drug or its metabolite in blood or urine, and detection or measurement in blood of a biologic marker added to the drug formulation are examples of direct methods of measures of adherence. Direct approaches are expensive, burdensome to the health care provider, and susceptible to distortion by the patient. However, for some drugs measuring drug levels is a good and commonly used means of assessing adherence. For instance, the serum concentration of antiepileptic drugs such as phenytoin or valproic acid within therapeutic concentration range probably reflect adherence

to regimens with these medications and subtherapeutic levels probably reflect poor adherence or suboptimal dose strengths.

Indirect methods of measurement of adherence include asking the patient about how easy it is for him or her to take prescribed medication, assessing clinical response, performing pill counts, ascertaining rates of refilling prescriptions, collecting patient questionnaires, using electronic medication monitors, measuring physiologic markers, asking the patient to keep a medication diary, and assessing children's adherence by asking the help of a caregiver, school nurse, or teacher.

Due to their relative low-cost and simplicity, the most commonly used measures of ART adherence are pill counts (Muyingo *et al.*, 2008), pharmacy records of dispensed medication (Low-Beer *et al.*, 2000; Orrell *et al.*, 2003), and patient self-reports (Oyugi *et al.*, 2004; Simoni *et al.*, 2006).

Measuring a patient's adherence remains a difficult task, and several approaches to measure adherence have been evaluated and compared showing specific limitations. Reliable and consistent ways of measuring adherence to therapy would help detect patients with poor adherence and guide appropriate interventions (Chalker *et al.*, 2008; Heidenreich, 2004). Previous studies have shown that available adherence measures have limitations, raising questions about how best to measure drug-taking behavior. A single isolated method of adherence assessment seems to be inadequate and impractical (Arnsten *et al.*, 2001; Bangsberg *et al.*, 2000). Although certain methods of measuring adherence may be preferred in specific clinical or research settings, a combination of measures maximizes accuracy (Liu *et al.*, 2001; Spilker, 1991). Adherence measurements in HIV patients in recent research have suggested that use of multiple measures will be of greater benefit than continue search for single defining adherence measure (Ostrop *et al.*, 2000).

Adherence measurement in children is more complicated than measurement in adults due to two principal reasons. First, the goal of the measurement is to learn the behavior of the child; however, that behavior is often influenced to varying degrees by the behavior of one or more

caregivers, medical providers, or others. For self-reported measures, the child may be too young to provide information directly and the report must come through the caregiver. Even when the child can provide primary information, studies have shown poor concordance of the data, and the accuracy of both measurements is unclear. Second, most existing adherence measures are designed for adults who take pills with infrequent regimen changes. They do not account for the role of the caregiver(s), the complexity of pediatric regimens, or the measurement of syrups. Objective measures, such as pharmacy refill, that do not depend on these factors are therefore appealing, but do not actually confirm that pills were ingested. Additional research and tools are needed (Mellins *et al.*, 2004).

2.3.2 Patient self-report (Patient Questioning)

Patient self-report of ART adherence is generally elicited using either direct querying about the ingestion of pills each day over a specific and usually short time frame or a visual analogue scale (VAS) with a numerical or pictorial anchor which the respondent uses to indicate the proportion of pills taken of the total prescribed during the indicated time period.

Substantial variability exists in how these two approaches are implemented, including in the method of administration (self or by trained practitioner/interviewer); modality (in-person, by phone, on-line); recall period (2, 3, 7 or 30 days), number of questions (single vs. multiple items); and type of questions (open or closed-ended). Despite this variability, self-reported data on ART adherence have been shown to correlate with other objective measures of adherence including pill count and viral load (Oyugi *et al.*, 2004; Simoni *et al.*, 2006).

Potential limitations of this method include social desirability and recall biases (Boileau *et al.*, 2008; Simoni *et al.*, 2006), although the impact of these biases on ART adherence estimation has yet to be assessed (Wagner and Miller, 2004).

Patient self-report about recent adherence is a common assessment method due to its relative ease and low cost of data collection, but self-reports tend to overestimate adherence (Ammassari *et al.*, 2002; Liu *et al.*, 2001). In addition, self-report adherence measures have been operationalized in different ways (Simoni *et al.*, 2003).

A recent meta-analysis showed that self-report adherence measures are predictive of clinical outcomes (Nieuwkerk and Oort, 2005), a finding that has been replicated in resource poor settings. However, no studies have validated whether routine self-report data in medical or pharmacy records are predictive of clinical outcomes (Oyugi *et al.*, 2004; Wools-Kaloustian *et al.*, 2006).

2.3.3 Pill Counts

The most common method used to measure adherence, other than patient questioning, has been pill counts (i.e., counting the number of pills that remain in the patient's medication bottles or vials). Although the simplicity and empiric nature of this method are attractive to many investigators, the method is subject to many problems, because patients can switch medicines between bottles and may discard pills before visits in order to appear to be following the regimen. For these reasons, pill counts should not be assumed to be a good measure of adherence. In addition, this method provides no information on other aspects of taking medications, such as dose timing and drug holidays (i.e., omission of medication on three or more sequential days), both of which may be important in determining clinical outcomes (Cramer *et al.*, 1989; Pullar *et al.*, 1989).

Pill counts, like self-reports, can overestimate adherence when compared with electronic medication monitoring (Ammassari *et al.*, 2002; Liu *et al.*, 2001). Collecting pill count data requires a separate recording process in the pharmacy that is often not part of routine dispensing operations. Nevertheless, pill counts have also been shown to be associated with viral load and CD4 counts (Liu *et al.*, 2001).

Although pill counts are often seen as objective measures of adherence, patient disposal of unused pills can lead to an overestimate of adherence when pill counts are scheduled in advance with the patient's knowledge (Miller and Hays, 2000). Unannounced pill counts at the

patient's place of residence have been shown to more accurately estimate adherence (Bangsberg *et al.*, 2000).

2.3.4 Pharmacy Records

Pharmacy records are a convenient and low-cost source of ART adherence information. Assessment can be done in a variety of ways: by comparing the number of monthly medication insurance claims or ART pick-up dates against the number of months on ART (Bisson *et al.*, 2008 and Miller and Hays, 2000), or by measuring medication possession ratios using the number of pills dispensed in the pharmacy and patient pill count data (Muyingo *et al.*, 2008).

Rates of refilling prescriptions are an accurate measure of overall adherence in a closed pharmacy system, provided that the refills are measured at several points in time. The use of pharmacy prescription refill data, however, requires that patients obtain their medications within a closed pharmacy system.

A medical system that uses electronic medical records and a closed pharmacy can provide the clinician or research scientist with readily available objective information on rates of refilling prescriptions that can be used to assess whether a patient is adhering to the regimen and to corroborate the patient's responses to direct questions or on questionnaires. Electronic pharmacy data are becoming more widely available, and this is one of the more frequently used methods in the literature. The act of obtaining refills and the frequency with which the refills are acquired reflect different aspects of a patient's adherence behavior, and adherence based on pharmacy refill data has been correlated with a broad range of patient outcomes (Christensen *et al.* 1997; Lau *et al.*, 1997).

Pharmacy refill records are commonly used in settings with electronic pharmacy data systems to calculate adherence indicators (Osterberg and Blaschke, 2005) either percentage of days within a defined period covered by medicines dispensed or occurrence of gaps between dispensings. Several studies have shown associations between dispensing-based adherence measures and clinical outcomes, including viral load and CD4 counts. Pharmacy refill

approaches have not been extensively tested in settings with manual dispensing records, where data completeness and quality may be problematic (Fairley *et al.*, 2005; Farley *et al.*, 2003).

2.3.5 Consistency of clinic attendance

Consistency of clinic attendance is potentially another way to assess continuity of care and risk for poor adherence. Because failure to attend clinic when expected is objective and easy to ascertain in most record systems, inconsistency of attendance may identify patients in need of outreach or adherence counseling.

One important way to address ART adherence problems is to follow up with patients who fail to appear for clinic visits and to target them for outreach by telephone or through community case workers. Virtually all clinics (98%) recorded the date of the next appointment, although less than one third actually monitored and recorded the discrepancy between when patients were expected to appear and when they actually did so (Chalker *et al.*, 2008).

2.3.6 Electronic Monitors

Electronic monitors capable of recording and stamping the time of opening bottles, dispensing drops (as in the case of glaucoma), or activating a canister (as in the case of asthma) on multiple occasions have been used for approximately 30 years. Rather than providing weekly or monthly averages, these devices provide precise and detailed insights into patients' behavior in taking medication, but they are still indirect methods of measuring adherence; they do not document whether the patient actually ingested the correct drug or correct dose.

Patients may open a container and not take the medication, take the wrong amount of medication, or invalidate the data by placing the medication into another container or taking multiple doses out of the container at the same time. The cost of electronic monitoring is not covered by insurance, and thus these devices are not in routine use. However, this approach provides the most accurate and valuable data on adherence in difficult clinical situations and in

the setting of clinical trials and adherence research and has advanced our knowledge of medication-taking behavior (Paterson *et al.*, 2002 and Urquhart, 1997).

2.3.7 CD4 cell count and Viral Load Determination

Laboratory assays such as CD4 cell count and viral load are often used as indirect, but more objective, measures of ART adherence. However, changes in CD4 count can lag behind other clinical markers of therapeutic success or failure and have not correlated consistently with other measures of adherence (Bisson *et al.*, 2008 and Simoni *et al.*, 2006).

Viral load has demonstrated a strong association with patient self-report of ART adherence but viral load tests are costly and not part of routine care in most resource-limited settings (Fletcher *et al.*, 2005; Nieuwkerk and Oort, 2005).

2.3.8 Therapeutic Drug Monitoring

TDM is considered a good tool for estimating ART adherence. It is one of direct measurement of patient adherence to ART, which is a major challenge to the successful treatment of HIV disease (Gerber and Acosta, 2003; Hugen *et al.*, 2002). Determination of plasma drug concentrations can be a valuable tool to directly measure adherence, though results can vary between patients based on rates of absorption and drug interactions (Back *et al.*, 2001). It is apparent that use of drug plasma concentration is the most accurate and objective way to assess adherence (Rakmanina and Anker, 2004). Mghamba *et al.* (2012) recommended the use of nevirapine plasma concentration in assessment of adherence in HIV infected children as nevirapine plasma concentration is a good predictor of adherence.

Patients with a trough concentration of antiretroviral in plasma within the therapeutic range are considered to be adherent (therapeutic range for nevirapine is 3000 to 8000 ng/mL). Subtherapeutic TDM concentration may indicate poor adherence in the days immediately preceding sampling. TDM can be used to identify non-adherent patients and thereby prevent unnecessary switching to more expensive ART regimens. A recommendation to alter ARV therapy based on a low concentration should have a means of assessing drug adherence at the

time of blood sampling (e.g. pill count, pharmacy refill, electronic monitoring). Dose adjustments made in the face of unidentified poor adherence may put the patient at risk of increased drug concentrations and hence toxicity (Hugen *et al.*, 2002; Kappelhoff *et al.*, 2005).

TDM for NNRTIs is most commonly used in the evaluation of adherence due to the long half-life ($t_{1/2}$) of evirapine and efavirenz (Gonzalez de Requena *et al.*, 2005; La Porte *et al.*, 2006). A prospective cohort study in Cameroon to compare adherence of fixed-dose combination of nevirapine, stavudine and lamivudine using nevirapine plasma level monitoring and self-report by patients found that self-reported adherence was significantly higher than adherence measures by nevirapine level monitoring. The authors concluded that nevirapine plasma concentration monitoring provides an accurate measurement of adherence compared to self-report, but cautioned that it is not feasible in most clinical settings especially in resource-limited areas due to cost (Kouanfack *et al.*, 2008). Murphy and Montaner (1996) applied TDM in measuring adherence to nevirapine; adherence was judged adequate if the nevirapine concentration reached 4000 ng/mL, corresponding to the threshold of nevirapine efficacy. Because of the long plasma half-life of nevirapine, a concentration 4000 ng/mL suggested probable adherence for several days. This was a clear advantage over drugs having a short plasma half-life, whose drug level only provides information on adherence in the previous hours, and are therefore more vulnerable to the “white-coat adherence” phenomenon (improvement of patient’s adherence before a medical visit). Nevertheless, it should be noted that plasma drug level is not predictive of adherence behavior in all patients, because nevirapine concentration may be altered (increased or decreased) for reasons other than adherence (eg, drug interactions, individual metabolism variation, poor quality of the drug)

2.4 Core Indicators for Assessment of Adherence at Health Facilities

2.4.1 INRUD-IAA Group Initiative on Antiretroviral Adherence

In 2006 the International Network for Rational Use of Drugs (INRUD) and national HIV/AIDS programs in five East African countries began the five-year Initiative on Antiretroviral Adherence (IAA) to develop practical interventions to improve adherence to

ART in routine treatment settings. They found wide variations in definitions and practice in measuring and reporting adherence (Chalker *et al.*, 2008). To address this gap, the INRUD-IAA group has developed and pilot tested methods and indicators to assess adherence at health facilities using patient interviews and the types of routine data available in these settings, which are reported in a companion publication. These indicators can be used to measure the success of health facilities in maintaining patients on treatment and to evaluate the impact of interventions (Chalker *et al.*, 2010).

2.4.2 Core Indicators of Adherence

The five core indicators of adherence with the alternate attendance indicators are:

Self report-based adherence measures from exit interviews

- Percentage of patients with full adherence to ART (i.e., no doses missed in the recall period, which is three days in the INRUD-IAA methodology).

Dispensing-based adherence equals measures

- Average percentage of days covered by ARVs dispensed for a sample of patients for a defined period (180 days).
- Percentage of patients who experienced a gap in ARV availability of more than 30 days in a row during the same defined period.

Patient attendance and defaulting

- Percentage of patients who attend on or before the day of their appointment.
- Percentage of patients who come within three days of their appointment.

Alternate attendance indicators

- Percentage of all visits in the last six months made before the days of medicine supplied at the previous visit have been consumed.
- Percentage of all visits in the last six months made within three days of when the medicine supplied at the previous visit have been consumed.

Source: World Health Organization and Management Sciences for Health. (2011). How to Investigate Adherence to Antiretroviral Treatment: An Indicator-Based Approach.

2.4.3 Self report-based adherence measures from exit interviews as indicator of adherence

A clinician or pharmacist can easily collect data for this indicator by asking patients whether they have missed any doses of pills in the last three days, and if so, how many. For valid answers to this question the interviewer must appear non-judgmental. The recommended way of asking this is; many patients have troubles in taking their ARV doses as prescribed, how many of the ARV doses did you miss in the last three days?

2.4.4 Patient Attendance and Defaulting Measure as a core indicator of adherence

The purpose is to look at a visit the patient made, note when the next appointment was made for, and then see if the patient kept the appointment. Because some programmes give certain patients three months of medicine, it is necessary to review the records to see the patient's attendance four months before, see the date of the next appointment, and then note whether the patient's next visit was on or before that date, or within three days of that date.

The two core performance indicators related to attendance are:

- Percentage of patients who attend on or before the day of their appointment.
- Percentage of patients who attend within three days of their appointment.

Sometimes the appointment dates are not available in the pharmacy notes making the two indicators above impossible to collect. Alternative attendance indicators are:

- Percentage of all visits in the last six months made before the medicine supplied at the previous visit have been consumed.
- Percentage of all visits in the last six months made within three days of when the medicine supplied at the previous visit have been consumed.

3. STATEMENT OF THE RESEARCH PROBLEM

One of the foremost concerns of ARV programs is the ability of people living with HIV/AIDS (PLHA) to maintain near perfect adherence over the long term. The need for near-perfect adherence to lifelong therapy from an early age has been identified as a major challenge in the administration of ART to HIV-infected children (Eley and Nuttall, 2007; Watson and Farley, 1999). There is concern about the extent to which this is achievable for children in resource-limited settings, particularly in the context of the rapid scale-up of pediatric treatment programs required to address the HIV burden on children in Africa (Eley and Nuttall, 2007; Orrell, 2003).

Although orphans may have access to health care and antiretroviral treatment, adhering to the treatment still remains a matter of concern (Havens *et al.*, 2002; Mellins *et al.*, 2003). Lack of adherence to ART may lead to therapeutic failure, deterioration of the immune system and/or emergence of medicine-resistant HIV strains, rapid disease progression, increased risk of mortality and escalating costs of care (Hogg *et al.*, 2002; Friedland and Williams, 1999).

Mghamba *et al.* (2012) recently determined adherence to ART by using medication return, caretaker's reporting and plasma NVP levels in children ranging between 2-14 years of age; but the study did not address the level of adherence to ART attained by orphaned children and the factors which affect it.

Evidence-based data from developing countries regarding ART adherence rates, predictors, and the effectiveness of support interventions are limited. The implication is that there is urgent need for systematic data collection and analysis to estimate the prevalence of nonadherence and to make strong evidence-based recommendations on the best ways to improve medication adherence (Paterson *et al.*, 2000).

To our knowledge, no study in Tanzania has been done to establish ART adherence level in orphaned children infected with HIV by multi-method approach. It was therefore important to

investigate the level of adherence in orphaned children who are infected with HIV by using nevirapine plasma concentrations as complementary indicator to conventional adherence measuring methods. The study also explored the factors affecting ART adherence in this pediatric population.

4. STUDY JUSTIFICATION

In view of challenges facing HIV-infected orphaned children in maintaining ART adherence, orphans need more care and attention to promote their adherence to ART than other categories of children. There is insufficient research to understand adherence practices in orphans under antiretroviral medications in Tanzania; scientific investigation is needed so as to gain in-depth information on adherence practices to antiretroviral medications prescribed to orphans to bridge existing knowledge gap and to make evidence-based recommendations for improving ART adherence of orphans.

Most available reports on adherence in Tanzania are mainly in adults but limited data is available on the extent of adherence in orphaned children. Even the reported adherence studies were mostly obtained by self-reported interviews and pill counts. The use of plasma drug concentrations as a predictor of adherence has not been adequately employed in the country. The application of nevirapine plasma concentration as a marker of adherence is a relative new approach in our HIV settings and this will provide added advantage for reliability of adherence data in HIV-infected orphans.

Findings of this study will be useful in the planning, implementation and monitoring of programs and interventions aimed at enhancing adherence of orphaned children. In addition, this information can be used to support the establishment and strengthening of structures, systems, and policies to support orphaned children living with HIV and are on treatment by relevant authorities including CTCs administrations, Ministry of Health and Social Welfare (MoHSW) and National Control AIDS Programme (NACP).

5. RESEARCH QUESTIONS

- What is the proportion of HIV-infected orphan children aged 2-14 years who are adherent to ART attending CTCs in Dar es Salaam?
- What factors influence levels of adherence to ART in HIV-infected orphan children in Dar es Salaam?
- How do parents/guardians and clinic visits reported adherence correlate with plasma levels of ARVs.

6. RESEARCH OBJECTIVES

6.1 Broad Objective

To determine ART adherence levels and factors influencing adherence to ART among HIV-infected orphan children attending selected Care and Treatment Centres (CTCs) in Dar es Salaam.

6.2 Specific Objectives

1. To determine the proportion of HIV-infected orphan children with adequate ART adherence levels in the selected CTCs in Dar es Salaam by caregiver self-report.
2. To determine the proportion of HIV-infected with adequate ART adherence levels in the selected CTCs in Dar es Salaam by clinic attendance records.
3. To determine the proportion of HIV-infected with adequate ART adherence levels in the selected CTCs in Dar es Salaam by determination of nevirapine plasma concentration.
4. To make comparisons of adherence measures determined by caregiver self-report, clinic attendance records and nevirapine plasma concentration determination.
5. To assess ART responses in HIV-infected orphan children attending the selected CTCs in Dar es Salaam.
6. To explore factors influencing ART adherence in HIV-infected orphans and assessment of caregivers' knowledge on HIV/AIDS

7. HYPOTHESIS

7.1 Null hypothesis

There is no significant proportion of HIV orphaned children (2-14 years) who attended selected CTCs in Dar es Salaam with inadequate ART adherence.

7.2 Alternative hypothesis

There is significant proportion of HIV orphaned children (2-14 years) who attended selected CTCs in Dar es Salaam with inadequate ART adherence.

8. RESEARCH METHODOLOGY

8.1 Description of the study area

The study conducted in HIV/AIDS Care and Treatment Centres (CTCs) integrated in Dar es Salaam Municipal Hospitals; Temeke, Amana and Mwananyamala.

8.2 Study population

The HIV-infected orphan children aged between 2 and 14 years receiving ART in Dar es Salaam Municipal Hospitals CTCs and their caretakers were recruited for this study.

Table 1: HIV-infected children receiving ARVs in Dar es Salaam Municipal Hospital CTCs as per 20th February, 2013

CTC Name	Children		Total for CTC
	Male	Female	
Temeke	572	562	1,134
Amana	614	668	1,282
Mwananyamala	461	512	973
Grand Total	1,647	1,742	3,389

Source: CTC administration in Temeke, Amana and Mwananyamala

8.3 Sampling procedure and sample size

Convenience sampling was used in which three CTCs located in Dar es Salaam Municipal Hospitals were picked for the study. These CTCs were chosen as study sites since the centers enroll a high number of pediatric ART patients and run a high percentage of Tanzania's pediatric ART services. In addition, these CTCs provide free ART services on routine basis and therefore were used to represent all CTCs based in Dar-es-Salaam.

The sample size for this study was calculated based on expected proportion of adherence to ART among People Living with HIV (PLHIV) based on previous study (Mghamba *et al.*, 2012) with a relative precision of 10% and a confidence interval (CI) of 95%.

The formula used for the calculation of sample size:

$$\text{Sample size (n)} = \frac{Z^2 PQ}{d^2}$$

Z = Value of constant at 95% confidence interval

P = Estimated Pediatric adherence to ART in Tanzania is 85% (Mghamba *et al.*, 2012)

Q = Complementary probability = (1-P)

d = Level of error allowed

For this study:

$$P = 85\% = 0.85$$

$$Z = 1.96$$

$$Q = (1-P) = 0.15$$

$$d = 5\% = 0.05$$

$$\begin{aligned} \text{Thus } n &= \frac{1.96^2 \times 0.85 \times 0.15}{0.05^2} \\ &= 195.92 \approx 196 \end{aligned}$$

10% of calculated figure is added in order to take care of attrition rate, thus overall sample size is rounded to 216. A total of 216 participants were planned to be included in this study.

Inclusion Criteria

- HIV-infected orphans aged 2-14 years
- HIV-infected orphans who had been on nevirapine based regimen for at least 6 months
- HIV-infected orphans whose caretakers provided informed consent to participate in the study.
- The selected CTCs have regular supply of medications with no additional resources beyond those provided to most CTCs clinics in the country.

Exclusion Criteria

- HIV-infected orphans below the age of 2 years old or above 14 years old
- HIV-infected orphans who had been on ARV treatment for less than 6 months
- HIV-infected orphans whose caretakers refuse to consent for participation in the study.

8.4 Study design

This was hospital-based, cross-sectional study which was conducted between June 2013 and September 2013 at pediatric HIV clinics of Dar es Salaam Municipal Hospitals in Tanzania.

8.5 Study setting

Dar es Salaam region has three Municipal hospitals; Mwananyamala, Temeke and Amana. This study was conducted in three pediatric HIV Clinics in these municipal hospitals.

A total of 216 HIV-infected orphans were recruited from Dar es Salaam municipal hospitals for this study; 86, 82 and 46 from Temeke, Mwananyamala and Amana municipal hospitals respectively. The “first-in, first-taken” principle was applied in recruiting research subject. Every patient who came first to the clinic was selected as long as the patient met the inclusion criteria.

The data collection process followed the routine system so as not interfere with the normal patient flow at the CTC. This approach reduces attrition rates observed when additional new

step in patient flow is introduced. In addition, using existing routine systems in CTC is cost-effective as it involves personnel who are experienced in running HIV clinics.

Nurse counselors were selected and trained as research assistants. Caretakers of HIV-infected orphaned children were identified by nurse counselors and requested to participate in the study during counseling sessions at paediatric HIV clinics. Nurse counselors administered the pretested questionnaire to primary caregivers of HIV-infected orphaned children in the counseling rooms to caretakers. The questionnaire-based interviews were convened in Kiswahili (Appendix IIIa or Appendix IIIb).

As the study was conducted in the context of cross sectional design, study population was assumed to be homogenous before blood samples collection. This assumption enabled determination of the estimated prevalence of entire population with at least nevirapine trough concentration. The blood samples were collected from these patients at phlebotomy section of CTC. Two millilitres (2 ml) of venous blood were drawn into an ethylenediaminetetraacetic acid tube. The blood samples centrifuged immediately to obtain plasma. The obtained plasma samples were kept at -80°C until drug assay.

8.5.1 Determination of Adherence to ART by Nevirapine plasma concentration

Nevirapine in human plasma concentrations was determined by High-Performance Liquid Chromatography based on method described by Kappelhoffa *et al.*, 2003. This was carried out in the MUHAS–Sida (Swedish International Development Cooperation Agency) Bioanalytical Laboratory, Unit of Pharmacology and Therapeutics, School of Pharmacy, MUHAS in Dar es Salaam, Tanzania.

Nevirapine plasma concentration was determined by RP-HPLC method. Before analysis of patient samples, the method was validated as per recently published FDA guidelines for validation of bioanalytical assays.

Stock solution for nevirapine was prepared by dissolving in methanol, to obtain a concentration of 1mg/ml whereas stock solution for carbamazepine (IS) was prepared by dissolving in acetonitrile to achieve a final concentration of 300 μ g/ml. Working solution for nevirapine was prepared by diluting suitably stock solution of nevirapine with methanol to get the concentration of 1000 μ g/ml. Similarly, dilutions were made for stock solution of carbamazepine using acetonitrile to prepare working solution of 30 μ g/ml. The calibration curve for nevirapine was obtained using seven calibration standard levels (0.5, 1.0, 2.5, 4, 7.5, 12 μ g / ml). Quality Control (QC) samples for nevirapine were prepared by spiking drug free plasma to give final concentration of low (LQC – 1.5 μ g / ml), medium (MQC – 5 μ g / ml) and high (HQC – 12.5 μ g / ml). The resulting peak area ratios between the internal standard (carbamazepine) and analyte (nevirapine) were plotted versus concentration to obtain a linear regression equation (model), linear regression analysis was done, considering the ratio of the peak area of analyte to internal standard versus concentration applied. A correlation coefficient of more than 0.99 was obtained for calibration curve.

The specificity of the RP-HPLC method was determined by comparison of the chromatogram of standard and sample solution. The parameters like retention time (Rt), resolution (Rs), tailing factor (Tf) and theoretical plates were calculated (figure 3). The accuracy and precision of the method were evaluated using the Q.C. samples. Intra-day accuracy and precision was measured by consecutively analyzing Q.C. samples in one single day. The procedure was repeated for five different days to test the inter-day accuracy and precision. Accuracy was calculated as percentage accuracy, whereas precision was measured in terms of relative standard precision (R.S.D.) of each calculated concentration. Recovery for Nevirapine was evaluated at three concentration levels corresponding to three Q.C. samples (1.5, 5 and 12.5 μ g/ml) analyzed in triplicate. Recovery was determined by comparing the ratio of the peak area of nevirapine obtained after the application of the processed plasma calibration samples with those achieved by working standard solution in the methanol (Figure 5).

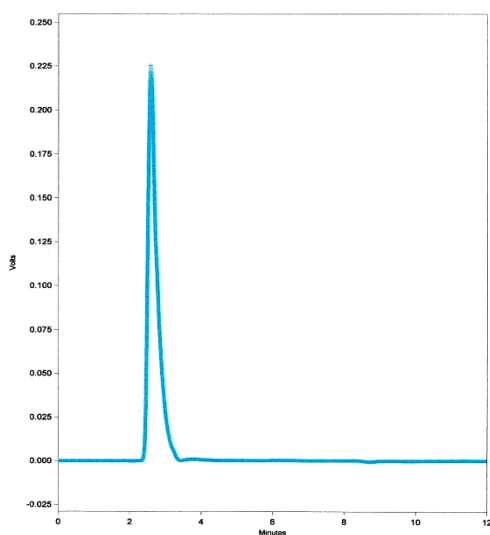


Figure 3: A Chromatogram of blank plasma obtained after analysis of plasma sample collected from a volunteer.

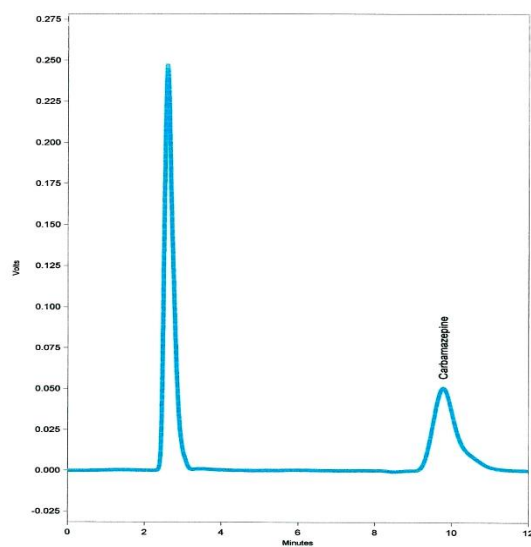


Figure 4: A Chromatogram of Internal Standard (IS)

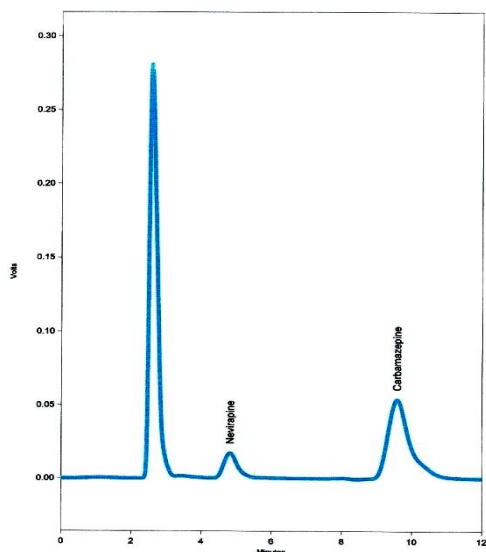


Figure 5: A Chromatogram obtained during validation in day 2 from C6.

The procedure for determination of nevirapine plasma levels from collected patient samples started with extraction of nevirapine in which 400 μ l of carbamazepine was added to 200 μ l of patient plasma samples in eppendorf tube, vortexed for two seconds and the contents mixed for 20 minutes on a shaker machine. It was centrifuged for 10 min at 4000rpm. After centrifugation, 200 μ l of clear supernatant transferred into autosampler vial and the aliquots of 25 μ l of solution was injected, flow rate of 0.5 ml/min, and wavelength of 275. The chromatographic analysis was done on Zorbax Extend C18 analytical column with a mobile phase composed of 25Mm triethylamine in water – acetonitrile (65:35 v/v). Each run of patient test samples included quality control and standard curve samples. Nevirapine concentrations in patient test samples were calculated by a linear regression equation (model) from calibration curve (Figure 6).

Nevirapine plasma concentration > 3 μ g/ml was categorized as good adherence This cut-off point is based on the steady-state trough concentration reached in the pharmacokinetic curve for nevirapine at a dose of 200 mg twice daily (Duong *et al.*, 2005).

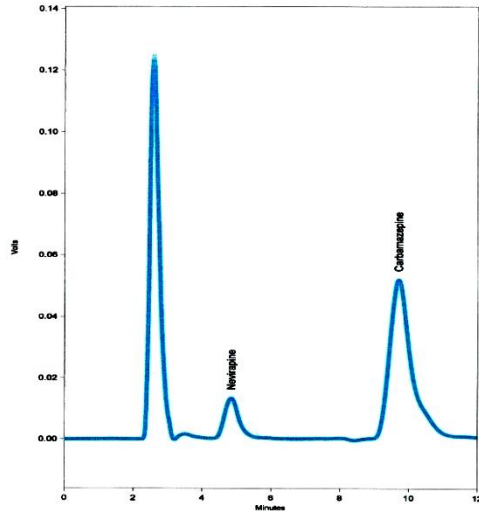


Figure 6: Chromatogram showing peaks of nevirapine and carbamazepine and their retention times from plasma of one HIV infected child (P1) undergoing ART

8.5.2 Determination of Adherence to ART by Caregiver report and Clinic attendance

Modified Indicator based approach as described in WHO manual (World Health Organization and Management Sciences for Health, 2011) was used to measure adherence in HIV-infected orphaned children. Using this approach, the two core adherence indicators were used to measure adherence in orphans; caregiver self report and clinic appointment records.

Caregiver self report involved questioning the primary caregiver the number of doses of antiretroviral therapy had been omitted in the past 3 days in non-judgmental manner. Missing one dose is equivalent to less than 95% adherence.

Retrospective Record Review on Patient Record Form (CTC2) (Appendix Va and Appendix Vb) was employed to retrieve data on pattern of clinic attendance of selected patients; it involves noting whether the patient comes back for follow-up on the assigned date. Adequate adherence to ART is when patients attend on or before the day of their appointment or patients

attend within three days of their appointment (World Health Organization and Management Sciences for Health, 2011).

8.5.3 Investigation into factors influencing ART adherence and assessment of caregivers' knowledge on HIV/AIDS

This investigation was carried out by a nurse counsellor using a questionnaire (Appendix IIIa or Appendix IIIb).

The persons in charge of routinely administering antiretroviral drugs to enrolled HIV-infected orphan children was identified as primary caregivers; written informed consent (Appendix IIa or Appendix IIb) was obtained from these primary caregivers (parents or guardians) of the children. A structured pre-tested questionnaire in Kiswahili version (Appendix IIIa or Appendix IIIb) was administered to these primary caretakers by trained nurse counselors during counseling session. The filling of the questionnaire included: socio-demographic characteristics of children and their primary caregivers, subdivisions of orphans; paternal, maternal and complete orphans, occupation of caregivers, caregiver-child relationship, caregivers' knowledge about HIV/AIDS and ART. The other issues examined include caretakers' understanding on how HIV is transmitted and doses missed in the past three days.

The other information was extracted from Patient Record Form (CTC2) (Appendix Va and Appendix Vb) maintained at the clinic which includes initial and latest recorded CD4 counts, WHO clinical stage, clinic attendance and duration of ART.

8.5.4 Performance of caretaker report and clinic attendance consistency as compared with Nevirapine plasma concentration for predicting inadequate adherence

The usefulness of diagnostic tests is their ability to detect a person with disease or exclude a person without disease and is usually described by terms such as sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV). When evaluating the feasibility or the success of a screening program, one should also consider the PPV and NPV values.

The whole purpose of a diagnostic test is to use its results to make a diagnosis, so we need to know the probability that the test result will give the correct diagnosis. PPV and NPV describe a patient's probability of having disease once the results of his or her tests are known.

The performance of adherence measurement methods in this study (caretaker report, clinic attendance and nevirapine plasma concentration) was assessed by determination of sensitivity, specificity, PPV and NPV.

8.5.5 Changes in CD4 counts as a measure of ART response

Antiretroviral therapy is recommended for all patients with symptomatic HIV disease. For patients without symptoms, therapy should be initiated at some point after the CD4 cell count declines below 350/ μL but before it reaches 200/ μL (Yeni *et al.*, 2004).

A CD4 count measures the number of T cells expressing CD4 and is used to assess the immune system of a patient. Immune status is categorized as good immune status if CD4 % >30% for children below 5yrs or CD4 count >500 for children above 5yrs. An individual whose CD4 is below these values is categorized as immunodeficient according to WHO immunological classification (NACP-T, 2009). A CD4 counts between 200 and 350 indicates that some damage to immune system has occurred. CD4 > 350 cells/ μl^3 indicates clinical improvement of the patients whereas body's immune system in a patient with less than 200 cells per microliter is no longer strong enough to prevent illness and infection and are at high risk of contracting AIDS defined illnesses (Hogg *et al.*, 2001; Palella *et al.*, 2003).

The pattern of CD4 counts over time is more important than any single CD4 because the values can change from day to day. Understanding the way CD4 change over time provides insight into the way patients respond to treatment and how effective treatment is with time.

The pattern of changes in CD4 counts was studied to determine response to ART. On the other hand association between changes in CD4 counts and ART adherence measures was assessed.

8.6 Ethical Considerations

Ethical clearance was obtained from Muhimbili University of Health and Allied Sciences senate, Research and Publication Committee (Appendix I). The permission to collect data sought from District Medical Officers for Ilala, Kinondoni and Temeke. Written informed consent obtained from caregivers of HIV-infected orphan children (Appendix IIa or Appendix IIb).

All the information collected in this research is treated as confidential and unauthorized persons do not have access to the data collected. Each subject was assigned a study identification number, and these subject identifiers were not released outside the research group. Codes were used and no identification made for the responders. All data collectors were asked to observe confidentiality for any patient information they receive. They were instructed not under any circumstances to divulge any of that information to anyone else outside the survey. Respondents were informed that their data will be used anonymously and that the aim of the study is to understand better the problems and how ARVs users can better be supported.

8.7 Pre-testing of the data collection methods

The questionnaire and blood sampling procedure were pretested at Mbagala Rangi Tatu Hospital. Pre-testing was carried out with a selected sample of the target population not from the actual targeted study facilities. The pre-testing was done prior to conducting the actual main study and necessary adjustments/corrections of the research tools were made accordingly. This important step provided an opportunity to identify and solve unforeseen problems.

8.8 Data Collection

Data were be collected by trained research assistants under supervision of the principal investigator (PI). Thorough check up was done to make sure that all the questionnaires are properly filled. Data were also abstracted from Patient Record Form (CTC2) (Appendix Va and Appendix Vb) by PI.

Research Assistants (RAs) participated in this study were trained. One day of training was conducted by principal investigator (PI). They underwent a brief protocol training session of one day that included understanding the study objectives, research ethics, data collection methods and familiarization with the study data collection tools.

Prior to data collection, CTC administration visited to explain the purpose of the study; permission sought from in-charges to collect required data by the study from HIV-infected orphans attending these CTCs. Furthermore, request submitted to CTC administration on using CTC resources and personnel for assisting with data collection exercise including identification of HIV-infected orphans by adherence counselors and laboratory technicians in drawing blood from selected patients and storage of blood samples before being taken to MUHAS for HPLC analysis.

8.9 Statistical Data analysis

Each questionnaire/data collected from each study sample was coded. Data checking and cleaning was done before entry into the computer statistical programme. Data entry and analysis was executed using SPSS software version 20. The significance level for this study was set at p value of < 0.05 . Data entry was validated by random review of 10% of the data entered.

8.9.1 Univariate Analysis

Descriptive analysis was employed on the overall cohort; descriptive statistics such as frequencies, percentages and means with standard deviations was carried out to explore all variables of the children and the caregivers. Data collected from the study were analyzed, categorized and summarized interpreted in terms of tables and histograms.

8.9.2 Bivariate analysis

The correlation between adherence measures and demographic characteristics was analysed by Chi-square test and logistic regression. On the hand, kappa statistic was used to assess agreement between adherence measures (caretaker report, clinic attendance and nevirapine plasma concentration).

9. RESULTS

This chapter presents the findings of this study. The qualitative and quantitative findings are translated into meaningful and descriptive information. The demographic description of the samples is given in section 9.1. Thereafter, ART adherence in orphans, findings related to the challenges of ART in orphans and investigations into knowledge regarding HIV/AIDS among caregivers of orphans are presented.

9.1 Description of study population

The 216 research participants were recruited from three Municipal Hospitals in Dar es Salaam with distribution of Temeke 88, Mwananyamala 82 and Amana 46. Temeke CTC has the highest number of patients taking ARVs of all CTCs in Dar es Salaam which corresponds to highest proportion of research subjects in this study (Table 2).

Table 2: The recruited research subjects

CTC	Frequency	Percentage
Temeke	88	40.7
Mwananyamala	82	38.0
Amana	46	21.3
Total	216	100

The HIV-infected orphan children studied were on antiretroviral treatment for at least six months; males 108 (50%) and females 108 (50%). The HIV-infected orphan children were aged between 2 and 14 years old, with a mean age was 9.3 ± 3.3 years. Over 50% of the children in this study were between 10 and 14 (Table 3). Each of the children was under the care of a relative or parent; 42 males (19.4%) and 174 female (80.6%). The youngest and oldest caregivers were aged 14 and 70 respectively and mean age was 43.1 ± 12.9 . Majority of the

caregivers were aged 30-49, and of note is that a large number of caregivers were below 50 (Table 4). This age is for various reasons unstable and extremely mobile due to the search for employment, family life, further study and other forces.

Table 3: Orphan age grouping

Age group	Frequency	Percentage
2-4	23	10.6
5-9	84	38.9
10-14	109	50.5
Total	216	100

Table 4: Caretaker age grouping

Age group	Frequency	Percentage
14-19	7	3.2
20-29	13	6.0
30-39	68	31.5
40-49	69	31.9
50-59	27	12.5
60-69	31	14.4
70-79	1	0.5
Total	216	100

Table 5 presents socio-demographic characteristics of caregivers; almost 7% had never attended school, only 0.5% of the caregivers have earned a tertiary education, with the majority (72.2%) stated to have completed primary school education. It is therefore safe to state that over 79.1% of the caregivers may be met with challenges resulting from the effects

of the low levels of education acquired or no education at all. Employment is a major source of income; a stable income is crucial to meet caring responsibility of orphans, a total of 48.1% indicated to be involved in rudimentary businesses, 26.9% were house wives and 17.1% were salaried workers who are deemed to have regular and stable incomes. The majority of the caregivers were legally married couple (51.9%), however a significant proportion of caregivers (21.3%) were widowed. It was found that 41.2% of orphans were in setting other than that of families. Furthermore the study revealed that 61.6% of the children on treatment are not cared for and supported by a surviving parent either mother or father which might be associated with poor giving situation. The previous studies have shown that degree of care taking influence levels of ART adherence in orphans.

Table 5: Sociodemographic characteristics of the caretakers (N=216)

Variable	Frequency	Percentage
Education		
Primary education	156	72.2
Secondary education	44	20.4
College/University	1	0.5
No formal education	15	6.9
Occupation		
Businessman	104	48.1
Salaried worker	37	17.1
House wife	58	26.9
Farmer	6	2.8
Student	1	0.5
Other	10	4.6
Relation		
Parental relationship	83	38.4
Non-parental relationship	133	61.6
Marital Status		
Married	112	51.9
In relationship	4	1.8
Single	43	19.9
Widowed	46	21.3
Divorced	11	5.1

Closer examination of sociodemographic and clinical characteristics of orphans recruited in this study revealed that 35.2% had undisclosed HIV status, 65.3% had advanced HIV

infection (WHO Stage III-IV). It was also established that most of children (94.4 %,) were on ART for more than 1year and 75.9% had a least one surviving parent (Table 6).

Table 6: Characteristics of HIV -infected children (N=216)

Variable	Frequency	Percentage
Child's orphan status		
Paternal orphan	80	37.0
Maternal orphan	84	38.9
Double orphan	52	24.1
Child's HIV Status		
Disclosed	140	64.8
Not Disclosed	76	35.2
Baseline WHO clinical stage		
Stage I	11	5.1
Stage II	64	29.6
Stage III	125	57.9
Stage IV	16	7.4
Duration of ART		
≤1 year	12	5.6
>1 year	204	94.4

9.2 ART adherence in orphans

9.2.1 The proportions of ART adherence measures

The proportion of good adherence as assessed by caretaker's self report, clinic attendance consistency and nevirapine plasma determination were 79.6%, 82.9% and 72.2% respectively as shown in Table 7 and Figure 7.

Table 7: Adherence measures by different approaches

Variable	Frequency	Percentage
Caretaker self report		
Good	172	79.6
Poor	44	20.4
Clinic Attendance		
Good	179	82.9
Poor	37	17.1
Nevirapine conc		
≥3µg/ml (Good)	156	72.2
<3µg/ml (Poor)	60	27.8

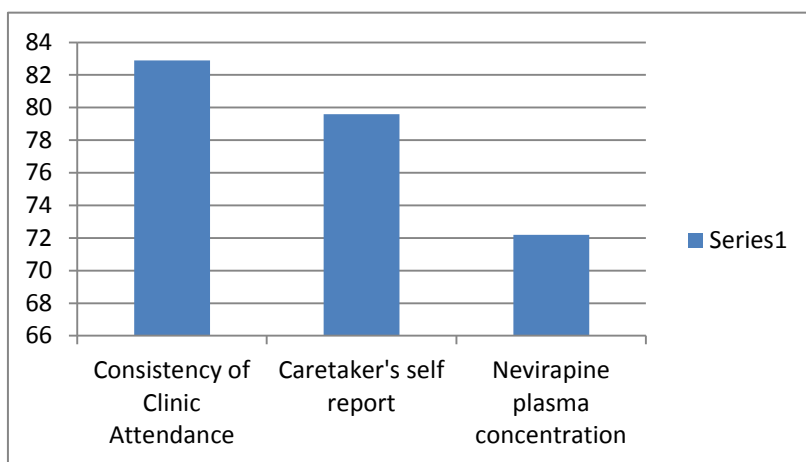


Figure 7: ART levels by different adherence assessment methods

9.2.2 Bivariate analysis among ART adherence measures

The association between nevirapine plasma concentrations and other adherence measures was assessed. Bivariate analysis among adherence measures showed statically significant correlations between nevirapine plasma levels and clinic attendance (P=0.02), others had no statistically significant correlation (Table 8).

Table 8: The association between nevirapine plasma concentrations and other adherence measures

		Nevirapine plasma concentration		Measure of association	
		≥3µg/ml n(%)	<3µg/ml n(%)	Pearson's χ^2	P-value
Self-reported adherence	Good	128(74.4)	44(25.6)	2.03	0.15
	Poor	28(63.6)	16(36.4)		
Clinic attendance	Good	135(75.4)	44(24.6)	5.32	0.02
	Poor	21(56.8)	16(43.2)		

Significantly at P<0.05

The relationship between nevirapine plasma concentrations and other adherence measures was measured by univariate logistic regression (Table 9). Multivariate logistic regression presents adjusted association between nevirapine plasma levels and other adherence measures (Table 10).

Table 9: Univariate logistic regression for association between nevirapine plasma concentrations and other adherence measures

		Nevirapine plasma concentration		Crude association		
		$\geq 3\mu\text{g/ml}$ n(%)	$< 3\mu\text{g/ml}$ n(%)	Odds ratio (95% CI)	P-value	
Adherence measure	Self-reported adherence	Good	128(74.4)	44(25.6)	0.60(0.298, 1.21)	0.16
		Poor	28(63.6)	16(36.4)	1	
Clinic attendance		Good	135(75.4)	44(24.6)	0.43(0.21, 0.89)	0.02
		Poor	21(56.8)	16(43.2)	1	

Significantly at $P < 0.05$ **Table 10: Multivariate logistic regression for association between nevirapine plasma concentrations and other adherence measures (adjusted association between nevirapine plasma levels and adherence measures).**

Adherence measure		Adjusted association	
		Odds ratio (95% CI)	P-value
Self-reported adherence	Good	0.62(0.30, 1.27)	0.19
	Poor	1	
Clinic attendance	Good	0.45(0.21, 0.95)	0.04
	Poor	1	

Significantly at $P < 0.05$

9.2.3 Agreement among adherence measures

The agreement between nevirapine plasma concentration and other adherence measuring methods was assessed using kappa statistic and it was weak in all cases except between nevirapine plasma concentration and clinic attendance ($k = 0.15$, $P = 0.02$) as shown in Table 11.

Table 11: Agreement between nevirapine plasma concentration versus caretaker report, clinic attendance consistency and clinician estimated adherence

Variable	Nevirapine conc.		Kappa value	P-value
	<3 μ g/ml n(%)	\geq 3 μ g/ml n(%)		
Caretaker report				
Missed	16	28	0.05	0.22
Never	44	128		
Clinic Attendance				
Good	44	135	0.15	0.02
Poor	16	21		

Significantly at $P < 0.05$

9.2.4 Performance of caretaker report and clinic attendance consistency as compared with Nevirapine plasma concentration for predicting inadequate adherence

In order to validate caretaker report and clinic attendance consistency against the actual detected plasma drug concentrations the sensitivity, specificity, and negative and positive predictive values were calculated.

Notable findings include the same sensitivity for both caretaker report and clinic attendance consistency (26.7%). The results imply that these two adherence assessment methods are able to identify only about 27 out of every one hundred non-adherent patients. Given a sensitivity of 26.7% or 0.27, we can safely conclude that 27% of the individuals with the disease will have a positive screening test. The specificity was 86.5% for clinic attendance and 82.1% for caretaker report. Negative predictive value was highest with clinic attendance (75.4%)

followed by caretaker report (74.4%). Given a specificity of 86.5% or 0.87, we can safely conclude that 87 percent of the individuals without the disease will test negative. Positive predictive value calculated were 36.4% and 43.2% for caretaker report and clinic attendance consistency respectively. A positive predictive value of 36.4% means that only 36.4% of the people with a positive test actually have the disease. In other words, individuals with a positive test have a 36.4% chance of having disease (Table12).

Table 12: Performance of caretaker report and clinic attendance consistency as compared with nevirapine plasma concentration for predicting inadequate adherence

Variable	Nevirapine conc.		Total	Sensitivity	Specificity	PPV	NPV
	<3µg/ml n(%)	≥3µg/ml n(%)					
Caretaker report							
Good	44(25.6)	128(74.4)	172	26.7%	82.1%	36.4%	74.4%
Poor	16 (36.4)	28(63.6)	44				
Clinic Attendance							
Good	44(24.6)	135(75.4)	179	26.7%	86.5%	43.2%	75.4%
Poor	16(43.2)	21(56.8)	37				
Total	60	156	216				

9.2.5 Immunological Response to ART

The changes in CD counts were assessed and as expected majority of patients showed increase in CD4 (72%), it was also noted that about 23% had decreased CD4 and about 6% had the same CD4 counts between initial count and latest recorded CD4 counts (Table 13 and Figure 8). On the other hand, the influence of initial CD4 counts and the duration of ART on changes in CD4 counts were assessed. The analysis revealed insignificant association between baseline

CD4 counts and changes in CD4 counts (Table 14) and duration of ART was found to be significant determinant of changes in CD4 counts (Table 15).

Association between changes in CD4 counts and ART adherence measures was studied. The analysis showed about 70% of patients with good adherence based on nevirapine plasma concentration showed increased CD4 counts, however 77.1% of recorded to be poor adherents by this method had positive response in CD4 counts. In all cases it was found that changes in CD4 counts were not significant determinant of adherence measures at $P < 0.05$ (Table 16).

Table 13: Trends in CD4 counts (N=164)

Trend in CD4	Frequency	Percent
CD4 increased	118	72.0
CD4 decreased	37	22.6
No change in CD4 counts	9	5.5
Total	164	100

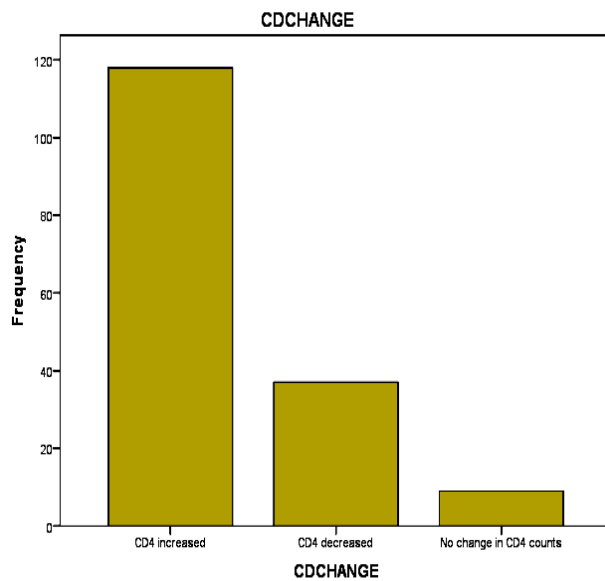


Figure 8: Trends in CD4 counts

Table 14: Association between Changes in CD4 counts and Initial CD4 counts (N=164)

Initial CD4 counts	Change in CD4 count			Measure of association	
	CD4 increased n(%)	CD4 decreased n(%)	No change in CD4 counts n(%)	Pearson's χ^2	P-value
Initial CD4 above 200 cells/mm ³	91(77.1)	34(94.4)	8(80.0)	5.41	0.07
Initial CD4 below 200 cells/mm ³	27(22.9)	2(5.6)	2(20.0)		

Table 15: Association between changes in CD4 counts and duration of ART (N=164)

ART duration	Change in CD4 counts			Measure of association	
	CD4 increased n(%)	CD4 decreased n(%)	No change in CD4 counts n(%)	Pearson's χ^2	P-value
Below 1 year	2(1.7)	1(2.8)	3(30.0)	21.06	0.000
Above 1 year	116(98.3)	35(97.2)	7(70.0)		

Table 16: ART adherence measures and its association with change in CD4 counts (N=164)

Adherence measure		Change in CD4 counts			Measure of association	
		CD4 increased n(%)	CD4 decreased n(%)	No change in CD4 counts n(%)	Pearson's χ^2	P-value
Nevirapine plasma conc	Good	81(69.8)	27(23.3)	8(6.9)	1.77	0.412
	Poor	37(77.1)	10(20.8)	1(2.1)		
Self-report adherence	Good	91(68.4)	34(25.6)	8(6.0)	4.39	0.112
	Poor	27(87.1)	3(9.7)	1(3.2)		
Clinic attendance	Good	102(72.3)	31(22.0)	8(5.7)	0.23	0.891
	Poor	16(69.6)	6(26.1)	1(4.3)		

9.2.6 Relationship of Demographic/Clinical variables to nevirapine plasma concentrations

Table 17 summarizes the association between caregivers' and patients' socio-demographic / clinical characteristics and nevirapine plasma concentrations. The association was investigated using Chi-square test (χ^2). Results from analyses to assess for bivariate associations between independent variables and outcome variable (nevirapine plasma concentrations) revealed lack of statistically significantly association at $P < 0.05$.

Table 17: The association between caregivers' and patients' demographic/clinical characteristics and nevirapine plasma concentrations

Variable		Nevirapine plasma concentration		Measure of association	
		≥3µg/ml n(%)	<3µg/ml n(%)	Pearson's χ^2	P-value
Caretaker gender	Male	33(78.6)	9(21.4)	1.05	0.31
	Female	123(70.7)	51(29.3)		
Caretaker age groups	14-49	114(72.2)	44(27.8)	0.001	0.97
	50-79	42(72.4)	16(27.6)		
Caretaker employment status	Businessman	74(71.2)	30(28.8)	0.11	0.74
	Not Businessman	82(73.2)	30(26.8)		
Caretaker marital status	Married	82(73.9)	29(26.1)	0.31	0.58
	Not married	74(70.5)	31(29.5)		
Caretaker education	Some education	147(73.1)	54(26.9)	1.20	0.27
	No education	9(60.0)	6(40.0)		
Disclosure status	Disclosed	97(69.3)	43(30.7)	1.71	0.19
	Not disclosed	59(77.6)	17(22.4)		
Orphan parental status	Parent	60(72.3)	23(27.7)	0.000	0.99
	Guardian	96(72.2)	37(27.8)		
Orphan age group	2-9	81(74.3)	28(25.7)	0.48	0.49
	10-14	75(70.1)	32(29.9)		
Orphan status	Double orphan	35(67.3)	17(32.7)	0.83	0.36
	Not double orphan	121(73.8)	43(26.2)		
Orphan sex	Male	81(75.0)	27(25.0)	0.83	0.36
	Female	75(69.4)	33(30.6)		
WHO clinical stage	Stage I and II	53(70.7)	22(29.3)	0.14	0.71
	Stage III and IV	103(73.0)	38(27.0)		
ART duration	≤1 year	10(83.3)	2(16.7)	0.78	0.38
	>1 year	146(71.6)	58(28.4)		

Significantly at P<0.05

The relationship between nevirapine plasma concentrations (categorical dependent variable) caregivers' and patients' demographic/clinical characteristics was measured by univariate logistic regression (Table 18). Disclosure status and orphan status presented notable associations. It was found that patients with disclosed HIV status were 1.5 times more likely to have within or above therapeutic plasma nevirapine concentrations, compared to undisclosed patients (UOR: 1.54, 95% CI: 0.81 to 2.94). Having at least one surviving parent was associated with 1.4 times more likely to have within or above therapeutic plasma nevirapine concentrations, compared to double orphans (UOR: 1.37, 95% CI: 0.70 to 2.69).

Table 18: Univariate logistic regression for caregivers' and patients' demographic/clinical characteristics associated with nevirapine plasma concentrations

Variable		Nevirapine plasma concentration		Crude association	
		≥3µg/ml n(%)	<3µg/ml n(%)	UOR (95% CI)	P-value
Caretaker gender	Male	33(78.6)	9(21.4)	0.66(0.29, 1.47)	0.31
	Female	123(70.7)	51(29.3)	1	
Caretaker age groups	14-49	114(72.2)	44(27.8)	1.01(0.52, 1.99)	0.97
	50-79	42(72.4)	16(27.6)	1	
Caretaker employment status	Businessman	74(71.2)	30(28.8)	1.11(0.61, 2.01)	0.74
	Not Businessman	82(73.2)	30(26.8)	1	
Caretaker marital status	Married	82(73.9)	29(26.1)	0.84(0.47, 1.53)	0.58
	Not married	74(70.5)	31(29.5)	1	
Caretaker education	Some education	147(73.1)	54(26.9)	0.55(0.19, 1.62)	0.28
	No education	9(60.0)	6(40.0)	1	
Disclosure status	Disclosed	97(69.3)	43(30.7)	1.54(0.81, 2.94)	0.19
	Not disclosed	59(77.6)	17(22.4)	1	
Orphan caregiver relationship	Parent	60(72.3)	23(27.7)	0.995(0.54, 1.84)	0.99
	Guardian	96(72.2)	37(27.8)	1	
Orphan age group	2-9	81(74.3)	28(25.7)	0.81(0.45, 1.47)	0.49
	10-14	75(70.1)	32(29.9)	1	
Orphan status	Double orphan	35(67.3)	17(32.7)	1.37(0.695, 2.69)	0.37
	Not double orphan	121(73.8)	43(26.2)	1	
Orphan sex	Male	81(75.0)	27(25.0)	0.76(0.42, 1.38)	0.36
	Female	75(69.4)	33(30.6)	1	
WHO clinical stage	Stage I and II	53(70.7)	22(29.3)	1.13(0.61, 2.09)	0.71
	Stage III and IV	103(73.0)	38(27.0)	1	
ART duration	≤1 year	10(83.3)	2(16.7)	0.50(0.11, 2.37)	0.39
	>1 year	146(71.6)	58(28.4)	1	

Significantly at P<0.05 AOR-Adjusted odds ratio CI- Confidence Interval

Confounding bias introduced in the crude association between plasma concentrations and caregivers' and patients' demographic/clinical characteristics in Table 18 was assessed in multivariate logistic regression models. Table 19 presents results from multivariate models to determine the association between nevirapine plasma concentration and caregivers' and patients' demographic/clinical characteristics after adjusting for each demographic and clinical characteristic. Adjusted associations of disclosure status and nevirapine concentration was: AOR: 1.56, 95% CI: 0.78 to 3.09 and for orphan status was: AOR: 1.38, 95% CI: 0.62 to 3.05.

Table 19: Multivariate binary logistic regression for caregivers' and patients' demographic/clinical characteristics associated with nevirapine plasma concentrations

Adjusting variable	AOR(95%CI)	P-value
Caretaker gender		
Male	0.65(0.27, 1.59)	0.35
Female	1	
Caretaker age groups		
14-49	0.95(0.42, 2.12)	0.899
50-79	1	
Caretaker employment status		
Businessman	1.05(0.54, 2.02)	0.89
Not Businessman	1	
Caretaker marital status		
Married	0.96(0.496, 1.87)	0.91
Not married	1	
Caretaker education		
Some education	0.58(0.17, 1.95)	0.38
No education	1	
Disclosure status		
Disclosed	1.56(0.78, 3.09)	0.21
Not disclosed	1	
Orphan caregiver relations		
Parent	1.19(0.56, 2.54)	0.65
Guardian	1	
Orphan age group		
2-9	0.83(0.44, 1.58)	0.57
10-14	1	
Orphan status		
Double orphan	1.38(0.62, 3.05)	0.43
Not double orphan	1	
Orphan sex		
Male	0.72(0.39, 1.35)	0.30
Female	1	
WHO clinical stage		
Stage I and II	1.11(0.59, 2.11)	0.74
Stage III and IV	1	
ART duration		
≤1 year	0.54(0.11, 2.65)	0.45
>1 year	1	

Significantly at P<0.05

9.3 Findings related to the challenges on ART in orphans

The percentage of respondents facing a particular challenge is indicated in Table 20. A response on individual item gives a clue on the challenges facing caregivers in taking care of orphans on ART. The major challenges identified in this study include Transport costs (87.5%), Long travelling time of more than 1 hour (39.4%), Lengthy waiting times of more than 2 hours causing long queues at the outpatient HIV clinics (75.5%), Forgetting appointment date (90.7%) and Missing at least one dose (46.7%).

Table 20: Proportions of respondents facing challenges in care of orphans (N=216)

No	Item	Frequency	Percentage
1	Incur transport costs to get to the clinic	189	87.5
2	Travel time of more than 1 hour from home to getting to clinic	85	39.4
3	Spending more than 2 hours from arriving at clinic to getting ART	163	75.5
4	Forgetfulness is one of challenges facing caretakers in taking orphan child for drug refill	196	90.7
5	Busy with work is one of challenges facing caretakers in taking orphan child for drug refill	73	33.8
6	Financial constraints is one of challenges facing caretakers in taking orphan child for drug refill	73	33.8
7	Forgetfulness was a reason for the child to miss at least one dose	21	46.7
8	Busy with work was a reason for the child to miss at least one dose	10	22.2
9	Away from home was a reason for the child to miss at least one dose	7	15.6
10	Being away from home is one of challenges facing caretakers in taking orphan child for drug refill	23	10.6
11	System used by HIV clinic administration for distribution of ART to patients	3	1.4
12	Inadequate information on how to administer ART	1	0.5

9.4 Investigation into knowledge regarding HIV/AIDS among caregivers of orphans

Table 21 indicates response on individual item related to the caregivers' knowledge regarding HIV/AIDS. Although the knowledge of the caregivers on HIV/AIDS was above average in some aspects, there was still lack of knowledge on isolated issues. It is matter of great concern as a total of 91.2% of respondents did not know that emergence of drug resistance might result for not taking ART as per instructions. It is not encouraging to note that fewer than half (44.4%) of the respondents could indicate contribution of ART in decreasing incidence of opportunistic infections. Only 24.1% realized that poor immunologic response result from inappropriate ART administration. It has been established that 74.5% of the respondents were aware on the major mode of HIV transmission; however 25.5% of respondents had insufficient knowledge on how HIV is transmitted.

Table 21: Percentages of HIV/AIDS knowledge questions (N=216)

No	Knowledge	Yes n(%)	No n(%)
1	Sexual means is a major way for transmission of HIV	161(74.5)	55(25.5)
2	ART contribute to improvements in the health and longevity through reduced plasma HIV RNA levels	67(31)	149(69)
3	ART contribute to improvements in the health and longevity through increased CD4 cell counts	63(29.2)	153(70.8)
4	ART contribute to improvements in the health and longevity through decreased incidence of opportunistic infections	96 (44.4)	120(55.6)
5	ART contribute to improvements in the health and longevity through decreased mortality	51(23.6)	165(76.4)
6	Harmful effects of not taking ART as instructed by dispenser include increased risk of mortality	136(63)	80(37)
7	Harmful effects of not taking ART as instructed by dispenser include rapid progression into AIDS	66(30.6)	150(69.4)
8	Harmful effects of not taking ART s as instructed by dispenser include poor immunologic response/ decreased CD4 cell counts	52(24.1)	164(75.9)
9	Harmful effects of not taking ART s as instructed by dispenser include increased drug resistance	19(8.8)	197(91.2)
10	Inadequate understanding of HIV transmission contribute to discrimination/stigma of HIV-infected orphans	170(78.7)	46(21.3)

10. DISCUSSION

The present study was designed to assess ART adherence practices in HIV-infected orphan children attending selected CTCs in Dar es Salaam. The focus of the study was to determine proportion of HIV-infected orphans with adequate ART adherence levels and factors influencing this pediatric category to adhere to antiretroviral medications by ART multiple measures.

10.1 The proportion of HIV-infected orphans with adequate ART adherence levels

In the present study, ART adherence levels determined using three different measurement tools; caretaker self report, clinic attendance consistency and nevirapine plasma levels determination. The adherent proportions established by these approaches were 79.6% by caretaker self report, 82.9% by clinic attendance consistency and 72.2% by nevirapine plasma levels determination.

The ART adherence levels established in this study are generally encouraging as high levels of adherence are necessary for viral suppression, prevention of resistance and disease progression (Bangsberg *et al.*, 2001). A meta-analysis of studies on ART adherence indicated that 77% of patients in Africa achieved adequate adherence of 95% compared to just 55% of patients in North America (Mills *et al.*, 2006). The reasons for high adherence levels attained in resource limited countries include free provision of drugs and care, use of fixed dose combinations (FDCs) and support through educational and counseling sessions (Batavia *et al.*, 2010).

The ART adherence in orphans determined in the present study is relatively low compared to pediatric adherence levels in studies which did not consider orphan status. Nabukeera-Barungi *et al.* (2007) reported pediatric adherence level of 94% using clinic-based pill counts, Reddington *et al.* 2000 and Van Dyke *et al.* (2002) found ART adherence levels in children by self reports to be 83% and 79% respectively. ART adherence levels of HIV-infected children from similar settings with current study were found to be 98% by caretaker report and 85% by nevirapine plasma levels determination (Mghamba *et al.*, 2012).

Self-report which is simple and inexpensive, is the most frequently used method but is notably susceptible to memory and social desirability biases leading to usual overestimation of adherence. Due to these shortcomings its accuracy is limited and tends to be biased toward greater adherence (Mills *et al.*, 2006). This is in agreement with findings by Minzi and Naazneen (2008), who validated self reported adherence and hospital pill count using unannounced home based pill count and found an overestimation of adherence by 35-40% compared to home pill count. The ART adherence levels established in present study by 3-day caregiver recall significantly exceeded adherence measured by nevirapine plasma level determination (Table 7). The finding has been explained to be in part due to tendency of patients to provide socially acceptable responses even if these responses are not truthful. On the other hand self reported adherence is exaggerated to please the provider or because of recall bias (DiMatteo and DiNicola, 1982; Sackett *et al.*, 1991).

The sensitivity of self reported adherence calculated in the present study was 26.7% which is relatively low and this method may fail to identify nonadherent patients. Thus, most persons requiring intervention would not be identified by self-report (Table 12). The analysis revealed that 74.4% of patients with good self reported adherence were found to have within or above normal therapeutic concentrations of nevirapine ($P=0.15$). However, 25.6% of patients reporting good adherence actually had levels below therapeutic drug concentrations. This would constitute the category of intrinsic poor adherence. These may have higher risks of contributing to the emergence of resistance to antiretroviral therapy. These results reveal that, whereas high self-reported adherence (S-RA) rates to ART correlated well with normal therapeutic plasma drug levels among these patients, S-RA as a tool may have some limitations in identifying non-adherent patients (Table 8). A study in Uganda showed ART adherence to be 93% for both orphans and nonorphans using caregiver 3-day prior report (Ntanda *et al.*, 2009). The same ART adherence level observed in the study is likely attributed to the low sensitivity of self-report method used in assessing adherence levels in the two pediatric categories.

ART adherence measured based on clinic attendance was 82.9%. This figure is closer to the one revealed by Rutayuga *et al.*, 2011 who found attendance based adherence in Mwananyamala Hospital in Dar es Salaam to be 80.9%. In clinical trials in which patients may only be able to obtain specific medications from the investigators, clinic attendance may be a more reliable measure of nonadherence. A study on HIV-infected persons did find that missed clinic visits were a strong independent predictor of worse virologic outcomes (Cramer *et al.*, 1990). Studies in other disease states suggest that missed clinic visits may not correlate well with medication adherence (Choo *et al.*, 1999; Milgrom *et al.*, 1996).

The assessment of adherence by nevirapine plasma levels was carried out in this study and found 27.8% of tested patients to be nonadherent (Table 7). These patients are standing a high risk of inadequate viral suppression and a subsequent potential of developing and accumulating resistant viral strains (Bossi *et al.*, 2004). The present findings are similar to that obtained in the study done in Netherlands among patients at a risk of treatment failure in a routine clinical care, in which 27.4% of the plasma concentrations were classified as having sub-therapeutic ARV plasma concentrations (de Maat *et al.*, 2003). In the other study conducted in North-Western Tanzania, 28.3% of adult HIV-infected patients were found to have sub-therapeutic plasma antiretroviral drug concentrations. Furthermore they found that the proportion of patients with sub-therapeutic ARV plasma concentrations was significantly higher in patients with high viral loads (≥ 400 copies/ μ l) than those with low viral loads (< 400 copies/ μ l) [39.7% versus 19.0%] (Gunda *et al.*, 2013). Mghamba *et al.* (2012) assessed ART adherence in HIV-infected children and found 15% to have sub-therapeutic nevirapine levels. Plasma levels of antiretrovirals provide unequivocal evidence that a medication has been ingested. Plasma levels have the potential to be highly objective adherence measures. Nevertheless, it should be noted that plasma drug level is not necessarily predictive of adherence behavior in all patients. This is due the fact that the pharmacokinetics of many antiretrovirals, especially protease inhibitors, can vary significantly from person to person. Thus, factors unrelated to adherence (eg, drug interactions, individual metabolism variation, poor quality of the drug) may push the plasma level up or drive it below a threshold considered to represent adherence. In addition, plasma levels only monitor adherence to a dose

prior to a study visit or clinic visit (Hoetelmans *et al.*, 1998; Hsu *et al.*, 1998). However, in this study, the measurement of ART adherence on nevirapine plasma levels provided information on adherence for several days due to long plasma half-life of nevirapine.

Bivariate analysis among adherence measures showed statically significant correlations between nevirapine plasma levels and clinic attendance ($P=0.02$), others had no statistically significant correlation (Table 8). The statistically significant correlation between adherence determined by nevirapine plasma levels and clinic attendance based adherence attests to the value of clinic attendance for measuring adherence. Similar but not statistically significant ART adherence levels were observed among patients on ART by caretaker self report and nevirapine plasma level determination, indicating that self reported adherence measure has a potential to be a reliable measurement tool in adherence provided it is improved and standardization is made to it. This will enhance ART adherence measurement particularly in resource limited settings such as Tanzania because self reporting can be conducted with simplicity and at low costs (Oyugi *et al.*, 2004; Simoni *et al.*, 2006). Multivariate logistic regression indicated that patients who reported good self reported adherence were 0.6 times more likely to have within or above therapeutic plasma nevirapine concentrations, compared to those who reported poor adherence (crude odds ratio: 0.62, 95% confidence interval (CI): 0.30 to 1.27). The calculated odds ratio is less than 1 ($OR < 1$), implying the effect of good or bad self reported adherence on nevirapine plasma concentration is low (Table 10).

The wide variation in adherence by the three different measurement approaches was noted. This demonstrates the critical importance of choice of adherence measure in clinical care. The lack of a perfect measure suggests that multiple measures should be used, possibly to create a composite measure. Different adherence measures may complement each other, it is therefore recommended to use more than one measure whenever possible. For example, plasma levels of antiretrovirals can supplement pill counts. Self-reported adherence or a survey that asks patients about behaviors (cacheing medications, weekend effects, etc.) that might result in underreporting adherence can complement electronic monitoring.

10.2 HIV-patients response to Anti-Retroviral Therapy (ART)

HIV infection is characterized by a gradual deterioration of immune function. CD4 counts is used to determine how well the immune system is working in people who have been diagnosed to have HIV and it is also used in monitoring response to ART in HIV-infected patients. World Health Organization (WHO) guidelines for monitoring HIV-infected individuals taking combination antiretroviral therapy (cART) in resource-limited settings recommend using CD4 count changes to monitor treatment effectiveness (Muzah *et al.*, 2012).

CD4 counts are routinely used world-wide in monitoring response to ART in HIV-positive patients. Effective ART typically results in increased CD4 count as observed in this study in which 72% of patients showed increase in CD4 counts. A number of studies reported the continuous increase of CD4 counts among the HIV/AIDS subjects who were receiving highly active antiretroviral therapy (HAART) (Bosch *et al.*, 2006; Crystal *et al.* 1997; Hunt *et al.*, 2003; Muzah *et al.*, 2012). CD4 cell counts generally decrease as HIV progresses in untreated HIV-infected subjects (Ogg *et al.*, 1998; Viviane *et al.* 2009)). Tiwari *et al.* (2008) observed among the subjects not receiving ART the mean CD4 count dropped from 281/mm³ to 214/mm³ in six months interval.

In the present study 28% of patients had blunted CD4 count responses. This phenomenon is referred to as discordant immune response and is associated with an increased risk of developing an AIDS event or death (Grabar *et al.*, 2000; Moore *et al.*, 2005). Most HIV-infected individuals realize CD4 response on ART. However, there exists a subset of HIV-infected adults who do not exhibit appropriate CD4 response on ART, despite virologic suppression. These CD4 non-responders have been shown to experience higher rates of HIV-associated morbidity and mortality (Wright *et al.*, 2011). Moore *et al.* (2005) found 20% - 40% of patients on ART do not show a significant increase in CD4 count despite viral suppression. Discordant immune response may arise either as a result of failed immune reconstitution or the excessive destruction of CD4 (Benveniste *et al.*, 2005). On the other

hand, Zanoni *et al.* (2012) revealed presence of chronic diarrhea; lower baseline hemoglobin and virologic failure to be significantly associated with poor CD4 recovery.

Initial CD4 counts were categorized into initial CD4 counts above 200 cells/mm³ and initial CD4 counts below 200 cells/mm³ in order to assess the impact of initial CD4 counts on immunological response. It was found that 77.1% and 22.9% of patients with increase in CD4 counts had initial CD4 counts above 200 cells/mm³ and initial CD4 counts below 200 cells/mm³ respectively. However, there was significant proportion of patients from the two categories with marked inappropriate CD4 response. The statistical analysis revealed insignificant association between baseline CD4 and immunological response observed (Table 14). The finding in the present study is different from what have been observed in some studies. Viviane *et al.* (2009) and Kulkarni *et al.* (2011) found a higher initial CD4 counts would result in a better rate of recovery of patients on ART. Several studies conducted in resource-rich settings have shown that a low baseline CD4 count is associated with discordant immune response (Kaufmann *et al.*, 2005; Schechter and Tuboi, 2006).

Duration of ART was found to be significant determinant of changes in CD4. Out of 118 of patients with increased CD4 counts, 116 (98.3%) were on treatment for more than one year and only 2(1.7%) were on treatment for less than one year (Table 15). Similar findings were revealed by Adams and Luguterah (2013); their mixed model results showed that the duration of treatment is a significant determinant of a Patients CD4+ cell count. In some studies, CD4 counts seemed to reach a plateau after the first 2–3 years of ART (Kaufmann *et al.*, 2003), whereas other studies found that, for selected groups of patients with a well-suppressed HIV-1 RNA load, there were continuous, albeit small increases in CD4 counts even after 3–4 years of ART (Hunt *et al.*, 2003). A study of 314 HIV-infected gay men for whom CD4 counts were determined for at least 2 years after the initiation of potent antiretroviral therapy revealed that, regardless of CD4 count at the time of initiation of HAART, CD4 counts increased significantly in the first 2 years after initiation. However, from 2 to 3.5 years after initiation, these cell count values neither increased nor decreased (Tarwater *et al.*, 2001). A study from

the Dutch AIDS Therapy Evaluation project Netherlands (ATHENA) cohort found that a lower CD4 count at the start of antiretroviral therapy was associated with a lower plateau CD4 count after 5–7 years (Gras *et al.*, 2006).

The current study has shown changes in CD4 counts are not significant determinant of adherence measures at $P < 0.05$ (Table 16). Lack of significant correlation between changes in CD4 counts and measures of adherence have been observed in a number of studies. This is likely due to the fact that changes in CD4 counts can lag behind other clinical markers of therapeutic success or failure and have not correlated consistently with other measures of adherence (Bisson *et al.*, 2008 and Simoni *et al.*, 2006).

A notable finding in the present study is the same proportion of patients (72%) with increased CD4 cell counts and those with nevirapine concentration within or above therapeutic window. The study indicated association between plasma nevirapine plasma concentrations and CD4 cells response among HIV-positive patients on ART. This observation is in agreement with findings by Mghamba *et al.* (2012) who reported that nevirapine plasma concentration could be a significant predictor of ART adherence and subsequent CD4⁺ cell count response.

10.3 Relationship of Demographic/Clinical characteristics to nevirapine plasma concentrations

Adherence is expected to relate to demographic factors such as sex, occupation, age and disclosure status. Education may impact adherence in several ways including facilitating communication with health care providers (Van Dyke *et al.*, 2002). Developed-country studies have shown no significant association between gender and virological response (Purkayastha *et al.*, 2005).

In the present study, bivariate associations between independent caretakers' sociodemographic characteristics and clinical characteristics of HIV-infected orphans versus nevirapine plasma concentrations were analyzed (Table 17). It is noticeable that no statistically significant correlation was observed. All variables measured in the survey were not independently associated with ART adherence. The lack of statically significant

correlation between demographic variables and ART adherence observed in the current study might be due to complex confounding and casual pathways that mask associations between these variables and ART adherence. On the other hand, a child's adherence to ART is strongly influenced by caregiver and family support (Haberer and Mellins, 2009); unfortunately this aspect was not investigated in the current study and might be responsible for masking influence of the variables on adherence investigated in the current study. HIV-infected orphans in HIV treatment centers can achieve superb therapeutic outcomes provided appropriate resources are dedicated to their care (Ntanda *et al.*, 2009). Even though these factors did not distinguish good adherers from poor adherers in this setting, they likely have an impact on the quality of life of people living with HIV and may influence patients' retention in ART programs (Bajunirwe *et al.*, 2009; Rosen *et al.*, 2007).

10.4 Identified challenges on ART in orphans

Although patients are highly motivated to take ART as prescribed, there are numerous constraints that challenge the optimal levels of adherence required to ensure positive treatment outcomes and prevent drug resistance. There are many barriers to adherence in both developed and developing countries. It is important to identify these barriers and develop strategies to improve long-term adherence. A number of reasons for defaulting from ART programmes have been identified or hypothesized. These include long distances to be travelled to ART sites, transport costs and waiting time (Mukherjee *et al.*, 2006; Murray *et al.* 2009; Roura *et al.* 2009).

The present study established main challenges facing caretakers in taking care of HIV-infected orphan children. Forgetfulness was indicated to be a challenge facing caretakers in taking orphan children for drug refill (90.7%) and it was also implicated for the child to miss at least one dose (46.7%). This is in agreement with findings by Ugwu and Eneh (2013) where they found the commonest caregiver-related reason was forgetfulness (55.2%). This has been observed in several other African studies as well as in studies conducted in resource-rich settings (Mills *et al.*, 2006). The majority of the participants who did not adhere to ART

provided varied motives for their defaults. A large proportion (46.1%) of people cited forgetfulness as reasons for missing therapy (Tiyou *et al.*, 2010).

Travel difficulty due to transport costs (87.5%) and travel time of more than 1 hour (39.4%) was reported in this study. Distance to treatment centres is of great concern to people living with HIV/AIDS (PLHIV) and costs associated with traveling may limit ART adherence. Studies have shown that patients who travelled more than one hour to hospital were more likely to be non-adherent (Alker *et al.*, 2004; Posse *et al.*, 2008 and Wasti *et al.*, 2011). Among patients who were not adherent to ART, 53.8% indicated to be due to distance to health facilities (Sasaki *et al.*, 2012). Thus, any new policy will need to address this issue and improve access to medical care services by integrating ART treatment into the mainstream of health care rather than concentrating treatment in a limited number of ART centres, which may be hard to reach for many patients.

It was also established that patients spend unnecessary long periods of time from arriving at clinic to getting ART; 75.5% of study population reported to spend more than two hours from arriving at the clinic to getting ARVs causing long queues at the outpatient HIV clinics. Almost half (42%) of health workers interviewed in Tanzania identified long waiting times as a problem and in Botswana, 57% of respondents reported that they spend four or more hours at the clinic, with the longest wait being 12 hours (Hardon *et al.*, 2007). Olowookere *et al.* (2012) assessed the waiting time and perceived satisfaction with care among PLHIV at an antiretroviral clinic in Nigeria. In their study majority (72.9%) of the respondents reported that the time elapsed between entry into the clinic and access to medical services (waiting time) was more than an hour and called for authority to take measures to reduce patient waiting time before access to medical services. Long waiting times may discourage patients from going to clinics which call for streamlined processes to assist patients remaining in care.

10.5 HIV/AIDS Knowledge among caregivers of orphans

Appropriate HIV/AIDS knowledge may play an important role in preventing further spread of HIV/AIDS among the general population. Basic knowledge about HIV/AIDS appears to be limited in African countries. Lack of practical knowledge on HIV/AIDS, ART and poor individual adherence to treatment are among the root causes of ineffective ART service delivery.

The present study evaluated HIV/AIDS knowledge among caretakers of HIV-positive orphans. In general, the study revealed a variable lack of knowledge about HIV/AIDS among caretakers.

Knowledge regarding HIV transmission modes was fairly good (74.5%). However, it was revealed that 25.5% of interviewed research subjects are not familiar with major means of HIV transmission which shows a great deal of work is to be done to create awareness on the methods for the transmission of HIV. Inadequate understanding of HIV transmission was cited by 78.7% of respondents to be a major factor contributing to discrimination/stigma of HIV-infected orphans. Nyablade *et al.* (2005) reported that lack of knowledge results in the fear that HIV could be transmitted through ordinary, daily interactions with people living with HIV/AIDS that involve exchange of body fluids was common. There is a reverse correlation between level of knowledge about HIV/AIDS and level of stigma. People who express stigmatising attitudes about HIV/AIDS often have retained misinformation about the transmission of HIV (Brown *et al.*, 2001). A similar study done in Uganda concluded that people with correct information on HIV transmission help in the reduction of misconception, myths, blame and discrimination (UAIDS, 2003).

The knowledge on how ARVs contribute to improvements in the health and longevity was assessed and 44.4% of the respondents indicated it is through decreased incidence of opportunistic infections. Harmful effects of not taking ARVs as instructed by dispensers were linked to poor immunologic response, increased risk of mortality and rapid progression into

AIDS by 24.1%, 63% and 30.6% of respondents respectively. Potent antiretroviral treatment has led to a dramatic decrease in human immunodeficiency virus (HIV)-associated morbidity and mortality (Palella *et al.*, 1998). Protease inhibitors became widely available in early 1996; many HIV clinical specialists have noted a marked decrease in the occurrence of AIDS-related opportunistic infections (Forrest *et al.*, 1998; Jacobson and French, 1998). The current study revealed that only 44.4% are knowledgeable on role of ART in decreasing AIDS-related opportunistic infections. This situation may influence the proportion of ART adherent patients.

Majority of respondents (91.2%) were not aware of likelihood for emergence of drug resistance from inappropriate ARVs administration. Inappropriate use and practices of antiretroviral therapy is one of the factors which contribute to the increased levels of HIV drug resistance (HIVDR) (Adje *et al.*, 2000; Yerly *et al.*, 1999). The occurrence of HIV drug resistance may result in failure by the first line regimens necessitating switching to the second line regimens which are expensive and not available in most resource-limited settings. In order to maximize long-term effectiveness of available ART, routine HIVDR Surveillance should be implemented and specific public health interventions must be taken on determinants of HIVDR to minimize further emergence and transmission of HIVDR (Bertagnolio *et al.*, 2012).

11. LIMITATIONS OF THE STUDY

There are important limitations to this research that should be pointed out:

- One is that being a cross sectional survey it captures data at one point in time. The study could not observe change in ART adherence over time nor could actual behavior be observed. Furthermore, a cross-sectional survey design may not fully control for threats to internal validity.

- These data are limited in their representativeness of the general population; there are limitations relating to the extent to which the study results can be generalized. The study group was urban so the study findings do not necessarily reflect practices in other settings. The findings may not valid to be generalised to the target population.

- The pharmacogenetic factors that may influence the functioning of enzymes involving in the biotransformation of nevirapine (CYP 2B6 and CYP3A4) were not investigated.

12. CONCLUSION

This study has demonstrated that significant proportions of HIV-infected orphans on ART have inadequate adherence and inappropriate CD4 response. This calls for interventions to be instituted to promote adherence in this pediatric category. There is wide variation in adherence levels measured by self-reported method, consistency of clinic attendance and nevirapine plasma determination indicating that efforts are needed to find out better methods of adherence measurement for both clinical care and research. Inadequate knowledge on several aspects of HIV/AIDS, unnecessary long waiting time and forgetfulness were identified to impair ART adherence of orphans; interventions to address these challenges urgently needed.

13. RECOMMENDATIONS

More attention should be paid on ART adherence in HIV-infected orphan children. An intervention should be initiated that will identify HIV-infected orphans receiving ART in HIV clinics and providing psycho-social support to these children. Counselors working at HIV clinics should be afforded additional skills training in adherence counseling so as to deliver their services effectively and efficiently. The capacity of home based caregivers should be strengthened to give optimal care to orphans on ART including providing social welfare grants, strengthening of follow-up mechanisms as a way of monitoring the progress of orphans on ART and the quality of care they receive from their caregivers.

Investigations should be carried out to identify root causes of discordant immune responses for some patients on ART and appropriate measures should be taken to address poor CD4 recovery.

Stigma needs to be addressed at the community level in order to minimize its impact; there is a need for programmes to be implemented in the community aimed at changing HIV related stigma, education is needed to develop realistic risk-perception to reduce stigma. Studies should be conducted to understand development of stigma and design reduction interventions on stigma. Operations research is required to develop and pilot interventions that reduce stigma.

Self-reported measures of adherence and clinic attendance based adherence were somehow compared with adherence measured by nevirapine plasma levels, providing insights into using these potential low-cost measures of adherence on routine service delivery provided improvement and standardization is done to it. Operational research is required to define how to improve the accuracy of the self-reported method because it is the most feasible method in clinical practice.

As most patients reported forgetfulness as the main reason for missing ART, specific counseling is needed to overcome this challenge. Strategies for addressing forgetfulness should be explored including using targeted reminders such as diaries, phone alarms and incorporating ART regimen in routine activities.

Measures should be put in place to address the challenge of patients spending unnecessary long time at clinics to avoid its detrimental effects including patients opting not to come to clinics for counseling and antiretroviral medication refills.

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LIST OF APPENDICES

Appendix I: Approval of Ethical Clearance

MUHIMBILI UNIVERSITY OF HEALTH AND ALLIED SCIENCES

Directorate of Postgraduate Studies

<p>P.O. BOX 65001 DAR ES SALAAM TANZANIA.</p> <p>Website: http://www.muhas.ac.tz</p>		<p>Tel: +255-(0)22-2150302 Ext 207. Tel (Direct): +255-(0)22-2151378 Telefax: 255-(0)22-2150465 E-mail: dpgs@muhas.ac.tz</p>
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Ref. No. MU/PGS/SAEC/Vol. VI/

27th May, 2013

Dr. Mopei, Nassoro Athumani
MSc. Clinical Pharmacology
MUHAS.

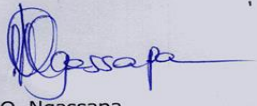
RE: APPROVAL OF ETHICAL CLEARANCE FOR A STUDY TITLED " ASSESSMENT OF ADHERENCE TO ANTIRETROVIRAL TREATMENT IN ORPHANED CHILDREN LIVING IN DAR ES SALAAM - TANZANIA"

Reference is made to the above heading.

I am pleased to inform you that, the Chairman has on behalf of the Senate approved ethical clearance for the above-mentioned study.

Thus ethical clearance is granted and you may proceed with the planned study.

Please liaise with bursar's office to get your research fund.


 Prof. O. Ngassapa
DIRECTOR, POSTGRADUATE STUDIES

/emm

cc Vice Chancellor, MUHAS
cc Deputy Vice Chancellor – ARC, MUHAS
cc Dean, School of Medicine, MUHAS

Appendix IIa: Consent Form- English Version

Study No

Title: Assessment of Adherence to Antiretroviral Treatment in Orphaned Children Living In Dar Es Salaam-Tanzania.

To Parent/Caretaker

Foreword

Hello! My name is, a nurse counselor working at this HIV clinic and also carrying out a research on Assessment of Adherence to Antiretroviral Treatment in Orphaned Children Living In Dar Es Salaam-Tanzania.

Purpose of study

The aim of this research is to assess adherence to antiretroviral therapy among Orphan HIV-infected children between 2-10 years old attending Care and Treatment Centres (CTCs) in Dar es Salaam, Tanzania. The findings of this research will be used to improve treatment of HIV infected patients using Highly Active Antiretroviral Therapy (HAART).

Participation in the Study

Information will be gathered through questionnaire administered by research assistant to Parent/guardian/caretaker. The study also involves taking blood samples from some HIV-infected orphaned children. All the information gathered will be strictly confidential and used for research purposes only.

Right to refusal or withdrawal

Participation in this study is purely voluntary and you have the right to decline from participating in the study. You can also withdraw your participation any time during the course of study without giving reasons.

Risks

No anticipated risk or harm that may result from participating in this study.

Benefits

Your participation in this study is very beneficial to HIV infected patients and the country at large as this will help in improving treatment of such patients. The findings will give ideas to health professionals on how to improve adherence to ART particularly in orphaned children. You are also informed that there will not be any payment in the form of cash money or any other form.

Contact Person

The principal investigator Dr. Nassoro A. Mopei (Mob. 0713-596363) is a key contact person with regard to any queries about this study. However, in the event of questions about your rights as a participant, you may call the Chairman of the University Senate Research and Publications Committee, MUHAS P.O. Box 65001, Dar es Salaam. Telephone; 2150302-6.

Signing the Consent

I would like to discuss with you few issues on your child modality of taking antiretroviral therapy. I ask for your participation in this study by responding to my questions. It shouldn't take more than ten minutes. Would you mind speaking with me?

If you agree to participate in this study please sign in this consent form.

I have read and understood the contents of this form and I have been given satisfactory explanation with all my questions answered. I therefore consent to participate in this study.

Signature of Participant Date

Signature of Research Assistant Date

Appendix IIb: Consent Form- Kiswahili Version

FOMU YA RIDHAA

Namba ya utafiti

Kichwa cha habari: Tathmini ya matumzi thabiti ya dawa za kukata makali ya virusi vya ukimwi (ARVs) kwa watoto yatima wanaoishi Dar es Salaam.

Kwa Mzazi/Mlezi

Utangulizi

Habari za leo! Mimi naitwa, mshauri wa masuala ya tiba ya ukimwi katika kliniki hii na vilevile ninafanya utafiti kuhusu matumizi thabiti ya ARVs kwa watoto yatima wanaoishi Dar es Salaam.

Dhumuni la utafiti

Dhumuni la utafiti huu ni kubaini hali ilivyo kuhusiana na matumizi thabiti ya dawa za ARVs kwa watoto yatima katika Jiji la Dar es Salaam. Matokeo ya utafiti huu yatasaidia kuboresha matibabu ya watu wanaoishi na virusi ya ukimwi.

Kushiriki katika utafiti huu

Taarifa juu ya matumizi thabiti ya dawa za ARVs kwa watoto yatima katika Jiji la Dar es Salaam zitakusanywa kupitia dodoso litakalotolewa kwa mzazi/mlezi wa mtoto. Utafiti huu utahusisha pia kuchukua damu toka kwa watoto yatima wanaotumia madawa haya.

Usiri wa Taarifa za Mshiriki

Unahakikishiwa kuwa taarifa zozote zitakazopatikana kutoka kwako wakati wa utafiti huu zitapewa usiri mkubwa sana na hazitatumika kwa malengo mengine yoyote tofauti na utafiti husika.

Athari za Utafiti huu kwa Mshiriki

Hakuna athari au madhara yoyote yatakayokupata kutokanana na kushiriki katika utafiti huu.

Faida ya kushiriki katika utafiti huu

Ushiriki wako katika utafiti huu una faida kubwa sana kwa mtoto wako na watumiaji wengine wa dawa hizi. Pia utakuwa umeisaidia kutambua hali halisi ya watumiaji wa dawa hizi wanavyozingatia maelekezo ya matumizi ya dawa, hii itawawezesha wataalam wa afya kuchukua hatua muafaka za kuboresha matibabu haya. Unafahamishwa pia kuwa hakutakuwa na malipo yoyote kwa njia ya fedha au njia nyingine yoyote ile.

Haki ya kushiriki au kutokushiriki katika utafiti huu

Ushiriki wako katika utafiti huu ni wa hiari kabisa. Unayo haki ya kushiriki au kutokushiriki bila kulazimika kutoa taarifa. Pia unayo haki ya kukataa kuendelea kushiriki/kuacha kujibu maswali wakati wowote utakapojisikia kufanya hivyo na hakutakuwa na hatua yoyote itakayochukuliwa dhidi yako au kulaumiwa kwa kufanya hivyo.

Mawasiliano

Wasiliana na mtafiti mkuu, Dk. Nassoro A. Mopei kwa simu namba 0713-596363 wakati wowote utakapokuwa na maswali au jambo lolote lenye kuhitaji ufafanuzi kuhusu utafiti huu. Hata hivyo, endapo utakuwa na maswali kuhusu haki yako kama mshiriki unaweza pia kuwasiliana na Mwenyekiti wa Baraza la utafiti na Uchapishaji wa Chuo kikuu cha sayansi ya tiba Muhimbili, S.L.P. 65001, Dar es salaam. Simu namba 2150302-6

Kukubali kushiriki

Ningependa kujadiliana nawe juu ya matumizi thabiti ya dawa za ARVs kwa mtoto wako. Ninakuomba kuchukua muda wako ili niweze kujadiliana nawe juu ya matumizi ya dawa hizi kwa mwanao. Kwa hivyo ninakuomba ushriki katika utafiti huu kwa kujibu maswali nitakayo kuuliza katika dodoso. Majadiliano yenyewe hayatachukua zaidi ya dakika kumi. Je upo tayari kwa majadiliano hayo?

Iwapo unakubali kushiriki katika utafiti huu, tafadhali thibitisha kwa kujaza na kusaini sehemu ya fomu hii hapa chini.

Mimi nimesoma/nimesomewa na kuelewa yaliyomo kwenye fomu hii na maswali yangu yote yamejibiwa vizuri. Hivyo ninakubali mwenyewe kwa hiari yangu bila kushurutishwa au kushawishiwa kushiriki katika utafiti huu.

Sahihi ya Mshiriki Tarehe

Sahihi ya mtafiti msaidizi Tarehe

Asante sana

Appendix IIIa: Questionnaire to Caretaker for Assessment of ART Adherence in Orphans (English Version)

Study No.

1. Date of interview/...../.....
2. Initials of interviewer
3. Name of Care and Treatment Centre (CTC)
4. Unique CTC ID no.
5. Sex of Primary Caregiver: a. Male () b. Female ()
6. What is your date of birth/...../.....
7. What is your marital status? a. Married () b. Single () c. Others ()
8. What is your highest level of Education? a. Primary education ()
b. Secondary education () c. College/University () d. No formal education ()
9. What is your occupation: a. Businessman () b. Salaried worker ()
c. House wife () d. Farmer () e. Student ()
f. Other ().....
10. What is child's orphan status: a. Paternal orphan () b. Maternal orphan ()
c. Double orphan ()
11. How are you related to the child a. Parental relationship () b. Non-parental relationship ()
12. Have you disclosed child's HIV status outside your family members?
a. YES () b. NO ()
13. What challenges do you face in taking orphan child for drug refill?
a. Forgetfulness () b. Away from home () c. Busy with work ()
d. Financial constraints () e. Others ()
14. Have you incurred any transport costs to get to the clinic today?
a. YES () b. NO ()
15. How much time did you spend from where you stay to getting to clinic today?
16. In your previous visit to clinic, how much time did you spend from arriving at clinic to getting ARVs?

17. Do you think information/instruction given by drug dispenser is enough for the caregiver to know how to administer ARVs to child correctly? a. YES () b. NO ()
18. Are you satisfied with the system put in place by HIV clinic administration for distribution of ARVs to patients? a. YES () b. NO ()
19. What is the dosage form of ARVs given to your child?
 a. Tablets () b. Syrups () c. Others ()
20. How drugs given to your child are formulated?
 a. Combined product () b. Separate components ()
21. What is type of ART combination regimen given to your child?

Drug 1	Drug 2	Drug 3
<u>Nevirapine</u>		

22. What is the total daily dose of each drug given to your child?

Drug	Total Daily Dose
<u>Nevirapine</u>	

23. What time has elapsed since the last ARV dose was administered to child?
24. In your opinion, how ARVs contribute to improvements in the health and longevity?
 a. Reduced plasma HIV RNA levels () b. Increased CD4 cell counts ()
 c. Decreased incidence of opportunistic infections () d. decreased mortality ()
 e. Others ()
25. What do you consider to be harmful effects of not taking ARVs as instructed by dispenser?
 a. Increased risk of mortality () b. Rapid progression into AIDS ()
 c. Poor immunologic response/ Decreased CD4 cell counts ()
 d. Increased drug resistance ()
 e. Others ()

26. Many patients have troubles in taking their ARV doses as prescribed, how many of the ARV doses did the child miss in the last three days? a. None () b. One ()
c. Two () d. More than Two ()

If the child missed at least one dose in Qn. 26; ask Qn. 27

27. I know it is difficult to take medication on daily basis, please tell me what causes the child to miss at least one dose. a. Forgetfulness () b. Away from home ()
c. Busy with work () d. Being too ill () e. Side effects ()
f. Others ()

28. There a number of ways in which HIV is transmitted; what do you consider to be a major way for transmission of these viruses? a. Sexual means () b. Non-sexual means ()

29. Which factors do you think contribute to discrimination/stigma of HIV-infected orphans?
a. Religious Beliefs ()
b. Cultural Beliefs ()
c. Inadequate understanding of HIV transmission ()
d. Incurability of HIV/AIDS ()
e. Fears Associated With HIV/AIDS ()
f. Manifestation of HIV/AIDS in Cultures with an Extensive Communal Life ()
g. Other ()

THANK YOU FOR YOUR COOPERATION

Appendix IIIb: Dodoso kwa Mzazi/Mlezi wa Mtoto Yatima kwa Ajili ya Kutathmini Matumizi Thabiti ya Dawa za Kukata Makali ya Virusi Vya Ukimwi (ARVs)

Namba ya utafiti

1. Tarehe ya usaili/...../.....
2. Utambulisho wa Muhojaji
3. Jina la Kituo cha Huduma na Matibabu (CTC)
4. Namba ya utambulisho ya mgonjwa
5. Jinsia ya mzazi/mlezi a. Mme () b. Mke ()
6. Umezaliwa tarehe gani?/...../.....
7. Hali yako ya ndoa ni ipi? a. Nimeoa/ Nimeolewa () b. Sijaoa/sijaolewa ()
c. Nyingine ()
8. Je una kiwango gani cha elimu?
a. Elimu ya Msingi () b. Elimu ya Sekondari () c. Elimu ya Juu () d. Sijasoma ()
9. Unafanya kazi gani? a. Mfanya biashara () b. Mfanyakazi wa mshahara () c. Mama wa nyumbani () d. Mkulima () e. Mwanafunzi () f. nyingineyo ()
10. Hali ya uyatima wa mtoto wako ni ipi? a. Yatima wa mama () b. Yatima wa baba ()
c. Yatima wa mama na baba ()
11. Una uhusiano gani na huyu mtoto mgonjwa: a. Mzazi () b. Mlezi ()
12. Je umewaeleza watu wengine nje ya familia juu ya hali ya mambukizi ya virusi vya Ukimwi (VVU) ya huyu mtoto? a. NDIYO () b. HAPANA ()
13. Ni changamoto gani zinazokukabili katika zoezi la kumleta mtoto kuchukua dawa?
a. Kusahau () b. Kusafiri () c. Kuwa na shughuli nyingi ()
d. Matatizo ya kifedha () e. Nyinginezo ()
14. Kuna gharama yoyote ya usafiri uliyoingia kwa wewe kumleta mtoto hapa kliniki?
a. NDIYO () b. HAPANA ()
15. Umetumia muda gani toka unapoishi mpaka kufika hapa kliniki?
16. Ulipokuja kliniki mara iliyoita, ulitumia muda gani toka ulipofika kliniki mpaka kupata ARVs kwa ajili ya mtoto wako?

17. Unafikiri maelekezo yanayotolewa na mtoa dawa yanatosha kumuwezesha mzazi/mlezi wa kutoa dawa kwa mtoto kwa usahihi? a. NDIYO () b. HAPANA ()
18. Je unaridhika na utaratibu uliowekwa na kliniki katika kutoa ARVs kwa wagonjwa? a. NDIYO () b. HAPANA ()
19. ARVs anazopewa mtoto wako zipo katika hali gani? a. Vidonge () b. Maji maji () c. Nyingineyo ()
20. ARVs anazopewa mtoto wako zipo ktika mfumo upi? a. Zimechanganywa pamoja () b. Hazijachanganywa pamoja ()
21. Ni aina gani za dawa anazopewa mwanao kwa ajili ya kukata makali ya virusi vya ukimwi?

<u>Dawa 1</u>	<u>Dawa 2</u>	<u>Dawa 3</u>
<u>Nevirapine</u>		

22. Ni dozi kiasi gani kwa kila dawa ya ARVs kwa siku anayopewa mtoto wako?

<u>Dawa</u>	<u>Jumla ya dozi kwa siku</u>
<u>Nevirapine</u>	

23. Ni muda gani umepita toka mtoto wako alipopewa dozi ya mwisho ya ARVs.....
24. Kwa maoni yako, dawa za kupunguza makali ya ukimwi (ARVs) zinachangia vipi katika kuongeza muda wa kushi na kuboresha afya kwa ujumla? a. Zinapunguza idadi ya virusi katika damu () b. Zinaongeza idadi ya chembechembe za CD4 () c. Zinapunguza maambukizi nyemelezi () d. Zinapunguza idadi ya vifo () e. Nyingineyo ()
25. Unafikiri ni madhara gani yanaweza kutokea iwapo mgonjwa hatozingatia kutumia dawa kwa mujibu wa maelekezo anayopewa? a. Hatari ya kupoteza maisha () b. Kujitokeza kwa haraka kwa ugojwa wa UKIMWI ()

- c. Kupungua kwa idadi ya chembechembe za CD4 ()
- d. Kutokea kwa usugu wa virusi vya ukimwi dhidi ya ARVs ()
- e. Nyingineyo ()
26. Nafahamu kuwa ni vigumu wagonjwa wengi kutumia dozi za ARVs kama inavyoelekezwa na daktari, kwa upande wa mtoto wako amekosa dozi ngapi za dawa hizi katika siku tatu zilizopita? a. Hajakosa dozi hata moja () b. Amekosa dozi moja () c. Amekosa dozi moja mbili () d. Amekosa dozi moja zaidi ya mbili ()
- Iwapo mtoto amekosa angalau dozi moja ya ARVs katika swali namba 26, uliza swali namba 27.
27. Najua ni vigumu kunywa dawa kila siku ni sababu gani zilizokufanya ushindwe kumpa mtoto dawa? a. Kusahau () b. Ulisafiri () c. Ulikuwa na shughuli nyingi () d. Ulikuwa unaumwa sana () e. Kutokana na madhara ya dawa () f. Sababu nyingineyo ()
28. Kuna njia nyingi za kupata maabukizi ya virusi vya ukimwi; wewe unafikiri ipi ni njia kuu ya kuambukizwa na virusi hivi? a. Njia ya kujamiana () b. Njia zisizohusiana na kujamiana ()
29. Je unafikiri ni sababu/mambo gani yanachangia katika unyanyapaa/kutengwa kwa watoto yatima wanoaoishi na virusi vya uimwi?
- a. Imani za dini ()
- b. Mila na desturi ()
- c. Uelewa mdogo wa njia za kuambukizwa na VVU ()
- d. Kutokuwepo kwa tiba ya ugonjwa wa Ukimwi ()
- e. Hofu kubwa ya watu juu ya Ukimwi/kuishi na VVU ()
- f. Kuwekwa wazi kwa ugonjwa wa Ukimwi/kuishi na VVU katika jamii inayoishi maisha ya kushirikiana kwa pamoja ()
- g. Nyingineyo ()

ASANTE KWA USHIRIKIANO

Appendix IVa: Patient Record Review Form (English Version)

Study No.

1. Name of CTC: 2. Unique CTC ID no.:

.....

3. Reviewed date:

4. Sex of Patient: a. Male () b. Female ()

5. Date of Birth:

6. Date started ART:

7. Status at start of ART

WHO Stage	CD4		Body Weight (in kg)
	Count	%	

8. Recent status of ART

CD4			Body Weight (in kg)	Date recorded
Count	%	Date recorded		

9. What is current ARV use status of patient?

a. Continue () b. Change () c. Stop () d. Restart ()

10. For change or stop in use of ARVs (In Qn. no. 9); reason cited:

a. Start TB treatment () b. Adverse reaction () c. Treatment failure ()
 d. Other ()

11. For restart in use of ARVs (In Qn. no. 9); recorded dates:

Stop date:

Re-start date:

12. Is the patient under Tuberculosis therapy? a. Yes () b. No ()

13. Patient's attendance record in selected scheduled clinic visit:

Scheduled appointment date	Actual date of clinic attendance	Attendance Category 1= Good 2= Poor

14. ARV adherence estimated by Clinician in the clinic visit in Qn. no. 13

- a. Good () b. Poor () c. Not shown ()

15. In case of poor adherence in Qn. no. 14; reason cited by Clinician:

- a. Toxicity () b. Stigma () c. Felt better () d. Too ill ()
 e. Other ().....

APPENDIX IVb: FOMU YA MAPITIO YA KUMBUKUMBU ZAMGONJWA

Namba ya utafiti

1. Jina la CTC: 2. Namba ya utambulisho.:
3. Tarehe ya kufanyika kwa mapitio ya kumbukumbu za mgonjwa:
.....
4. Jinsia ya Mgonjwa a. Mme () b. Mke ()
5. Tarehe ya kuzaliwa ya mgonjwa:
6. Tarehe ya kuanza matibabu ya ARVs :
7. Hali iyokuwepo mwanzoni mwa matibabu ya ARVs

Ngazi ya maambukizo	CD4		Uzito wa mgonjwa (kg)
	Namba	Asilimia	

8. Hali ya mgonjwa ya siku za karibuni

CD4		Tarehe	Uzito wa mgonjwa (kg)	Tarehe
Namba	Asilimia			

9. Hali ya matumizi ya ARVs ipo vipi?
 - a. Anaendelea () b. Amebadilishiwa () c. Ameanza tena ()
10. Iwapo kuna mabadiliko ya ARVs; sababu iliyotolewa:
 - a. Matibabu ya TB () b. Madhara ya dawa () c. Kushindwa kwa dawa ()
 - d. Nyingineyo ()

11. Iwapo mgonjwa amelazimika kuanza tena kutumia ARVs; kumbukumbu za tarehe:

Tarehe ya kuacha/...../.....

Tarehe ya kuanza tena/...../.....

12. Je mgonjwa yupo kwenye matibabu ya kifua kikuu? a. NDIYO () b. HAPANA ()

13. Rekodi za mahudhurio ya kliniki

<u>Tarehe alvopangiwa</u>	<u>Tarehe alivofika</u>	<u>Kundi la mahudhurio</u> 1= Nzuri 2= Dhaifu

14. Hali ya uthabiti wa ARV kwa mujibu wa mganga kwa tarehe za mahudhurio hapo juu (Swali na. 13)

a. Nzuri () b. Dhaifu () c. Haikuonyeshwa ()

15. Iwapo kuna udhaifu wa uthabiti wa ARVs (Swali na. 14); sababu iliyotolewa na mganga:

a. Madhara ya dawa () b. Unyanyapaa () c. Kujisikia nafuu () d. Kuwa mgonjwa ()

e. Nyingineyo ().....

Appendix Va: CTC2 (Front Pages)

THE UNITED REPUBLIC OF TANZANIA **MINISTRY OF HEALTH AND SOCIAL WELFARE**

CTC2 Card No: _____

FACILITY NAME: _____ **FACILITY CODE:** _____ **DISTRICT:** _____

UNIQUE CTC ID NUMBER: _____ **HEALTH FACILITY FILE NUMBER:** _____

NAME (incl. middle int): _____ **SEX:** Female Male

DATE OF BIRTH*: _____ **MARITAL STATUS (see code 1):** _____

AGE: _____ (Years/months) _____ on (Adults)

HEIGHT: _____

PATIENT REFERRED FROM (tick appropriate):

OPD / INPATIENT

BTI

TB DOTS

RCH / PMCT / SD

PMVH GROUP

SELF REFERRAL (incl VCT)

HOME BASED CARE

OTHER (specify) _____

PATIENT ADDRESS:

DISTRICT / DIVISION / WARD: _____

STREET / VILLAGE: _____

STREET / VILLAGE / GHOSHANI: _____

NAME OF TEN CELL LEADER: _____

NAME OF HEAD OF HOUSEHOLD: _____

CONTACT OF HOUSEHOLD HEAD: _____

PARENT'S TELEPHONE No: _____

TRANSFER IN (tick those applicable):

WITH RECORDS (infect and CTC 1 forms)

NO RECORDS AVAILABLE

IN CARE

ON ART

PATIENT SUPPORT:

NAME OF TREATMENT SUPPORTER: _____

TELEPHONE No. OF TREATMENT SUPPORTER: _____

PROXY JOINED COMMUNITY SUPPORT ORGANIZATION Yes No

NAME OF ORGANIZATION / GROUP: _____

(I)	(II)	(III)	(IV)	(V)	(VI)	(VII)	(VIII)	(IX)	(X)	(XI)	(XII)	(XIII)
WGT BIRTH (kg)	WGT 1 YRS (kg)	WGT 2 YRS (kg)	LENGTH BIRTH (cm)	HGT CLINICAL STAGE (cm)	HAIR COLOR (black 1, other 2)	FUNCTIONAL STATURE (cm)	PREVIOUS TB (1=1 yr, 2=1-2 yrs, 3=2-5 yrs, 4=6-10 yrs)	TB No. 1 (No. 2)	ANY TB (No. 3)	ART Status (code 4)	ART Reason (code 5)	

CTC 2: PATIENT RECORD FORM **NATIONAL HIV CARE AND TREATMENT**

DRUG ALLERGIES: _____

FROM ANY EXPOSURE (tick appropriate):

NONE

PNCB THERAPY (transfer in without records)

PMCT MONOTHERAPY

PMCT COMBINATION THERAPY

PEP

DATE CONFIRMED HIV: _____

DATE ENROLLED IN CARE: _____

DATE MEDICALLY ELIGIBLE: _____ **WHO STAGE:** (1-4) **CD4 COUNT / %:**

DATE ELIGIBLE & READY: _____

DATE START ART: _____

STATUS AT START ART: **AGE:** **WHO STAGE:** (1-4) **CD4 COUNT / %:** **FUNCTIONAL STATURE:** (see notes 6) **BCVT:** **WEIGHT:**

(I)	(II)	(III)	(IV)	(V)	(VI)	(VII)	(VIII)	(IX)	(X)	(XI)
HIV COMBINATION (code 1)	ART ADHERENCE (code 2)	DRUGS PROPHYLAXIS & OTHER (code 3)	ART OTHER (code 4)	ART TB (code 5)	ART TB (code 6)	ART TB (code 7)	ART TB (code 8)	ART TB (code 9)	ART TB (code 10)	ART TB (code 11)

See handbook (national version) to reference code of each status (for transfer only) * see notes of WHO TB, infectious IDI codes Revised September 2013

Appendix Vb: CTC2 (Back Pages)

CTC 2 DISCUSSION TOPICS AND CODES

Discussion topics for follow-up education and counseling (group or individual, pre- and post ART)
Choose one to three priority topics each visit

Topic	Date	Comments	Date	Comments	Date	Comments	Date	Comments
Basic HIV/AIDS and prevention								
Disclosure and identifying treatment supporter								
Coping and planning the future								
Promoting testing within households								
Pregnancy, MTCT and family planning								
Adherence issues								
Disease progression and role of ART								
Prevention of disease, environmental hygiene, hand washing, bednets and nutrition								
CTX and HIV prophylaxis								
Importance of adherence, how to measure, plan, what to do when failed, etc. Refer to checklist								
How to use CTC2 for consultations								
Importance of HBC and PLHA support group								
Self Care								
Importance of appointments, dates and time, planning transport								
Provide patient leaflets / brochures								

FAMILY INFORMATION

	NAME	RELATION	AGE	HIV STATUS + / - / unknown	HIV CARE Y/N	LINKAGE CTC2 ET No.	HEALTH FACILITY FLE No.
1							
2							
3							
4							
5							
6							
7							

1. MARITAL STATUS

1 = SINGLE
2 = MARRIED
3 = COHABITING
4 = OVERLOOKED/SEPARATED
5 = MISCARRIAGED

2. ART TYPE

1 = Scheduled visit at this site
2 = Unscheduled visit at this site
3 = Unscheduled visit at other site
4 = Unscheduled visit at home
5 = Unscheduled visit at other location

3. OTHER SYMPTOMS AND DRUGS

1 = DYSURICIA
2 = GASTROINTESTINAL
3 = HEADACHE
4 = RASH
5 = OTHER (SPECIFY)

4. FUNCTIONAL STATUS

1 = NORMAL
2 = MODERATELY IMPAIRED
3 = SEVERELY IMPAIRED

5. ADHERENCE/PLANNING

1 = NOT TAKING
2 = PARTIAL ADHERENCE
3 = FULL ADHERENCE
4 = PLANNING
5 = NOT PLANNING

6. TB SCREENING / DIAGNOSIS

1 = Screened
2 = Not screened
3 = Screened and negative
4 = Screened and positive
5 = Screened and unknown
6 = Not screened and unknown

6. ART ADHERENCE

1 = NOT TAKING
2 = PARTIAL ADHERENCE
3 = FULL ADHERENCE
4 = PLANNING
5 = NOT PLANNING

7. TB TREATMENT (PREV. OR CURRENT)

1 = NOT TAKING
2 = TAKING
3 = COMPLETED
4 = INCOMPLETE
5 = UNKNOWN

8. ART RESISTANCE

1 = NO RESISTANCE
2 = RESISTANCE TO ONE DRUG
3 = RESISTANCE TO TWO DRUGS
4 = RESISTANCE TO THREE DRUGS
5 = RESISTANCE TO FOUR DRUGS
6 = RESISTANCE TO ALL DRUGS
7 = UNKNOWN

9. TB TREATMENT (PREV. OR CURRENT)

1 = NOT TAKING
2 = TAKING
3 = COMPLETED
4 = INCOMPLETE
5 = UNKNOWN

10. TB TREATMENT (PREV. OR CURRENT)

1 = NOT TAKING
2 = TAKING
3 = COMPLETED
4 = INCOMPLETE
5 = UNKNOWN

11. TB TREATMENT (PREV. OR CURRENT)

1 = NOT TAKING
2 = TAKING
3 = COMPLETED
4 = INCOMPLETE
5 = UNKNOWN

12. TB TREATMENT (PREV. OR CURRENT)

1 = NOT TAKING
2 = TAKING
3 = COMPLETED
4 = INCOMPLETE
5 = UNKNOWN

13. TB TREATMENT (PREV. OR CURRENT)

1 = NOT TAKING
2 = TAKING
3 = COMPLETED
4 = INCOMPLETE
5 = UNKNOWN

14. TB TREATMENT (PREV. OR CURRENT)

1 = NOT TAKING
2 = TAKING
3 = COMPLETED
4 = INCOMPLETE
5 = UNKNOWN

15. TB TREATMENT (PREV. OR CURRENT)

1 = NOT TAKING
2 = TAKING
3 = COMPLETED
4 = INCOMPLETE
5 = UNKNOWN

16. TB TREATMENT (PREV. OR CURRENT)

1 = NOT TAKING
2 = TAKING
3 = COMPLETED
4 = INCOMPLETE
5 = UNKNOWN

17. TB TREATMENT (PREV. OR CURRENT)

1 = NOT TAKING
2 = TAKING
3 = COMPLETED
4 = INCOMPLETE
5 = UNKNOWN

18. TB TREATMENT (PREV. OR CURRENT)

1 = NOT TAKING
2 = TAKING
3 = COMPLETED
4 = INCOMPLETE
5 = UNKNOWN

19. TB TREATMENT (PREV. OR CURRENT)

1 = NOT TAKING
2 = TAKING
3 = COMPLETED
4 = INCOMPLETE
5 = UNKNOWN

20. TB TREATMENT (PREV. OR CURRENT)

1 = NOT TAKING
2 = TAKING
3 = COMPLETED
4 = INCOMPLETE
5 = UNKNOWN

21. TB TREATMENT (PREV. OR CURRENT)

1 = NOT TAKING
2 = TAKING
3 = COMPLETED
4 = INCOMPLETE
5 = UNKNOWN

22. TB TREATMENT (PREV. OR CURRENT)

1 = NOT TAKING
2 = TAKING
3 = COMPLETED
4 = INCOMPLETE
5 = UNKNOWN

23. TB TREATMENT (PREV. OR CURRENT)

1 = NOT TAKING
2 = TAKING
3 = COMPLETED
4 = INCOMPLETE
5 = UNKNOWN

24. TB TREATMENT (PREV. OR CURRENT)

1 = NOT TAKING
2 = TAKING
3 = COMPLETED
4 = INCOMPLETE
5 = UNKNOWN

25. TB TREATMENT (PREV. OR CURRENT)

1 = NOT TAKING
2 = TAKING
3 = COMPLETED
4 = INCOMPLETE
5 = UNKNOWN