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**Examining Critical Issues Associated with Human Immunodeficiency Virus
Antiretroviral Therapy Administration in Resource-Limited Settings:
Adherence and Drug Quality**

by

Jayne Byakika Tusiime

A dissertation submitted in partial satisfaction of the

requirements for the degree of

Doctor of Philosophy

in

Epidemiology

in the

Graduate Division

of the

University of California, Berkeley

Committee in charge:

**Professor Arthur Reingold, Chair
Associate Professor Alan Hubbard
Professor Mark Wilson**

Spring 2010

Steady State Bioequivalence of Generic and Innovator Formulations of Stavudine, Lamivudine, and Nevirapine in HIV-Infected Ugandan Adults

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Longitudinal Antiretroviral Adherence in HIV+ Ugandan Parents and Their Children Initiating HAART in the MTCT-Plus Family Treatment Model: Role of Depression in Declining Adherence Over Time

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DEDICATION

I dedicate this dissertation to my wonderful family. Particularly to my understanding and patient husband, Patrick, who has put up with these many years of study and research, and to our precious children Natasha, Samson, Sonia and Nathaniel who are the joy of our lives and the reason for taking this extra mile. I must also thank my loving mother and my terrific in-laws who have helped so much with caring for the children in my absence and have given me their fullest support. Finally, I dedicate this work to my late father, Gershom Samson Kasajja Byakika who believed in diligence, hard work and the pursuit of academic excellence.

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ABSTRACT

Examining Critical Issues Associated with Human Immunodeficiency Virus Antiretroviral Therapy Administration in Resource-Limited Settings: Adherence and Drug Quality

by

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Human Immunodeficiency Virus (HIV) infection remains a global health problem affecting many lives especially in sub-Saharan Africa (SSA). The push by the global community towards universal access to HIV prevention, treatment, care, and support has enabled millions of individuals to start on antiretroviral therapy (ART) in resource-limited settings. However, there is concern that widespread antiretroviral use could lead to widespread drug resistance. Suboptimal adherence leading to incomplete viral suppression is the primary predictor of HIV drug resistance. Concerns have been raised about the ability of patients in resource-limited settings to maintain the high level of adherence required to produce adequate viral suppression and hence prevent the emergence of resistant strains of HIV.

The cost of antiretroviral medications is the most frequently cited barrier to adherence in resource-limited settings. Even with the recent substantial reductions in drug prices, many patients cannot afford to sustain their therapy over time. Generic antiretroviral medications (ARVs) today form the backbone of first-line regimens in developing countries. However, there are limited data on the bioavailability and bioequivalence of generic ARVs with branded pharmaceutical equivalents. While substantial effort has been put into increasing access to ART in resource-limited settings, less attention has been paid to the responsibilities of governments and international agencies to address the threat of substandard and counterfeit ARVs. The prevalence of counterfeit ARVs in resource-limited settings is not known. The Beck Depression Inventory (BDI) is a commonly used instrument to measure depressive symptoms in HIV-infected populations in SSA but it has never been validated in these populations.

This dissertation is an attempt to address some of the critical issues associated with administration of ART in resource-limited settings. I explored the issues of adherence to HIV medications and ARV drug quality using data collected from patients participating in three prospective cohort studies and one cross sectional study conducted in two different HIV-infected populations in Uganda between September 2002 and December 2006. Specifically, I assessed i) the effect of source of payment for ARVs (no cost vs. self-pay) on adherence; ii) the impact on adherence of treating all HIV-infected members in a household; iii) the psychometric properties of the Beck Depression Inventory (BDI) when used in an HIV-infected population, and iv) the

bioequivalence of a fixed dose combination generic drug (Triomune®) with the brand name pharmaceutical equivalents (Zerit®/Epivir®/Viramune®).

Patients receiving no cost ART had 3.8 percentage points higher adherence than those patients paying for their treatment. Excellent adherence was observed when all household members infected with HIV were treated, however adherence declined over time. Depression was associated with poor adherence. The BDI had good psychometric properties with Cronbach's alpha of 0.79 and the expected a posteriori reliability coefficient (EAP) of 0.86. Comparing generic and brandname ARVs, we found that the generic formulation was not statistically bioequivalent to the brand formulations during steady state, although exposures were comparable. Fifty percent of the total variability in maximum plasma concentration (C_{max}) and the area under the plasma concentration-time curve (AUC) was due to between-subject variability.

These results suggest that removing a financial barrier to treatment with ART by providing no cost HIV treatment may significantly improve adherence to ART. Our results also indicate that providing free ART to all eligible members in a household is associated with excellent adherence in both parents and children. Adherence to ART among new parents declines over time, even when patients receive treatment at no cost. Depression should be addressed as a potential adherence barrier. These findings support the use of the BDI-II in assessing depressive symptoms for HIV-infected patients in sub-Saharan Africa, especially women. Finally, these findings provide support for the use of Triomune in resource-limited settings, although identification of the sources of between-subject variability in AUC and C_{max} in these populations is critical.

CHAPTER 1

Critical Issues Associated with Human Immunodeficiency Virus Antiretroviral Therapy Administration in Resource-Limited Settings: Adherence and Drug Quality

INTRODUCTION

The epidemiologic features of HIV infection

HIV infection remains a global health problem affecting many lives. Globally, there were an estimated 33.4 million [31.1 million–35.8 million] people living with HIV in 2008.[1] Nevertheless, recent epidemiological data show a decline in new HIV infections in some countries and a decline in the number of deaths due to HIV/AIDS. This is in part a result of success in expanding access to antiretroviral drugs in resource-limited settings. The overall number of people living with HIV has steadily increased as new infections occur each year, HIV treatments extend life, and new infections outnumber AIDS deaths. Sub-Saharan Africa is the region that is worst affected by HIV/AIDS, with 67% of all people living with HIV. Women and children comprise a large proportion of all people living with HIV. Women account for half of all people living with HIV worldwide and nearly 60% of those with HIV infection in sub-Saharan Africa. The number of children living with HIV has steadily risen in recent years, from 1.6 million [1.4 million–2.1 million] in 2001 to 2.1 million [1.2 million–2.9 million] in 2007. Almost 90% of these children live in sub-Saharan Africa [1].

Access to antiretroviral therapy in resource-limited settings

The push by the global community towards universal access to HIV prevention, treatment, care, and support has enabled millions of individuals to start on antiretroviral therapy (ART) in resource-limited settings. The proportion of people receiving antiretroviral drugs in low- and middle income countries has increased from 7% in 2003 to 42 % in 2008 [1]. More women than men are receiving ART.

While expanding access to HIV/AIDS treatment in resource-limited settings is recognized as a global health priority, there is concern that widespread antiretroviral use could lead to widespread drug resistance[2-4]. An increased prevalence of HIV drug resistance has been observed in high income countries where antiretroviral drugs have been in use the longest [5, 6] In the low- and middle-income countries where antiretroviral drugs were introduced more recently, resistance to these medications is much less common , but may increase as HIV-positive people on therapy live longer. Data from the developed world indicate that up to 10% of individuals with incident HIV infections and 50% of individuals with prevalent HIV infections carry resistant HIV strains [7-9], compromising treatment response[10]. Development of HIV drug resistance is of great concern in resource-limited settings, where the availability of second-line treatment regimens is very limited.

Adherence to antiretroviral therapy

Suboptimal adherence leading to incomplete viral suppression is the primary predictor of HIV drug resistance[11-16]. A high level of treatment adherence is needed to avoid or delay the emergence of drug resistance, which is closely associated with treatment failure. A growing body of data associates treatment interruptions—including those guided by CD4 count—with viral rebound, poorer clinical outcomes, and diminished quality of life[17-19]. Some have suggested that extreme poverty in resource-limited settings will lead to suboptimal adherence and the global transmission of drug resistant strains of HIV [20]. Efforts to promote treatment adherence

have been helped by the simplification of antiretroviral drug regimens in recent years, including the development of once-daily dosing [21, 22]. Although high levels of adherence can be achieved in resource-limited settings and in various vulnerable populations [23], many people living with HIV find it difficult to adhere to antiretroviral drug regimens.

Adherence in resource-rich settings

Most of our understanding of adherence to HIV antiretroviral therapy comes from studies in resource-rich settings. While adherence to early single protease-inhibitor regimens available in 1996 required >95% adherence for reliable viral suppression[24], most populations studied fall short of this goal. Suboptimal adherence has been reported in: a large multicenter clinical trial (85% adherence by self-report)[25], patients from a veterans and university hospital (75% by electronic medication monitoring)[24], the marginally housed (73% by pill count, 67% by electronic medication monitoring)[26], those with serious mental illness (66% by electronic medication monitoring)[27], minority women (64% by electronic medication monitoring)[28], and two different groups of inner-city residents with a history of injection drug use (80% by pill count, 53.5% by electronic medication monitoring in one group[29], and 53% by electronic medication monitoring in the other group)[30]. Studies from Canada and developed countries in Latin America and Europe demonstrate similar levels of suboptimal adherence[31-33]. In all, average adherence to antiretroviral therapy, using the best measures is approximately 70% [34] and declines over time on treatment [25-28].

Predictors of adherence in resource-rich settings

A number of factors have been associated with non-adherence to ART. Predictors of adherence may be related to the patient, the drug regimen, the stage of disease, and the setting in which health care is delivered (or provider characteristics)[35, 36]. Patient-related psychosocial determinants, such as depression/psychiatric morbidity, active drug or alcohol use, stressful life events, lack of social support, and poor health literacy, are better predictors of adherence than demographic determinants.[24, 37-49]. . Studies report conflicting evidence about the association between socio-demographic factors and adherence. When an association is found, the direction is towards lower levels of adherence among those of younger age, nonwhite race/ethnicity and lower income. Unstable housing is also associated with non-adherence in resource-rich settings. Gender, educational level, insurance status, and HIV risk factors generally are not associated with adherence [24, 25, 38-42, 47, 50-54] .

Antiretroviral treatment regimen factors associated with adherence include the number of pills prescribed, the complexity of the regimen (e.g. dosing frequency and food restrictions), the specific type of antiretroviral drugs, and short and long-term side effects of the medication. Greater regimen complexity (e.g. dosing frequency and food instructions), pill burden and side effects are associated with greater non-adherence[55, 56]. Once-daily regimens may improve adherence behavior, but this has not yet been adequately examined. Other studies report that the “fit” of the regimen into an individual’s daily routine is another important determinant of adherence [39, 57].

Disease-related predictors of adherence include stage and duration of HIV infection. A few studies show an increase in non-adherence with more HIV-related symptoms [39, 41, 53]. Other studies describe an association between a lower CD4 cell count and non-adherence, although this finding is seen less consistently across studies[24, 38, 39, 46, 52]. Two studies have described increased adherence in those with a history of opportunistic infections [58, 59]. The

authors postulate that experience with illness stokes the desire for health and a motivation to adhere.

Provider-related determinants of adherence include a strong and trusting provider-patient relationship and [60-63] provider experience treating HIV-related infections[64]. Ease of access to medication refills, presence of a social worker, and availability of afternoon clinics have all been associated with good adherence[65]. While several provider and health system factors are associated with good adherence, individual providers are poor at predicting individual adherence behavior[24, 66-68].

Adherence in resource-limited settings

Concerns have been raised about the ability of patients in resource-limited settings to maintain the high level of adherence required to produce adequate viral suppression and hence prevent the emergence of resistant strains of HIV[3, 4, 69]. Some have suggested that extreme poverty will be associated with poor adherence[70]. Others have argued that comprehensive adherence programs should be in place prior to expanding antiretroviral access in developing countries[20, 69].

Levels of adherence in resource-limited Settings

Despite such concerns, adherence in developing countries has been found to be generally better than adherence in developed countries. Most studies done in resource-limited settings, with the majority of them from sub-Saharan Africa, have found adherence levels of greater than 90% [71-86]. Orrell found that 48 week adherence using clinic-based pill counts was 93.5% in 289 individuals attending a public hospital HIV clinic in South Africa and receiving free ART through Phase III clinical trials [72]. In a cohort of Senegalese adults, mean adherence was 91% by clinic-based pill count and self-report [76]. In a 12-week study of 34 ART-naïve, HIV-positive Ugandans purchasing therapy, mean adherence as measured by electronic medication monitoring caps, home-based unannounced pill counts, three-day self report and a thirty-day visual analogue scale ranged from 91-94%[71]. Similarly, Byakika-Tusiime et al found 97.3-99.8% unannounced pill count adherence in individuals receiving free therapy in the Mother-to-Child Transmission Plus program in Kampala, Uganda [87]. A multicenter trial of 60 ARV naïve patients in Cameroon to determine the effectiveness and safety of a generic fixed dose combination of nevirapine, stavudine and lamivudine documented a mean self reported adherence of 99%[79], confirmed by drug level concentrations.

Other studies done in sub-Saharan Africa have used patient-reported measures of adherence. These studies generally find levels of adherence comparable to that seen in resource-rich settings. Laurent et al demonstrated the sustainability of clinical and biologic outcomes in a cohort of HIV-infected Senegalese adults followed for a median period of 30 months. Most patients reported good adherence (80%-90%), although adherence tended to decline with time[76]. In a prospective one-arm trial to determine the effectiveness of once-a-day HAART in 40 treatment-naïve Senegalese adults, 95% of the patients reported taking all their tablets in the previous three days[83]. In a prospective observational study in Senegal among 58 treatment naïve patients, 87.9% of the participants reported $\geq 80\%$ adherence, although, only those who had the capacity to adhere to ART and contribute to the cost of treatment, according to a social survey, were included in the study. In spite of the generally good adherence in this patient population, the general trend was declining adherence over time[88]. In a study conducted in a private sector HIV/AIDS disease management program in South Africa, adherence to ART was

greater than 70% for 3908 patients [89]. In a study of 66 patients at an adult clinic in Soweto, South Africa, 88% of the patients reported > 95% adherence[73]. In a cross sectional study of 304 self-paying patients in Kampala, Uganda, 68% of the patients reported greater than 95% adherence [75]. Results of the assessment of the first national program to provide ART in Uganda showed that patients took their drugs ‘about as prescribed’ in 88% instances[82]. In a randomized controlled trial in Mombasa, Kenya to determine the efficacy and acceptability of an alarm device for improving medication compliance among women in resource poor countries, 66% of women reported >95% compliance [90]. One of the few studies to find low levels of adherence, comparable to those reported in resource rich settings, was a cross sectional study of 109 self-paying patients on ART in Botswana, where only 54% of the patients reported \geq 95% adherence[77].

In addition to ART treatment for chronic HIV infection, a few studies have reported on adherence during treatment to prevent maternal-to-child transmission of HIV. In a randomized clinical trial in Kenya to study compliance with antiretroviral drug regimens to prevent perinatal HIV-1 transmission, 86% of the participants reported taking at least 80% of the ante partum doses of the Thai-CDC regimen, although only 44% reported taking at least 80% of the expected intrapartum doses. Ninety-one percent of the Kenyan women on the HIVNET012 regimen reported taking the maternal dose before delivery and 97% reported giving the infant dose[91].

Studies done in resource-limited settings outside Africa also have found levels of adherence generally equivalent to or better than those seen in resource-rich settings. In contrast, in a study of 182 HIV-infected Brazilians receiving free ART, Brigido et al found that only 41% of the patients had optimal adherence by self-report over 30 days[81]. In another study conducted in Brazil, Remien et al found that 82% of 200 individuals on free ART in public health care settings in Rio de Janeiro [32] reported greater than 90% adherence in an interview survey [92]. In a retrospective study of 161 HIV-infected Chinese patients who had been on ART for at least one year, over 95% of the patients reported taking more than 95% of their medications[86]. In a cross sectional study of 310 HIV positive patients on ART in India, mean four-day adherence for those paying for therapy was 96.4%, while that for patients receiving free therapy was 80.5% by self-report. Self paying participants reported lower adherence than those receiving free treatment (87% versus 97%). While overall adherence in this group of patients in India was good, free therapy was associated with lower adherence[85].

Adherence to antiretroviral therapy among children in resource-limited countries

Most studies on adherence to ART have been done among HIV-infected adults, but there are many HIV-infected children in resource-limited settings who are on ART. In a cross-sectional study of 170 children ages 2 to 18 years attending the pediatric HIV/AIDS clinic in Kampala, Uganda, Nabukera et al found a mean adherence of 72% by unannounced pill count[93]. This level of adherence is much lower than that observed among adults in a similar setting (>90% vs. 72%). However, achieving adherence in children is a great challenge. Many HIV-infected children are orphaned and are under the care of other relatives, like their grandparents, many of whom are illiterate. Many children may miss doses because of competing employment and childcare responsibilities of their caregivers. In some cases, the surrogate caretaker may not know about the child’s HIV status and may not know either how to administer medications or the implications of missing doses. Nabukera et al found that those children whose official caretakers were the only ones who knew about the child’s illness had worse adherence than those whose illness had been disclosed to other people in the home [100].

Barriers to ART adherence in resource-limited settings

Many of the factors associated with non-adherence with ART among adults in resource-rich settings also apply in resource limited settings. These include frequency of dosing[72], younger age [72, 85], being unmarried[75], depression[85], male gender[73], forgetfulness[77, 81, 86], being too busy[77, 86], being away from home[73], regimen complexity[73], alcohol use[81], and lack of belief in the efficacy of ART[81]. Nonetheless, there are many additional barriers to adherence that are unique to resource-limited settings, such as the cost of the medications, the long distance to the treatment centers, stigma, medication stock outs, and speaking a different language from the health care providers.

The cost of antiretroviral medications is the most frequently cited barrier to adherence in resource-limited settings[73-75, 77]. Even with the recent substantial reductions in drug prices, many patients cannot afford to sustain their therapy over time. Income is very low and unemployment is very common. In Oyugi's study in Uganda, for example, more than half of the participants had a monthly income of less than US \$50 (the median Ugandan income is US \$250 per year)[71]. Byakika-Tusiime et al showed that inability to purchase medications was the most important predictor of incomplete adherence[75]. Those earning less than \$50 a month were almost three times more likely to achieve less than 95% adherence. Financial constraints lead to treatment discontinuations that are beyond patients' control. In Botswana, non-adherence was characterized by prolonged gaps in treatment rather than day-to-day non-adherence[77]. Gaps in treatment were common and related to the cost of therapy and an inability to afford medications for varying periods of time. In Botswana, it was estimated that if cost of the medications were removed as a barrier to adherence, adherence would increase from 54% to 74%. In a study in Soweto, South Africa, many self-paying patients were on two drug therapy because they could not afford the recommended three drug regimen[73]. Among Senegalese patients, adherence rose from 83% to 93% when the cost of the drugs was reduced [74]. Van Oosterhout et al found drug shortages in the hospital and financial constraints to be the main barriers to adherence in Malawi[94].

There is conflicting evidence suggesting that provision of free therapy compromises adherence[85]. In a study of HIV positive patients on ART in India, adherence in the group receiving free therapy was lower than that in the group paying for therapy. Those receiving free therapy were four times more likely to have incomplete adherence than those paying for their therapy. Free therapy should, therefore, be accompanied by adequate adherence support.

Language barriers and illiteracy can also affect adherence. Many HIV-infected individuals in resource-limited settings are illiterate or have had very little education. They may not be able to communicate in English, yet this is the medium of communication in most health facilities in Anglophone countries. Patients, therefore, may misunderstand the instructions given to them about their medications, leading to missing their doses. Orrell et al found that speaking a different language from the healthcare facility staff was a predictor of incomplete adherence [72].

Until recently, there were very few HIV/AIDS treatment centers in resource-limited settings. HIV/AIDS treatment was available at only a few centers of excellence, meaning that patients might have to travel long distances to reach these centers and incur substantial costs for transport. Weiser et al found that distance from home to clinic was associated with non-adherence in Botswana, as did Brigido et al in Brazil [77, 81].

HIV-related stigma remains an important problem in many settings. Fear of stigma has been associated with non-adherence in South Africa[73] and Botswana[77]. In the study in South

Africa, the odds of achieving > 95% adherence decreased considerably with an increased fear of stigmatization (i.e. rejection or violence or both) by the patient's sexual partner. Fifteen percent of patients in the study in Botswana claimed that stigma interfered with their ability to take their medication. Stigma usually posed a barrier for patients who thought they could not take their treatments at home or at work due to fear of detection and for patients who felt uncomfortable going to the clinic for tests and medication refills as a result of concerns about the confidentiality of their HIV serostatus.

Differing levels of adherence in resource-rich and resource-limited settings

Why are levels of adherence in resource-limited settings higher than in resource-rich settings? While the reasons for this difference are largely unknown, it is clear from existing data that level of poverty does not explain level of adherence in a population. People receiving ART in resource limited settings generally start therapy at a later stage of disease and may have more dramatic clinical improvement with initiation of therapy. While there may be some illicit use of opiate drugs in some resource-limited settings, active use of illicit drugs, especially use of stimulants (which have an especially deleterious impact on adherence), is uncommon in resource-limited settings[95-99]. Providing care for large numbers of dependent children has been described as a central motivation for adherence among HIV+ parents who have lost spouses and other relatives due to HIV. Because patients on self pay therapy often secure funds to pay for treatment from extended family networks, there is, by necessity, strong social support to adhere to therapy[100].

Are the current levels of adherence reported in resource-limited settings sustainable? There are two reasons that levels of adherence may decline over time. First, current estimates of adherence are limited by substantial selection bias based on the few, highly selected individuals with early access to HIV therapy. Such individuals are unlikely to be representative of the larger HIV-infected population in resource-poor settings. As access to ART expands, estimates of adherence among HIV-infected individuals may fall. Second, most studies have looked at patients who had recently initiated HIV therapy. Adherence is often highest early in treatment, due to amelioration of HIV-related symptoms and improvement in health status. As treatment continues, improvements in health plateau and chronic complications of therapy, such as neuropathy or lipodystrophy, become more frequent and more severe. As a result, adherence in resource rich settings tends to decline over time, and some reports suggest similar declines of adherence with long-term treatment in resource-limited settings. Current high levels of adherence in such settings could represent a "honeymoon effect" on both a population level (as treatment access expands) and an individual level (as patients continue treatment for years and decades) [76, 88]. Levels of adherence in resource limited settings are likely to fall from these early estimates of average adherence in excess of 90%, but would have to decline substantially before they reach levels typically found in resource-rich settings.

Testing and validation of instruments used in epidemiologic studies

In many epidemiological studies, data are collected using standard instruments designed to increase the comparability of data between the study groups [101]. Instruments are simply devices for measuring the variables of interest. They can be in the form of record abstracting forms, questionnaires, physical examinations, bio-specimen collection or environmental samples [101]. They can also be in the form of observational schedules, structured logbooks or standard

forms for recording data from existing records [102]. Questionnaires are the mainstay for epidemiologists though the other methods can be just as useful and each has its strengths and limitations.

What is measurement?

Measurement can be defined as the assignment of numbers to categories of observations where the properties of the numbers become the properties of the measurement-nominal, ordinal, interval, ratio and so on [103]. The central purpose of measurement is to provide a reasonable and consistent way to summarize the responses that people make expressing their achievements and attitudes through instruments such as attitude scales, questionnaires, surveys and psychological scales [103].

The questionnaire is the most common instrument used in epidemiologic studies. Simply defined, a questionnaire is a standardized list of factual questions or elicited opinions [104]. There are three basic components of a questionnaire: the content, the form of the question and the level of data collected [105]. Every word in a question can influence the validity and reliability of the responses.

Reliability and validity

The adequacy of a measuring instrument is determined by its reliability and validity [103, 104]. Two fundamental questions should be asked when selecting a measuring instrument. First, does the instrument measure a variable consistently? And second, is the instrument a true measure of the variable? The first is an indication of reliability while the second raises the issue of validity [103, 104]. Psychometric validation is the process by which an instrument is assessed for reliability and validity through the mounting of a series of defined tests on the population group for whom the instrument is intended [103, 104].

Reliability refers to the reproducibility and consistency of the instrument, and the degree to which it is free from random error [103, 104]. There are several criteria that should be assessed before an instrument can be judged reliable. These include test-retest, alternate forms, inter-rater reliability and internal consistency. Test-retest refers to when the instrument is administered to the same population on at least two occasions and the results are correlated [103, 106]. Alternate forms reliability is a measure in which the measurer develops two sets of items for the instrument following similar steps. The two alternate copies of the instrument are administered and calibrated and the results of the two forms are correlated to provide the alternate forms reliability coefficient [103]. Inter-rater reliability is the extent to which results obtained by two or more raters agree for the same population [102, 103]. Internal consistency is the concordance between two variables that measure the same general characteristic [103, 105]. Cronbach's alpha is an estimate of internal consistency based on all possible correlations between all the items within the scale. Values range from 0 to 1. A reliability coefficient of 0.70 implies that 70% of the measured variable is reliable and 30% is due to random error, indicating that the item does not belong to the same conceptual domain [102].

Validity is an assessment of whether an instrument measures what it aims to measure. It should comprise evidence based on test content, response processes, internal structure, relations to other variables and consequences of testing [107].

Quality of ARVs in resource-limited settings

Role of generic ARVs

HIV/AIDS care has benefited tremendously from the availability of antiretroviral drugs, both brand name and generic. However, the cost of brand name medications is too high for most HIV/AIDS patients in resource-limited countries. Use of generic drugs dramatically lowers the cost of care; however, the safety and efficacy of generic drugs must be ensured and maintained. Proven bioavailability and bioequivalence, in addition to satisfactory manufacturing, distribution, and administration, are keys to successfully implementing the use of qualified generic ARVs. Generic drugs have the potential to cause harm if rigorous standards for their production and use are not followed, but generic drugs that are qualified (i.e. have undergone bioequivalence studies and been tested to satisfy appropriate pharmaceutical standards) offer great promise in the treatment of HIV/AIDS.

Generic ARVs today form the backbone of first-line treatment regimens in developing countries. Some generic ARV drugs are qualified by the U.S. Food and Drug Administration (FDA), the European Medicines Agency (EMA), and the World Health Organization (WHO); however, many other available generic products are not. These unqualified generic products are often distributed based on the record and findings of the innovator (brandname) drugs, along with the expectation that the unqualified generic products will perform similarly to the innovator products. Unfortunately, unqualified generic products are not necessarily bioequivalent to innovator products, even though bioequivalence is often the scientific basis on which generic and innovator drugs are compared. According to the FDA, if two products are said to be bioequivalent, then the bioavailability of the two drugs should not differ significantly when the two products are administered at the same dosage under similar conditions[108]. Bioequivalence can be expected when using innovator and qualified generic products. However, because of the urgent need for HIV/AIDS medications in many resource-limited countries, the practice of supplying unqualified generic drugs to patients has become common, and bioequivalence cannot be guaranteed.

Substandard/Counterfeit ARVs

While substantial effort has been put into increasing access to ART in resource-limited settings, less attention has been paid to the responsibilities of governments and international agencies to address the threat of substandard and counterfeit ARVs. According to the WHO, a substandard drug is 'a genuine drug product which does not meet quality specifications set for it' or a 'legally branded or generic product, but one that does not meet international standards for quality, purity, strength or packaging' while a counterfeit drug is "one which is deliberately and fraudulently mislabeled with respect to identity and/or source. Counterfeiting can apply to both branded and generic products and counterfeit products may include products with the correct ingredients or with the wrong ingredients, without active ingredients, with insufficient active ingredients or with fake packaging," [109]. Counterfeit drugs may have little or no therapeutic value, leading to illness and death from the condition supposedly being treated. The fake drugs may even contain toxic substances that directly cause illness and death [110]. Counterfeit drugs with the appropriate active ingredients in subclinical amounts can also lead to increased illness or death[111], and pose the further risk of encouraging the development and spread of drug resistant pathogens[112]. Although much counterfeit drug trade occurs in the unregulated market

of unofficial drug vendors, especially in developing countries, counterfeit drugs are also found extensively in licensed pharmacies.

The reality today is that the quality of drugs for export from developed to developing countries is still determined through a much less rigorous evaluation than that used for the domestic market [113]. Efficacy and safety are often not evaluated at all. Drugs destined for international aid and development programmes are also often exempt from regulatory control [114]. The expectation is that the recipient country will evaluate the quality of the imported drug. While this may be an acceptable expectation between rich countries, placing this burden of responsibility on countries that do not have the resources to do it is impractical.

The true extent of the problem of counterfeit drugs has not been fully documented. Much of what is known about counterfeit drugs is from investigative journalism and not scientific research. Some reviews on this issue have been published [115-117]. The US FDA estimates that counterfeit drugs account for 10% of the international market, and according to the WHO, the proportion may be as much as 25% of the market in developing countries [118]. In Nigeria, before 2006, health officials estimated that 70% of drugs in circulation in the country were either fake or adulterated [119].

The prevalence of counterfeit ARVs is not known. A number of factors make ARVs an attractive target for counterfeiters, including high unit costs and long-term, sustained demand. In addition, stigma and fear of loss of confidentiality in health care settings increase the demand for ARVs delivered through often poorly regulated private sector health care providers, pharmacies or other channels. In 2004, the Kenyan newspaper 'The Nation' documented an active informal market for ARVs, including AIDS cures and counterfeit drugs, on Tsavo Road in Nairobi [44]. A 2003 report from Ethiopia found illegally imported "concoctions" being marketed as ARVs [120]. The trafficking of counterfeit ARVs has been documented in two instances to date. In 2003, the WHO issued an alert that a product called 'Ginovir 3D', marketed in Cote d'Ivoire as a triple ARV combination product, contained only one of the drugs (zidovudine) listed on the label. It contained none of the other two drugs (lamivudine and indinavir) listed on the label, and contained an additional drug not on the label (stavudine) [121]. In 2004, Medecins Sans Frontieres (MSF) discovered counterfeit ARVs on the market in the Democratic Republic of Congo that contained an antidepressant and a muscle relaxant [122].

Need for ARV drug monitoring

In developing countries, the need for routine monitoring of drug concentrations is overshadowed by the need to decrease the cost of therapy. However, assaying of serum drug concentrations may be justified for several reasons [123]. First, in order to increase access to ARVs in Africa, the use of more affordable generic products is being encouraged. This creates the need to test and ensure that the generic products are bio-equivalent to the innovator ones. Second, it is recognized that even if extensive studies are being carried out in the developed countries, there might be population and race-related differences that warrant additional pharmacokinetic or pharmacogenomic studies in developing countries. Third, drug concentration measurement may be needed to determine patient adherence to therapy in instances of therapeutic failure, as an alternative to the currently unavailable HIV viral load, CD4 lymphocyte counts and resistance testing in the resource limited environment. Drug levels might also need to be determined in order to differentiate concentration-dependent versus non-concentration dependent drug toxicities. Fourth, environmental conditions such as temperature, humidity, and light, which are known to affect the stability of pharmaceuticals, may affect products either

during transportation or storage, leading to instability and resulting in possible decreased efficacy of the products. Therefore, with the scarcity of laboratory support in developing countries, there may be a need for drug concentration determination for the purpose of differentiating pharmacological from pharmaceutical failures. Pharmacological failures refer to inability of a drug to perform as expected in the body while pharmaceutical failures refer to poor formulation of the drug.

RATIONALE AND STUDY SIGNIFICANCE

Access to ART in resource-limited settings has greatly increased in recent years[1]. As access to ART expands, however, there are critical issues to deal with, which if not handled adequately, may negate the benefits of ART and the efforts of the global community. Among these critical issues are adherence to treatment and quality of the ARV drugs administered to patients. Adherence can be the Achilles' heel of successful antiretroviral therapy. It is therefore important to know what factors prevent and which ones promote good adherence, in order to design effective strategies for maximizing adherence.

Now that many more patients are being given ART, a major challenge is enhancing treatment retention and adherence [124]. Given that many patients receive HIV medications at no cost, whether they still value them as much as they would if they had to pay for them is an important question. Should patients contribute to paying for their HIV medications in order to preserve their [medication] perceived value and hence enhance adherence? These are questions to consider when designing policies that govern ART administration and subsequently affect adherence. There is a lack of definitive information to guide these discussions.

Most adherence studies have been conducted on individuals, without looking at other [infected] members of their households. HIV/AIDS is often a family disease and needs to be treated as such. Approaching HIV treatment from a family perspective encourages disclosure of HIV status, which promotes adherence. Treating all HIV-infected household members also prevents sharing of pills, as everyone has his/her own medication. However, there is little evidence to evaluate the effect on adherence of treating all HIV+ individuals in a household.

Generic ARVs today form the backbone of first-line regimens in developing countries. However, their quality is not guaranteed. Many generic products are not qualified by the leading drug regulatory bodies in the world and these products, including HIV/AIDS medications, find their way to developing countries. Because of the urgent need for HIV/AIDS medications in many resource-limited countries, the practice of supplying unqualified generic drugs to patients has become common, and bioequivalence [with the innovators] cannot be guaranteed. Aggressive monitoring of the quality of ARVs in developing countries is essential to ensure that good quality drugs are given to patients. Unfortunately, many countries cannot afford to undertake this monitoring because of financial limitations and lack of technical know-how. Findings of the present study will highlight the status of substandard/counterfeit ARV drugs in Uganda and encourage more aggressive monitoring of the ARVs brought into the country.

DISSERTATION GOALS AND OUTLINE

This dissertation is an attempt to address some of the critical issues associated with administration of antiretroviral therapy in resource-limited settings. Specifically, I explore the issues of adherence to HIV medications and ARV drug quality. The dissertation comprises three prospective cohort studies and one cross sectional study conducted in two different HIV-infected populations in Uganda.

SPECIFIC AIMS

Aim 1: Assess the effect of source of payment for ARVs (no cost vs. self-pay) on adherence.

Hypothesis 1: Patients on no cost treatment have better adherence than patients on self-pay treatment.

Aim 2: Assess the impact on adherence of treating all HIV-infected members in a household.

Hypothesis 2: Treating all HIV-infected patients in a household is associated with higher adherence.

Aim 3: Assess the psychometric properties of the Beck Depression Inventory when used in an HIV-infected population.

Aim 4: Assess the bioequivalence of a fixed dose combination generic drug (Triomune®) with the brand name pharmaceutical equivalents (Zerit®/Epivir®/Viramune®).

Hypothesis 4: There is no difference in the bioavailability of the generic drug and the brand name drugs.

Chapter 1 reviews the problem of non-adherence to antiretroviral therapy and substandard/counterfeit drugs in resource-limited settings. In chapter II, data from a cohort of HIV-infected individuals (Adherence Monitoring Uganda-AMU) that was assembled when ART was not widely available and many patients paid for their medications out-of-pocket, were used to assess the effect of source of payment for medications on adherence. Chapters III and IV use data from a cohort assembled from the mother-to-child transmission plus (MTCT+) program. Chapter III assesses the impact of providing ART to all HIV-infected members in a household and chapter IV examines the psychometric properties of the Beck Depression Inventory (BDI-II). In chapter V, data collected from a sub-cohort of the AMU cohort are used to assess the bioequivalence of a generic drug (Triomune®) with the brand name pharmaceutical equivalents (Zerit®/Epivir®/Viramune®). Finally, chapter VI integrates the results of these studies, discusses the implications for further research, and makes recommendations for the way forward.

All studies were approved by the Uganda National Council of Science and Technology, the Institutional Review Board (IRB) of Makerere University, Kampala and the University of California San Francisco. For part of this dissertation, I conducted secondary data analysis with

de-identified data. The Committee for the Protection of Human Subjects at the University of California, Berkeley reviewed the data and decided that this analysis did not constitute human subjects' research and hence did not require their approval. The other section of this dissertation comprises two co-authored articles with permission from my co-authors to use and reproduce these papers as part of my dissertation. Permission to use these papers as part of my dissertation at UC Berkeley was sought from the Dean of the Graduate Division.

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CHAPTER 2

HIV Antiretroviral Therapy at No Cost to Patients is Associated with Better Adherence to Treatment: A Marginal Structural Model Analysis using a Targeted Maximum Likelihood Estimator

ABSTRACT

Objective. To estimate the causal effect of source of payment for HIV medication on the mean of treatment adherence.

Methods. Data were obtained from an observational cohort of 97 HIV-infected individuals who were observed from 2003 to 2006 in Kampala, Uganda. Adherence was the primary outcome and it was measured using the 30-day visual analogue scale. Marginal structural models were used to estimate the effect of source of payment for HIV medication on adherence, adjusting for confounding by income, duration on antiretroviral therapy (ART), timing of visit, prior adherence, prior CD4⁺ T cell count and prior plasma HIV RNA. Traditional association models were also examined and the results compared.

Results. No cost HIV treatment was associated with a 3.8% improvement in adherence in the marginal structural model, while the traditional statistical models showed a 3.1-3.3 % improvement in adherence associated with no cost HIV treatment.

Conclusion. Removing a financial barrier to treatment with ART by providing no cost HIV treatment appears to significantly improve adherence to antiretroviral therapy. With sufficient information on confounders, MSMs can be used to make robust inferences about causal effects in epidemiologic research.

INTRODUCTION

Access to antiretroviral therapy continues to expand at a rapid rate(1-3). Of the estimated 9.5 million people in need of treatment in 2008 in low- and middle-income countries, 42% had access, up from 33% in 2007(3). The greatest progress was seen in sub-Saharan Africa, where two-thirds of all HIV infections occur. Prices of the most commonly used antiretroviral drugs have declined significantly in recent years, contributing to wider availability of treatment. In most cases HIV medications are provided at no cost to the patients. A major challenge in this effort to scale up HIV treatment is the concern about enhancing treatment retention and adherence (3). Now that patients receive HIV medications at no cost, will they still value them as much as they would if they had to pay for them? Should patients contribute to paying for their HIV medications in order to preserve their perceived value and hence enhance adherence? These are some questions to consider when evaluating ways to maintain adherence to these life saving drugs. There is a lack of definitive information to guide these discussions.

Many studies in resource limited settings have documented that the cost of medications is a major predictor of non-adherence to ART (4-8). All these studies were observational in design. While important associations between variables can be obtained from observational studies, such studies often are unable to adequately control for confounding, leading to biased estimates of causal effects.

In observational studies, estimation of the causal effect of an exposure on an outcome may be biased because of confounding, i.e. covariates associated with treatment may also be associated with the potential response, so that the observed response differences cannot be attributed directly to the exposure. Proper estimation of causal effects must account for confounding. In studies where the treatment/exposure does not change (i.e. point treatment), the traditional method of analysis is to model the probability of disease as a function of exposure and pretreatment covariates. However, with a time-varying exposure, these traditional methods may be biased if time-varying covariates are simultaneously confounders and intermediates-that is, if covariates are predictors of the outcome and also predict subsequent exposure, and past exposure history predicts resulting covariate level (9). Such covariates are called time-dependent confounders (9), and they pose unique analytical challenges requiring specialized methods.

Marginal structural models (MSMs), developed by Robins et al (9, 10) allow proper adjustment for time-dependent confounding. MSMs are being used more frequently to obtain causal effect estimates in observational studies (11-21), where causal effects are typically defined by a comparison of how a population mean outcome changes when the population exposure of interest changes. These models are appealing because the coefficients are directly interpretable causally and they provide unbiased marginal estimates, even in the presence of time-dependent confounding.

Inverse probability of treatment weight (IPTW), G-computation, double robust and targeted maximum likelihood estimation (TMLE) methods are techniques used for estimating parameters in a MSM (13, 14). Among these, the targeted MLE is the most robust to model specification (22). However, there are few simple examples that illustrate how to apply targeted MLE to obtain estimates of causal associations between exposures and outcomes.

In this paper, we describe the application of MSMs using targeted MLE to estimate the causal effect of source of payment for ART on treatment adherence in Uganda. Comparing adjustment methods yields useful insights into the advantages of MSMs. We compare the results obtained using MSMs with those obtained from traditional association models, discuss the

strengths and weaknesses of MSMs, and highlight key issues epidemiologists should recognize before and while undertaking such an analysis.

Our main objective is to estimate the causal effect of source of payment for HIV medication on the mean of treatment adherence. Another objective of this analysis is to demonstrate an application of MSMs to a real analytical problem to encourage use of this approach among epidemiologists.

MATERIALS AND METHODS

In this analysis, we utilized data from the Adherence Monitoring Uganda (AMU) study (23, 24). AMU was an observational prospective cohort study of adherence and treatment response among individuals on HIV generic antiretroviral therapy conducted from 2002–2007 in Kampala, Uganda. The cohort comprised both patients on self-pay and those on no cost treatment. During the study period some subjects switched from self-pay to no cost treatment. Details of recruitment and data collection have been published elsewhere (23, 24).

Briefly, data on adherence and other covariates like CD4 T cell count, viral load, income and source of payment for HIV antiretroviral medications were collected monthly for the first six months then every three months for the next 18 months for each patient. The outcome of interest was adherence at every visit. Adherence was measured by electronic medication monitoring (EMM), unannounced pill count, 3-day self report and 30-day visual analogue scale for the first six months and by 30-day visual analogue scale only for the next 18 months. Adherence was defined as the percentage of prescribed pills taken over a given period. The main exposure variable for this analysis [hereto referred as treatment] was source of payment for HIV medication. Subjects were asked at every visit who had paid for their medication for that month.

Confounders considered included prior adherence, prior CD4 T cell count, prior plasma HIV RNA level, income, duration on ART and time of visit.

Statistical analyses

Marginal structural models (MSM) were used to estimate the mean difference in adherence for a given month that would have been observed between the treatment group (those individuals that received subsidized therapy) and the control group (those individuals who paid for their therapy) if source of payment for therapy had been assigned randomly. The marginal treatment effect is the parameter of interest. A targeted maximum likelihood estimator (TMLE) was used to estimate this parameter. A data set was created that consisted of a data point for each person-month during follow-up for which source of payment for medication and subsequent adherence were measured. Thus, each person contributed several data points. That is, repeated measures of the outcome were used for each person. The same treatment effect was estimated for every time point and all the observations within an individual were pooled. The observed data for a given person-month consisted of a binary treatment (A = source of payment for medication for a given month), a continuous outcome (Y = adherence for the month), and a set of covariates (W).

Causal effect estimation

We estimated the marginal additive effect of a binary point treatment, A , on outcome Y . The MSM was

$$E(Y_a) = \beta_0 + \beta_1 a$$

where a was the actual treatment received and takes on the value 1 if a patient received subsidized treatment and a value 0 if a patient paid for their treatment.

The parameter of interest, the marginal additive treatment effect, was defined as

$$\psi_0 = E(Y_i(1) - E Y_i(0)),$$
 which in this case is equal to β_1 .

where $Y_i(1)$ corresponds to the outcome observed when subject i receives the treatment group ($A_i = 1$) and $Y_i(0)$ corresponds to the outcome observed when subject i is assigned to the control group ($A_i = 0$). Here $A_i = 1$ represents a patient receiving subsidized therapy while $A_i = 0$ represents one on self-pay therapy. The parameter of interest ψ_0 was estimated using the observed data as:

$$\hat{\psi} = \hat{E}_w(\hat{E}(Y | A = 1, W)) - (\hat{E}(Y | A = 0, W)).$$

Targeted maximum likelihood estimation

Targeted maximum likelihood estimation targets the parameter of interest in a way that reduces bias of the parameter of interest while possibly increasing the bias and/or variance of other parameters that are not of interest (nuisance parameters)(25). Usual maximum likelihood estimation may not be making the best trade-off between bias and variance for the parameter of interest. To conduct TMLE, two major assumptions were made: the first assumption, randomization assumption (RA), implies that conditional on measured covariates, treatment assignment is independent of the outcome. The second assumption, the experimental treatment assignment (ETA) assumption, requires that the conditional probability of receiving treatment conditional on the covariates is bounded away from 0 and 1. In other words, observations within strata defined by W have a probability greater than 0 of receiving treatment at all possible levels of the treatment assignment.

We define

$Q(A, W) = E(Y | A, W)$ the density/mean of the conditional distribution

$g(A, W) = P(A | W)$ the probability of receiving the treatment given the covariate history, also called the treatment mechanism. Both $Q(A, W)$ and $g(A, W)$ are estimable from the data.

The TMLE estimator is given by:

$$\hat{\psi}^{TMLE} = \frac{1}{n} \sum_{i=1}^n \hat{Q}^*(a = 1, W_i) - \hat{Q}^*(a = 0, W_i)$$

$\hat{Q}^*(A, W)$ is the targeted estimate of the density and to obtain it involves estimation of nuisance parameter $g(A, W)$ as well. Start with an initial estimate for the conditional mean of Y , $\hat{Q}^0(A, W)$

Q_n^* can be estimated using a data adaptive procedure like Super Learner (26). For this analysis, we used the Deletion/Substitution/Addition (DSA) algorithm (27, 28) with 10-fold cross validation. To account for the correlation between observations on the same subject, all observations on the same subject were put in the same fold. This estimate may also be obtained using standard multivariable regression.

The initial estimate is fluctuated in a manner designed to reduce the bias for the parameter of interest.

$$\hat{Q}^1 = \hat{Q}^0 + \hat{\varepsilon}h(A,W)$$

where $h(A,W)$, a function based on the parameter of interest, and in this context is a function of the treatment mechanism, is the inverse of the probability of receiving the treatment and is obtained from this formula

$$h(A, W) = \frac{I(A=1)}{p(A=1|W)} - \frac{I(A=0)}{p(A=0|W)}$$

where $I(A=k)$ where k is equal to 0 or 1, is an indicator function for the type of treatment received. An indicator function is a function defined on a set X that indicates membership of an element in a subset A of X , having the value 1 for all elements of A and the value 0 for all elements of X not in A . For this case, $I(A=1)$ is the indicator for receiving treatment $A=1$ (no cost treatment) and is thus represented by 1 in this equation for someone who received no cost treatment and 0 for $I(A=0)$. Likewise for someone who paid for their treatment $I(A=0)$ would be 1 and $I(A=1)$ would be 0. This function, $h(A,W)$, also called a “clever covariate” is specific for this particular parameter of interest in the MSM and is not the general clever covariate for the TMLE.

The estimate for ε is obtained by regressing Y on $h(A,W)$, with offset $\hat{Q}^0(A,W)$. The magnitude of ε represents the degree of residual confounding based on the initial fit with regards to the parameter of interest. This is referred to as the targeting step in TMLE. The targeting step is repeated until $\hat{\varepsilon} = 0$. In the current TMLE with the parameter of interest, this occurs after one step, therefore $\hat{Q}^1(A,W) = \hat{Q}^*(A,W)$

The treatment mechanism was fitted data-adaptively using the DSA algorithm based on multivariable logistic regression of source of payment for treatment on confounders (W). Time-lagged confounder measurements were used to ensure that confounders occurred before (and, therefore, could not be influenced by) payment source (Figure 1). HIV RNA values and income were log transformed to control outlier values. Missing confounder values were imputed by using the median value of that variable. Missing values for payment source and adherence were not imputed.

The final step was to estimate the parameter of interest-- the difference in mean adherence between when everyone receives no cost therapy and when everyone pays for their therapy. Using the final updated model, mean adherence was estimated assuming everyone had received no cost therapy and also assuming everyone had paid for their therapy. The difference between these two means provided our parameter of interest. This process is analogous to running an ideal experiment, in which the investigator first assigns each individual in the cohort to receive no cost treatment and observes the resulting adherence and then assigns the identical cohort to pay for their treatment and observes the resulting adherence. Instead, in this step, the investigator sets source of payment for treatment equal to 1 in the regression model and records the predicted adherence for all person-months and then sets source of payment for treatment equal to 0 and records the predicted adherence for all person-months.

Standard errors for calculating the 95% confidence intervals were estimated using clustered bootstrap (i.e. randomly sampling patients with replacement). Corresponding R code is provided in the Appendix.

Other assumptions were also used for this analysis. It was assumed that the covariates (W) were measured before the treatment (A) and the treatment was measured before the outcome (Y) [time ordering assumption]. This is implied by the data collection protocol. Existence of counterfactuals was assumed [counterfactual assumption]. That is, what was observed (Y) was one of the potential outcomes. Counterfactual outcomes are all the potential outcomes that a subject can experience given the available treatments although, s(he) can only experience one of them that results from the specific treatment received. It was assumed that there were no unmeasured confounders. That is, treatment was independent of the counterfactuals given the covariates [randomization assumption]. The ETA assumption was evaluated by looking at the predicted probabilities of treatment for the treatment mechanism. There was no observed violation of the ETA assumption, with the minimum predicted probability of treatment being 0.08 and the maximum being 0.89. All analyses were conducted using R software Version 2.7.2.

RESULTS

Participant characteristics. Ninety seven participants were enrolled into the study. Participants initiated therapy at advanced stages of HIV infection, with a mean CD4 cell count of 56 cells/ml [SD 130] and median \log_{10} copies RNA/ml of 5.53 (IQR 4.91-5.82). The majority of the cohort was female (63.9%). The mean age was 36 years (SD 7.5). Nearly one-quarter of participants were unemployed and paid for therapy by transfers (borrowing money) from other family members or friends. One third of the study participants had completed up to a primary level of education. Details of participant characteristics at study entry have been published elsewhere (24).

Seventy six of the 97 participants enrolled into the study were included in this analysis with a total of 251 observations. The 76 participants were observed for a total of 1669 person-months. Median follow up time was 22 person-months (IQR 18-27). Half of the participants switched from self-pay to no cost therapy (38/76). Of the 21 excluded from the analysis, 18 did not have follow-up data beyond 24 weeks and 3 were missing adherence data at visits beyond 24 weeks. This analysis analyzed data collected after 24 weeks of follow up because before this time data were not collected on source of payment for HIV medications.

Predictors of treatment. In the model for the treatment mechanism, receiving no cost treatment was more likely to occur among individuals with a lower prior CD4⁺ cell count and at later visits (Table 1).

Adherence and source of payment for HIV medication. Overall mean adherence (\pm SD) over the course of follow up was 95.68% \pm 16%. Mean adherence in the self-pay person-months was 93.50% \pm 19.16% while that in the no cost person-months was 98.56% \pm 9.78% (Figure 2). In the model selected by the DSA algorithm, current higher adherence was more likely to occur among patients with a higher rate of prior adherence and those with lower prior HIV RNA (Table 2).

Applying the targeted maximum likelihood estimator, receiving no cost HIV medication was estimated to increase adherence by 3.82% compared to self-pay treatment. This was slightly higher than the estimates from two traditional association models - generalized estimating equations (GEE) and ordinary least squares (OLS) (Table 3).

DISCUSSION

Observational cohort studies collecting comprehensive longitudinal data provide a valuable source of information supplementing efficacy measures from randomized trials. In the absence of data from randomized trials, prospective observational data are often the best available evidence for assessment of therapeutic effects. Using a marginal structural model, we estimated a 3.8% difference in mean adherence when HIV-infected patients receive no cost HIV treatment compared to when they pay for the treatment out-of-pocket. Our finding is modest compared to results from other studies(5, 7) that looked at the impact on adherence in absolute terms of no cost therapy and self-pay therapy. By how much does adherence change when patients switch from self-pay treatment to no cost treatment? Weiser and colleagues showed that if cost was removed as a barrier to adherence, the proportion of adherent individuals in Botswana would increase from 54% to 74% (7). In a cohort in Senegal, adherence rose from 83% to 93% when the cost of HIV medications was reduced(5). In Cameroon, Boyer et al found an inverse relationship between adherence and self-reported financial difficulties(29). While all of these studies, including our study, were observational studies, there are some important differences to note. The Botswana and Cameroon studies were cross sectional in design. Cross sectional studies generate hypotheses but they do not test hypotheses. Such studies lie at the bottom of the hierarchy for making causal inference. Our study and the study in Senegal were prospective in nature, which gives them more credibility to make causal inference. Another major difference is in the analytic techniques. Our study employed a marginal structural model while the other studies employed traditional association models.

Our analysis using traditional models gave effects of 3.3% and 3.1% using a repeated measures model and the ordinary least squares model respectively. These effect sizes were less than the one from the MSM, underestimating the net effect of payment source on adherence, with the estimate from the ordinary least squares models failing to meet statistical significance (95% CI -0.57 to 7.24) Other analyses have shown greater attenuation of effects and even reversal of effects when using traditional statistical models (11, 13).

There are situations in which traditional association model estimates are equivalent to MSM estimates and have a causal interpretation. This can occur only when the treatment groups are exchangeable—that is, when there is no confounding and the only difference between the treatment groups is that one received the treatment and the other did not (for a binary treatment). This is the situation in a well randomized study. It is difficult to achieve such a situation in an observational study and the best one can do is to collect data on all the important known or suspected confounders and use appropriate analysis techniques to obtain results similar to those from a randomized study. The results from our traditional association models were similar to those from the MSM model, suggesting that the confounders we considered for analysis were probably the actual confounders for this association and the analysis groups were almost exchangeable, fulfilling the assumption of “No Unmeasured Confounders” for both analyses. Use of a causal diagram when designing a study is a useful way to identify potential confounders and to identify which variables to adjust for in the analysis to avoid creating further confounding(30, 31).

Our findings are useful in the ongoing debate in low and middle-income countries whether to retain or remove user fees in health care systems. There is broad consensus that user fees are an important barrier to accessing health services, especially for poor people(32-34). In addition, they negatively impact adherence to long-term expensive treatments, among other

effects. With regard to adherence to HIV medications, self-pay for medications may result in poor adherence because of the possibility of rationing drugs to cover a longer period; sharing drugs among HIV-infected family members; or complete failure to purchase the drugs.

There are a number of limitations to our study. First, our study was conducted among ARV-naïve individuals who had just started on treatment at an advanced stage of disease. Because every participant's desire at this time was to not die, adherence to medications was high, irrespective of the source of payment for medication. The dramatic "Lazarus effect" (i.e. the miraculous improvement in health condition) inspired serious efforts to receive the treatment despite the challenges associated with acquiring the medications. The situation is different today. People have been taking these medications for some time and are generally healthier, and it is uncertain whether these results would be replicated in an ARV-experienced population. Our guess is that the treatment effect would be even greater in an ARV-experienced population in which temporary lack of money to purchase the medications might be welcomed as a "drug holiday". Second, our sample size was rather small. Our analysis included only 76 patients. This explains the rather wide 95% confidence intervals we observed. However, using repeated measures on patients increased the power of the study to detect differences between the treatment groups. Finally, we used a thirty-day visual analogue scale (VAS) to measure adherence. There have been mixed findings with self reported measures of adherence (35,36). Some studies suggest that the VAS is not a dependable measure of adherence. In one study in Malawi, adherence when measured with the VAS was not significantly associated with detectable viral load even after controlling for possible confounders (36). However in our study the VAS estimates of adherence had a significant association with viral load. For every unit increase in adherence there was a decrease in viral load of 550 cells/microliter controlling for CD4 cell count and income (P value < 0.0001).

Our study also had several strengths. Data were drawn from a well-studied cohort with high retention rates and well characterized adherence measures(23, 24, 37). In addition, the study was conducted at a time when the healthcare system in Uganda was transitioning from self-pay to no-cost treatment, which provided a "natural experiment" for study. It would be unethical at the present time to conduct a randomized trial to answer the question answered in this analysis. Furthermore, state-of-the-art data analyses and the use of alternative methods to control for confounding improved the robustness of the findings.

In summary, we found that receiving no cost HIV treatment was associated with better adherence among low income HIV-infected patients in a resource-limited setting. MSMs and other models for causal inference from longitudinal data are important in situations where the conduct of randomized trials is not possible because of cost and ethical concerns. With sufficiently rich information on confounders for exposure and outcome and few missing data, MSMs are a great tool for causal inference.

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Figure 1 Directed acyclic graph (causal diagram)

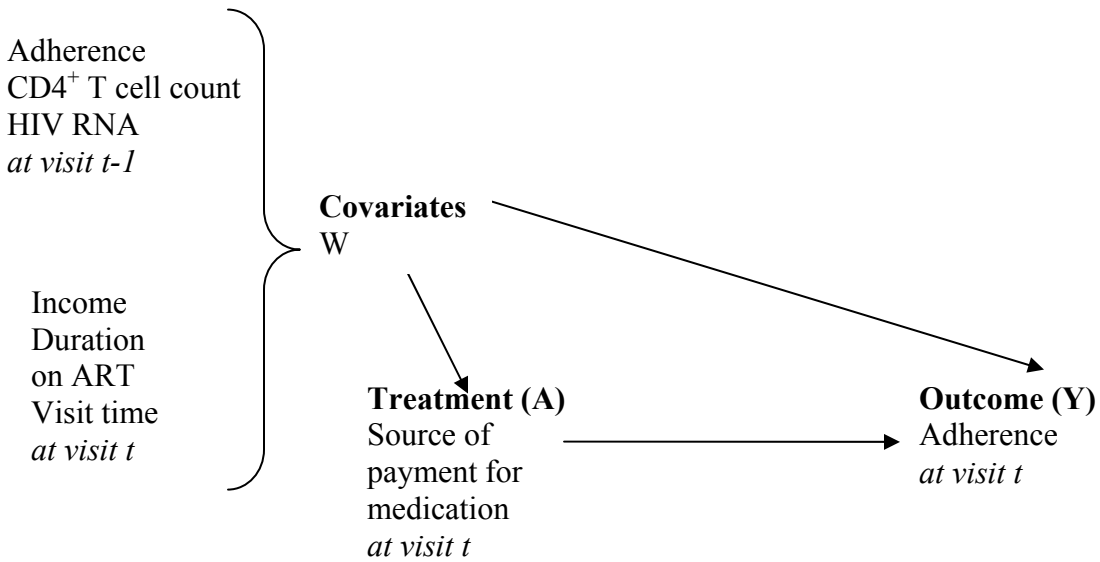


Figure 2 Comparing mean adherence rates for person-months on self-pay treatment and person-months on no cost treatment

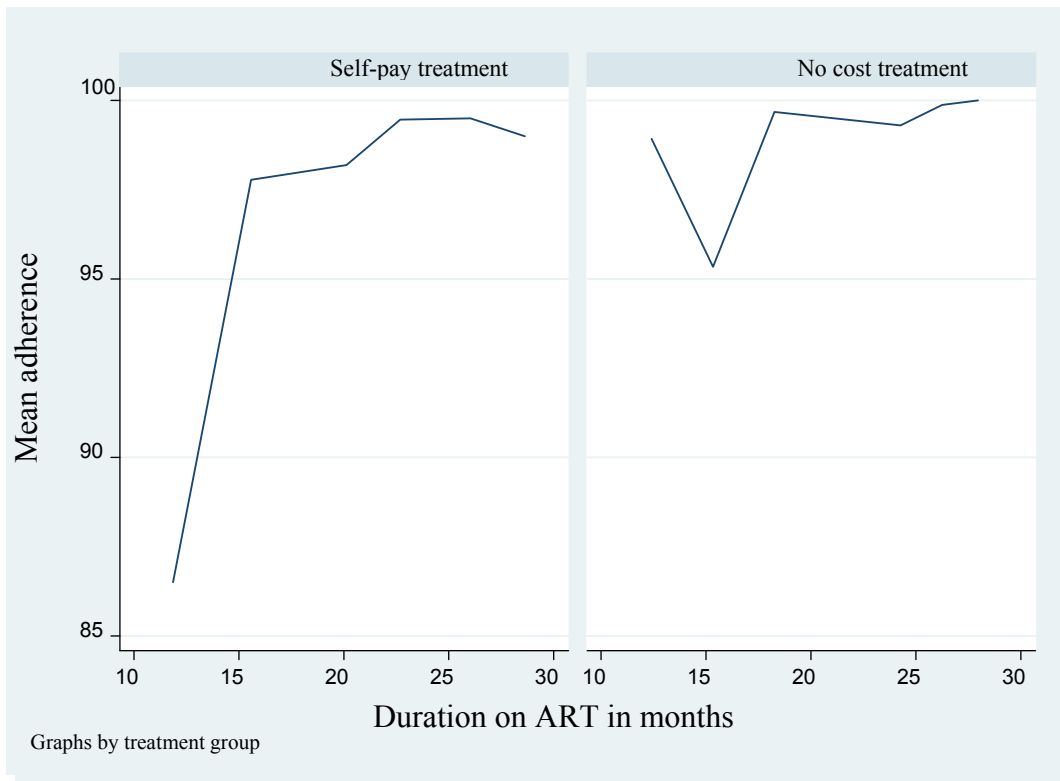


Table 1 Multivariable regression model of source of payment for HIV medication on confounders

Term in multivariable logistic regression model	OR (95%CI)
Prior adherence	1.002 (0.983 to 1.021)
Prior HIV RNA ^a	1.042 (0.878 to 1.235)
Prior CD4 ⁺ cell count	0.998 (0.997 to 0.999)
Income ^a	0.971 (0.906 to 1.041)
Duration on ART	1.000 (0.998 to 1.003)
Time of visit	1.905 (1.333 to 2.722)

NOTE: Model was selected using cross-validated deletion/substitution/addition algorithm.

^a log transformed

Table 2 Multivariable regression model of adherence percentage on source of payment for HIV medication and confounders

Term in multivariable linear regression model	Coefficient (95%CI)
Source of payment for HIV medication	3.332 (-0.575 to 7.239)
Prior adherence	0.232 (0.107 to 0.357)
Prior HIV RNA ^a	-1.413 (-2.546 to -0.279)
Prior CD4 ⁺ cell count	-0.003 (-0.014 to 0.007)
Income ^a	0.054 (-0.406 to 0.514)
Duration on ART	0.003 (-0.014 to 0.020)
Time of visit	1.337 (-0.964 to 3.637)

NOTE: Model was selected using cross-validated deletion/substitution/addition algorithm.

^a log transformed

Table 3 Causal mean difference estimates (MSM) vs. Traditional model estimates of the effect of source of payment for HIV medication on adherence

Method	Difference in mean adherence (95% CI)
Marginal Structural Model	3.82 (1.10-6.53)
Generalized Estimating Equations	
Crude	6.26 (2.66-9.85)
Adjusted*	3.10 (0.95-5.24)
Ordinary Least Squares	
Crude	5.06 (1.09-9.04)
Adjusted*	3.33 (-0.57-7.24)

- adjusted for income, duration on ART, prior adherence, prior CD4+ T cell count, prior HIV RNA

APPENDIX

R CODE FOR IMPLEMENTING TARGETED MAXIMUM LIKELIHOOD ESTIMATOR

DSA is available at <http://www.stat.berkeley.edu/~laan/Software/>

```
library(DSA)
```

```
mydsa1 <- DSA(adherence~pre.adh+tx+pre.CD4+log.pre.VL+log.income+time+ARTdur,data = data.impute[,  
c(1,6,8,9)],userseed=252,family=gaussian,  
maxsize=20,maxorderint=2,maxsumofpow=2,vfold=10,nsplits=1,id=as.numeric(data.impute$ID))
```

```
Q0 <- glm(mydsa1$model.selected,family=gaussian,data=data.impute)  
summary(Q0)
```

```
#Treatment Mechanism
```

```
mydsa2 <- DSA(tx~pre.adh+pre.CD4+log.pre.VL+log.income+time+ARTdur,data = data.impute[,  
c(1,3,6,8,9)],userseed=252,family=binomial,  
maxsize=20,maxorderint=2,maxsumofpow=2,vfold=10,nsplits=1,id=as.numeric(data.impute$ID))
```

```
glm1 <- glm(mydsa2$model.selected,family=binomial,data=data.impute)  
summary(glm1)
```

```
pred.tx <- predict(glm1,type="response",newdata=data.impute)  
summary(pred.tx)  
hist(pred.tx)
```

```
## Clever Covariate
```

```
cc <- ifelse (data.impute$tx==1, 1/pred.tx,-1/(1-pred.tx))
```

```
## Targeted MLE
```

```
pred.Q0 <- predict(Q0,type="response", newdata=data.impute)  
fit.E <- lm(adherence~ -1 + offset(pred.Q0) + cc, data=data.impute)
```

```
##
```

```
data.A1 <- data.impute  
data.A1$tx <- 1
```

```
data.A0 <- data.impute  
data.A0$tx <- 0
```

```
Q1.A1 <- predict(Q0,type="response",newdata=data.A1) + fit.E$coef*(1/pred.tx)  
Q1.A0 <- predict(Q0, type="response", newdata=data.A0) + fit.E$coef*(-1/(1-pred.tx))
```

```
## Parameter of interest
```

```
mean.effect <- mean((Q1.A1-Q1.A0),na.rm=TRUE)  
mean.effect
```

```
# Variance estimate based on clustered bootstrap
```

```
> n.boot <- 1000  
> mean.effect.boot <- rep(NA,n.boot)  
> full.ID <- as.numeric(data.impute$ID)
```

```

> n.ID <- length(unique(full.ID))
>
> for(bb in seq(n.boot)){
+ boot.ID <- sample(unique(full.ID),size=n.ID,replace=TRUE)
+ boot.data <- NULL
+ for(ii in seq(n.ID)){
+ boot.data <- rbind(boot.data,data.impute[full.ID==boot.ID[ii],])
+ }
+ boot.n <- nrow(boot.data)
+ # mydsa1 <- DSA(adherence~pre.adh+tx+pre.CD4+log.pre.VL+log.income+time+ARTdur,data = boot.data[,c(1,6,8,9)],family=gaussian, maxsize=20,maxorderint=2,maxsumofpow=2,vfold=10,nsplits=1)
+ # Q0 <- glm(mydsa1$model.selected,family=gaussian,data=boot.data)
+ Q0 <- glm(adherence~pre.adh+pre.CD4+log.pre.VL+log.income+time+ARTdur,family=gaussian,data=boot.data)
+
+ # mydsa2 <- DSA(tx~pre.adh+pre.CD4+log.pre.VL+log.income+time+ARTdur,data = boot.data[,c(1,3,6,8,9)],family=binomial, maxsize=15,maxorderint=2,maxsumofpow=2,vfold=10,nsplits=1)
+ # glmTx <- glm(mydsa2$model.selected,family=binomial,data=boot.data)
+ glmTx <- glm(tx~pre.adh+pre.CD4+log.pre.VL+log.income+time+ARTdur,family=binomial,data=boot.data)
+
+ pred.tx <- predict(glmTx,type="response",newdata=boot.data)
+ pred.tx[pred.tx < 0.01] <- 0.01
+ pred.tx[pred.tx > (1-0.01)] <- (1-0.01)
+
+ cc <- ifelse (boot.data$tx==1, 1/pred.tx,-1/(1-pred.tx))
+ pred.Q0 <- predict(Q0,type="response", newdata=boot.data)
+ fit.E <- lm(adherence~ -1 + offset(pred.Q0) + cc, data=boot.data)
+ data.A1 <- boot.data
+ data.A1$tx <- 1
+
+ data.A0 <- boot.data
+ data.A0$tx <- 0
+ Q1.A1 <- predict(Q0,type="response",newdata=data.A1) + fit.E$coef*(1/pred.tx)
+ Q1.A0 <- predict(Q0, type="response", newdata=data.A0) + fit.E$coef*(-1/(1-pred.tx))
+ mean.effect.boot[bb] <- mean((Q1.A1-Q1.A0),na.rm=TRUE)
+ # print(bb)
+ # print(boot.n)
+ # print(boot.ID)
+ }
>
> summary(mean.effect.boot)
> sd(mean.effect.boot)
> c(mean.effect-1.96*sd(mean.effect.boot),mean.effect,mean.effect+1.96*sd(mean.effect.boot))

```


CHAPTER 3

Longitudinal Antiretroviral Adherence in New HIV+ Ugandan Parents and Their Children in the MTCT-Plus Family Treatment Model: Role of Depression and Declining Adherence over Time.

ABSTRACT

To assess the effect of family-based treatment on adherence amongst HIV-infected parents and their HIV-infected children, we conducted a quantitative study with a qualitative component among members of the Mother-To-Child-Transmission Plus program (MTCT-Plus) in Kampala, Uganda. The MTCT-Plus program provided free antiretroviral therapy (ART) to all World Health Organization (WHO) stage III/IV individuals in a family of the index HIV+ mother. We collected home based unannounced pill counts and self reported adherence in 75 patients initiating antiretroviral therapy for 6 months and an additional 102 patients on stable antiretroviral therapy (mean =11.8 \pm 5.4 months). Mean adherence was 99.2% \pm 7.5 over 24 weeks for the newly treated individuals and was 94.1% \pm 10.7 for the chronically treated individuals. Depression was the only factor associated with incomplete adherence (< 95%) by multivariable analysis (OR= 0.32; 95% CI (0.11-0.93); p=0.04). Adherence in newly treated children was similar to that of newly treated adults but it was less in chronically treated children compared to chronically treated adults. Among those with average adherence \geq 95% by pill count, 83% (54/65) achieved virologic suppression (\leq 400 copies/ml) at 24 weeks of follow-up. There was a significant change in adherence over time (OR=0.93; 95% CI (0.88-0.97). Patients on ART for 1 month longer had 0.93 times the odds of achieving \geq 95% adherence. Number of children in the household was not associated with adherence to ART (p=0.99) Qualitative interviews revealed lack of transportation money, stigma, response to therapy, drug packaging and cost of therapy may impact adherence. Our results indicate that providing free ART to all eligible members in a household is associated with excellent adherence in both parents and children. Adherence to ART among new parents declines over time even when patients receive treatment at no cost. Depression should be addressed as a potential adherence barrier. Further study will be necessary to assess the long-term impact of this family model on adherence to ART in resource-limited settings.

INTRODUCTION

Globally, there were an estimated 33 million people living with HIV in 2007. [1] Women account for half of all people living with HIV worldwide, and nearly 60% of all HIV infections in sub-Saharan Africa.[1] About 800, 000 adults in Uganda live with HIV/AIDS giving an HIV prevalence rate of 6.1%. Of these, about 60% (480, 000) are women. [2] Among the 3 million people who were receiving antiretroviral therapy at the end of 2007, 61% were female[3].

While most studies in resource-constrained settings have shown high levels of adherence to antiretroviral therapy [4-24], there has been little discussion on how HIV+ women balance demands of adherence to HIV antiretroviral therapy, childcare, and antiretroviral adherence among their children who are HIV-infected. Motherhood potentially places particular stress on HIV-infected women, due to higher levels of depression, poorer family cohesion, less ability to perform daily functions, and care of HIV infected children. [25] In a study to evaluate whether living with children adversely affects adherence to ART in HIV-infected women, Merenstein et al (2008) found that adherence to ART was inversely associated with the number of children living in the household. [26] Each additional child reported living in the household was associated with a 6% decrease in the odds of $\geq 95\%$ adherence. The desire to share or ration therapy is a concern for individual programs that may not provide treatment to the entire family. [27] This is particularly important in Uganda due to the high rate of fertility. Uganda has the third highest total fertility rate in Africa, at 6.81, and the third highest worldwide, with a crude birth rate of 48.15 births per 1000 population. [28] The specifics of how HIV-infected women balance childcare, adherence to their own medication and adherence among their children need to be further elucidated. Using data from participants in the MTCT-Plus Program in Kampala Uganda, we examined whether having children in a household affected adherence to ART; the effect on adherence when all eligible HIV-infected members in a household are treated; and the factors associated with incomplete adherence in HIV-infected mothers.

METHODS

Participants and Setting

The study was conducted among patients attending the MTCT-Plus program at Mulago Hospital in Kampala, Uganda. Mulago Hospital is Uganda's largest teaching, referral, and research hospital. The MTCT-Plus program works through existing MTCT programs that use established treatments to prevent mother-to-child transmission of HIV. It is a practical model of family-centered, multidisciplinary care for HIV-infected patients. The "Plus" component provides an essential care package with appropriate therapies for mothers and their HIV-infected family members that include basic care for prevention and/or treatment of HIV-related opportunistic infections and treatment with antiretroviral drugs.

Study Description

We conducted a quantitative study to measure adherence among individuals attending the MTCT-Plus program and a qualitative study to understand reasons for incomplete adherence

among HIV-infected mothers. In order to look at adherence to initial and chronic antiretroviral therapy, we recruited individuals who were newly initiating antiretroviral therapy and individuals who were on chronic antiretroviral therapy. New initiates were followed for six months and individuals on chronic treatment received a single assessment.

Recruitment

New antiretroviral treatment

Patients initiating antiretroviral therapy were recruited if they were antiretroviral (ARV) naïve, residing within 15km of the city centre, planning to stay within the study radius for six months and consented to participate. We considered individuals ART-naïve if they had never taken ART, except for vertical transmission prophylaxis for the women and children. From April 2004 to March 2005, we enrolled a consecutive sample of patients initiating ARV therapy at the MTCT-Plus Program clinic. Newly treated individuals received monthly unannounced pill counts, quarterly structured interview, and quarterly phlebotomy.

Stable antiretroviral treatment

Patients on stable antiretroviral therapy for 2 – 42 months were identified from the MTCP-plus register. Stably treated individuals received a single structured interview and unannounced pill count.

Data Collection

After determining eligibility and obtaining written informed consent, the research assistants administered a structured questionnaire in either English or Luganda (the dominant regional language) to obtain socio-demographic characteristics of the participants. The Luganda questionnaire was translated from the original English version into Luganda, and then a second translator back-translated the questionnaire into English to ensure consistency between the two languages. Mothers or guardians consented for their children below 10 years and children above 10 and below 18 years gave assent to participate. In addition, the research assistants administered the Beck Depression Inventory (BDI) to detect depressive symptoms. This instrument has been used elsewhere in sub-Saharan Africa. [29-31]

For new initiates, the research assistant did a baseline count of the pills the patient had received from the pharmacy on the day of enrollment. Tracking information including contact address, telephone, friends and relatives' names and places where the participant usually spends their time was collected. Participants were escorted home to map their home location for future follow-up adherence visits.

Adherence Measures

Three measures of adherence were used: unannounced pill count, 3-day self report and 30-day visual analogue scale as previously described. [13] Adherence measures were collected monthly for patients initiating therapy and once for patients on stable antiretroviral therapy. At each visit, all three measures of adherence were administered and reasons for missed pills recorded. On the visual analog scale, participants were asked to mark off on a scale that run from

0% to 100%, a point that best described how well they had taken their pills over the previous 30 days. For children using liquid ARV formulations the amount of each medication taken between home visits was determined by weighing the medications in their containers at the beginning of the study and at the time of the visit to avoid excessive handling. We used a Proscale PR-500 digital pocket scale to measure the weights. The care takers of the children reported the children's adherence.

Biologic Measures

Plasma HIV-1 RNA (viral load) and CD4 T cell count were assessed at baseline and at 24 months for patients initiating antiretroviral therapy and once for the patients on stable antiretroviral therapy using Amplicor HIV-1 Monitor, version 1.5 (Roche Diagnostics), protocol (lower limit of detection, 400 copies per milliliter) and flow cytometry respectively.

Qualitative Study

Focus Group Discussions (FGDs)

Three FGDs were conducted to understand the factors associated with adherence. The groups were made homogenous to encourage free discussion among people with similar characteristics. Hence we formed groups of six HIV positive mothers, five HIV positive male partners and four HIV negative male partners, respectively. Discussions were conducted in the local language (Luganda) and facilitated by a research assistant well trained in qualitative research methods. The discussions were tape recorded and transcribed and then translated into English.

In-depth interviews

From the prospective study, 10 participants were purposively selected to participate in the one-on-one in-depth interviews. Interviews were conducted at participants' homes by a pair of researchers, one Ugandan and one American. Interviews were conducted in either English or Luganda, depending on the participant's preference. Luganda is the local language commonly used in Kampala. Patients were asked to describe how they initiated antiretroviral medication and the impact of HIV therapy on their health, their household finances, and their family. Interviews were coded according to theme and analyzed using an inductive method. [32] Results reported here focus on the themes of adherence and medication cost, and are presented in narrative form in order to highlight the complex social dynamics of medication access and adherence described by patients.

Data Analysis

Socio-demographic characteristics were summarized using medians and means with standard deviations (SD) for continuous variables and using proportions for categorical variables. Depression was defined as a BDI score of ≥ 14 . For the new ARV initiates, the level of adherence was determined by calculating the average of all individual adherence scores over six

months. Each measure of adherence was considered separately and an average score for each measure determined. Adherence was also dichotomized at $<$ or $\geq 95\%$. Logistic regression models were used to determine predictors of adherence and to adjust for the effects of potential confounders in ART treated adults. There were too few subjects to model predictors of adherence in children. Generalized estimating equations (GEE) were used to assess the longitudinal effect of time on adherence[33, 34]. Bootstrap standard errors were used to make inferences[35]. All statistical tests were two-sided and considered significant at $\alpha = 0.05$. Ninety five percent confidence intervals were estimated for the results.

RESULTS

Baseline Characteristics

One hundred seventy-seven participants were enrolled in the entire study: there were 75 people newly initiating antiretroviral therapy and 102 on stable antiretroviral therapy for a total of 177. Of the 177 patients 70% (n=124) were women and 23% (n= 41) were children below 18 years of age. Median age was 30 years. Among the adults, the majority of the patients (65%) were married. Details of each study are given in Table 1. About half of all the patients (49%, 74/149) had primary education as the highest level of education attained. Some children (n=25) and adults (n=3) had not had any formal education. Seventy one percent (n=126) of the patients reported having at least one other household member infected with HIV while 38% (n=68) reported having at least one other member of the household on ART. Median baseline CD4 count was 277 cells/L.

Adherence

New antiretroviral therapy

Mean (\pm SD) adherence was $98.0\% \pm 7.3$, $97.9\% \pm 6.4$ and $99.2\% \pm 7.5$ by 3-day self report, 30-day visual analogue scale and home based unannounced pill count for all observations, respectively. Median adherence by all measures was 100%. (Table 2) Sixty-six participants (88%) had $\geq 95\%$ adherence by pill count. Among those with average adherence $\geq 95\%$ by pill count, 83% (54/65) achieved virologic suppression (≤ 400 copies/ml) at 24 weeks of follow-up. (Table 4)

Stable antiretroviral therapy

The mean, median, and range of antiretroviral therapy prior to enrollment was 11.8 ± 4.5 , 11 (IQR= 8-15), and 2-42 months, respectively. Mean (\pm SD) adherence was $100\% \pm 0.0$, $100\% \pm 0.0$ and $94.1\% \pm 10.7$ by 3-day self report, 30-day visual analogue scale and pill count, respectively. All patients reported $\geq 95\%$ adherence, but pill count showed that only 60% of the patients (61/102) had $\geq 95\%$ adherence.

Adherence in children

Mean (\pm SD) adherence for new initiates was 98.0 ± 4.4 , 97.9 ± 5.3 and 100 ± 10.4 by 3-day self report, 30-day visual analogue scale and pill count, respectively. Among chronic patients, the mean, median, and range of antiretroviral therapy prior to enrollment were 13 ± 7.3 , 10.5 (IQR=8-17.5) and 5-42 months, respectively. Mean (\pm SD) adherence was 100.0 ± 0.0 , 100.0 ± 0.0 and 87.5 ± 14.5 by 3-day self report, 30-day visual analogue scale and pill count, respectively. All patients reported >95% adherence, but pill count showed that only 36% of the patients (10/28) had $\geq 95\%$ adherence.

Predictors of adherence

In a combined multivariable analysis of new ARV initiates and those on stable therapy among adults, only depression was found to be a predictor of adherence (OR= 0.32; 95% CI (0.11-0.93); $p=0.04$). Number of children in the household was not significantly associated with adherence (OR= 1.0; 95% CI 0.72-1.38); $p = 0.99$). In a repeated measures analysis using generalized estimating equations, there was a significant decline in adherence over time on therapy (OR=0.93; 95% CI (0.88-0.97); $p=0.003$). Patients on ART for one month longer had 0.9 times the odds of achieving $\geq 95\%$ adherence.

Reasons given for incomplete adherence were forgetfulness, side effects, traveling away from home and lack of money for transport to the clinic to refill prescriptions.

Qualitative findings

Twenty five individuals participated in the qualitative study. Similar to a previous report as patients on self-pay antiretroviral therapy [27], the most common motivator to adhere to ART was the desire to stay alive in order to care for and support one's children and other family members. Improvements in children's health status motivated their mothers/guardians to ensure that their children adhered to their prescribed medications. As one respondent indicated,

In my case, this virus made me worry about my child. I would fail to eat food, I would be crying all the time. Whenever I looked at my child he was diminishing, turning into bones. But since my child started taking medicine I became stronger, and I stopped worrying. (HIV+ mother)

Participants identified their spouses as important sources of adherence support. Spouses reminded them to take their medicines. Type of packaging of medicines was also a factor in adherence. Packaging that separated daily doses was very helpful in keeping track of whether or not that day's doses had been taken. This was preferred to tablets in bottles and liquid medications.

Self pay therapy has been associated with missed doses. [4] The MTCT-Plus program provides ART at no cost to its members. Many participants stated that they would not have been able to access antiretroviral medications without MTCT-Plus because they would not have been able to afford it. Provision of no-cost therapy to all eligible HIV-infected individuals in the household ensured sustained adherence.

They tell us that a dose for one month is 70,000/= [USD 35]; others say the cost reduced to 50,000/= [USD25]. But personally I couldn't afford 50,000 [USD 25]. Plus that of the child! (HIV+ mother)

We must thank the organizers of this program, because where we work if you are HIV-positive, your salary is cut 50% to go for treatment. But through this program the medications are free. (HIV-negative father)

As has been reported in other studies [11, 16, 36], perceived stigma was commonly reported as a barrier to access and adherence.

These days when people come to know that you have AIDS they don't want to come near you, as if you are an abominable thing ("bakwenyinyala"). You cannot feel free. Wherever you go they start talking, "See that one, she is sick." (HIV-positive mother)

Women who test HIV-positive through the MTCT-Plus program are required to inform their partners of their HIV status in order to access ART for themselves and their family members. Disclosing their status was often very difficult for women, who feared that their partners would blame and/or abandon them upon hearing the news. Some women dealt with this challenge by keeping their status a secret until they were able to bring their partner in for testing:

[After testing positive] I went back home and first kept quiet for two days. I asked myself, how can I approach him to tell him? One day when he came back I told him, they checked my blood but they refused to give me the results until I take my spouse in for testing. I convinced him and he accompanied me. (HIV-positive mother)

Clinical response to ART in children was an important factor in reducing stigma. One woman described how giving birth to a healthy baby caused her family to reverse their assumption that she was dying of AIDS and become supportive again:

You scare people so much that they take you out of their thoughts. They think you are dead even before your time, and count you among those who died long ago. And yet you are still alive... There was a time when I had a skin rash all over and one of my sisters asked, "What is it, what is it?" She said "You look like an infected person."Then when she saw that since giving birth my baby was not falling sick (the other children used to be sickly), that my baby was looking nice, did not have a rash, and was growing fast she said "I used to think you were infected. I had taken you out of all of my plans." I responded that "I am not infected, don't you see my baby?" So that's where I ended her suspicions about my being sick. Now she knows that I am not infected, which is not true. (HIV-positive mother)

Explaining antiretroviral use to children was cited as a challenge to long-term care of HIV+ children. Parents generally did not tell their children why they were giving them medicine, but some children started to inquire:

The thing that disturbs me is that I always think what will I tell my child when he

grows to a level of understanding and he asks me why he is taking drugs. Because even now he asks me, 'mummy I no longer cough but why am I still taking drugs every day?' What will I tell the child? (HIV+ mother)

DISCUSSION

Near perfect adherence to ART was observed in mothers and children when treatment was provided to all eligible members in the family. These high levels of adherence confirm findings in other resource-limited settings. [4-16] Our findings contrast with those of Merestein et al, who found an inverse relationship between adherence and number of children in the family. [26] The major difference between the two studies is that in our study all household members had an opportunity to be treated and there was complete disclosure of HIV sero-status. This and Merestein findings collectively suggest that adherence is supported by a family-based model that promotes disclosure and provides free therapy to all individuals in a household.

Depression was significantly associated with incomplete adherence. This is consistent with observations in resource-rich settings [37-41] and resource-limited settings [17, 42-44]. Lack of transportation money, and stigma were identified as other additional adherence barriers. Ware and Bangsberg have hypothesized that HIV+ individuals in sub-Saharan Africa achieve exceptional ART adherence, despite enormous adherence barriers, because of a synergistic cycle created by the support of close social relationships to overcome adherence barriers. [45] Based on this help, ART-treated individuals feel a responsibility to reciprocate this assistance, which is only possible with preserved health and excellent adherence. Reciprocation then strengthens these social bonds and the ability to ask for further assistance. Depression and stigma may impact adherence by disrupting the support, or social capital, provided by these reciprocal relationships.

New mothers are at risk for post-partum depression. [46] Early parenthood is also a vulnerable time for stigma, as many mothers first learn their HIV status during delivery. These factors may contribute to the decline in adherence we observed in this population. It is interesting that while declining adherence is common in resource-rich settings, it has not yet been observed in resource-limited setting. In fact, two recent longitudinal studies found no decline in adherence over time. [47, 48] This population of new parents may be particularly vulnerable to the impact of stigma and depression. Interventions to reduce depression and stigma among new HIV+ parents may be an important strategy to support adherence in resource-limited settings.

There have been concerns about development of viral resistance with increased use of a single dose of nevirapine (Sd-NVP) in women and infants who subsequently become infected. [49] In our study, where all women and children had been exposed to Sd-NVP, 83% of those with adherence $\geq 95\%$ achieved virologic suppression (≤ 400 copies/ml) at 24 weeks of follow-up. While we did not test for drug resistance, our results suggest that the majority of highly adherent women and children exposed to Sd-NVP at delivery achieved viral suppression. One limitation to this interpretation, however, is that we did not document the time from delivery to the time of initiating ART. Lockman et al (2007) found that Sd-NVP is not associated with treatment response when ART is initiated more than six months after Sd-NVP in the mother and child [50]

Two of our adherence measures were by self-report. This could introduce information bias and misclassification of participants into adherence categories with respect to these measures. The more objective unannounced pill counts in adults and bottle weights in children,

however, confirmed high adherence levels. We did not have a sufficient number of children to address predictors of adherence in this age group. Finally, our findings were in early recipients of the MTCT-Plus program, before free antiretroviral therapy was widely available in Uganda. As a result, the participants in this study may not be generalizable to the larger HIV+ population.

In summary, our results suggest that providing ART to all eligible members in a household promotes adherence. Further study will be necessary to assess the long-term impact of this family model on adherence to ART in resource-limited settings. However, we also found a significant decline in adherence over time. Interventions to sustain high levels of adherence should be designed and implemented, one of which is treatment of depression in patients on ART.

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Table 4 Patient Characteristics

Socio-demographic characteristics	All		Prospective		Cross sectional	
	N = 177	%	N = 75	%	N =102	%
Sex						
Female	124	70.1	55	73.3	69	67.7
Marital status						
Married	89	50.3	37	49.3	52	51
Separated	30	17.0	12	16.0	18	17.7
Widowed	14	7.9	10	13.3	4	3.9
Never married	44	24.9	16	21.3	28	27.5
Education						
Primary	74	49.7	29	48.3	45	50.6
Secondary	61	40.9	22	36.7	39	43.8
Tertiary	14	9.4	9	15.0	5	5.6
Household members with HIV						
0	51	29.0	30	40.0	21	20.8
1	88	50.0	31	41.3	57	56.4
2	30	17.1	12	16.0	18	17.8
>2	7	4.0	2	2.7	5	5.0
Household members on ART						
0	57	45.6	23	51.1	34	42.5
1	62	49.6	21	46.7	41	51.3
2	6	4.8	1	2.2	5	6.3
>2						
	Median		Median		Median	
Age (years)	30		30		30	
Household members #	4		4		5	
Monthly Income (\$ USD)	75		25		90	
Biological characteristics	Mean (SD)	Median	Mean (SD)	Median	Mean (SD)	Median
Baseline CD4 count	413 (± 422)	277	261 (± 377)	161	525 (± 421)	402
Baseline Viral Load	179936 (± 262834)	14012	394895 (± 279935)	305584	218792 (± 58508)	400
CD4 at 24 weeks	-	-	461 (± 569)	300	-	-
Change in CD4	-	-	197 (± 396)	136	-	-
Viral Load at 24 weeks	-	-	22041 (± 109053)	400	-	-
Change in Viral load	-	-	-371101 (± 275937)	-289872	-	-

Table 5 Adherence (%)

Measure	Prospective Study N=75		Cross sectional Study N=102	
	Mean \pm SD	Median	Mean \pm SD	Median
3- day self report	98.0 \pm 7.3	100.0	100 \pm 0.0	100.0
30-day visual analogue scale	97.9 \pm 6.4	100.0	100 \pm 0.0	100.0
Unannounced pill count	99.2 \pm 7.5	100.0	94.1 \pm 10.7	99.2

Table 6 Adherence in children

Measure	Prospective Study N=13		Cross sectional Study N=28	
	Mean \pm SD	Median	Mean \pm SD	Median
3- day self report	98.0 \pm 4.4	100.0	100 \pm 0.0	100.0
30-day visual analogue scale	97.9 \pm 5.3	99.8	100 \pm 0.0	100.0
Unannounced pill count	100.0 \pm 10.4	100.0	87.51 \pm 14.5	90.1

Table 7 Adherence and Virologic suppression

Adherence	Viral suppression		
	> 400 copies/ml	\leq 400 copies/ml	Total
\leq 0.95	3	6	9
> 0.95	11	54	65
$X^2 = 1.39, p = 0.24$			

CHAPTER IV

Examining the Psychometric Properties of the Beck Depression Inventory –II using an Item Response Modeling Approach in an HIV-infected Population in Kampala, Uganda

ABSTRACT

Background: Depression is prevalent among individuals living with HIV, with evidence suggesting that more than one third of people with HIV/AIDS may have mood disorders or clinically significant depressive symptoms. Sub-Saharan Africa bears the greatest burden due to HIV/AIDS and hence also has high rates of depression. The social, economic and health impact of depression in sub-Saharan Africa is great. However, there are few scales for measuring depression for which validity evidence has been collected in this population. The Beck Depression Inventory (BDI-II) is one of the most widely used instruments for assessing depressive symptom severity. Although the BDI-II has been used in sub-Saharan Africa, the psychometric properties of the BDI have not been well studied in this region especially among HIV infected patients. The purpose of this analysis therefore, was to examine the psychometric properties of the BDI-II in a low income HIV-infected population using an item response modeling (IRM) approach.

Methods: Data for this analysis were obtained from a cross-sectional survey of adult members of the Mother-To-Child-Transmission Plus Program in Kampala, Uganda. The BDI-II was administered to every study participant at enrollment. The sample was predominantly female. Data were analyzed using both the Classical Test Theory (CTT) approach and the Item Response Modeling (IRM) approach.

Results: Mean depression score was 8.86 ± 5.44 . Cronbach's alpha was 0.79 and the expected a posteriori (EAP) reliability coefficient was 0.86. Most of the items fit the analysis model reasonably well. The Wright map showed a good spread of the items over the entire span of the construct of depression. Differential item functioning was observed for some items. There was evidence for validity based on instrument content, internal structure and relations to other external variables.

Conclusion: This analysis demonstrated good psychometric properties of the BDI-II when used to screen for depression in a low income predominantly female HIV-infected population in Uganda. These findings support the use of the BDI-II in assessing depressive symptoms for HIV-infected patients in sub-Saharan Africa, especially women.

INTRODUCTION

Depression is prevalent among individuals living with HIV, with evidence suggesting that more than one third of people with HIV/AIDS may have mood disorders or clinically significant depressive symptoms[1-6]. A meta-analysis comparing rates of depression in HIV-positive and at risk HIV-negative patients demonstrated a twofold increase in the prevalence of major depression in patients infected with HIV [3].

Sub-Saharan Africa (SSA) is home to two thirds of all patients living with HIV, with AIDS being the leading cause of death [7]. In 2007, 76% of all deaths due to AIDS were in sub-Saharan Africa [7]. There is a dearth of literature on depression among individuals living with HIV/AIDS in sub-Saharan Africa. The sparse literature shows elevated rates of depression among HIV-infected individuals relative to community samples [5, 8-15] that is consistent with the developed world [16-19].

The social, economic and health impact of depression in sub-Saharan Africa is great. Depression is associated with mortality [20-23], work disability [21, 22, 24, 25], lower quality of life [21, 26-30], risk of heart disease [31] and high-risk behaviors for contracting or transmitting HIV infection [32]. Depression is also associated with lower adherence to antiretroviral medication [33, 34].

Depression in sub-Saharan Africa presents in forms (culture specific idioms, somatic, based on interpersonal relationships or spiritual in nature) that may obscure detection [35-37]. However, depression exists at possibly higher prevalence rates than in western countries [36] according to several studies [21, 22, 24, 25, 27, 28, 30, 37-46] of community and non-HIV specific clinic populations, with generally higher rates for women than men [36, 42]. Finally, though depression may present differently in sub-Saharan Africa compared to western countries, it [depression] is reasonably easy to detect when present and sought [36].

Depression in SSA has been measured using standard tools developed in western countries such as the Beck Depression Inventory (BDI) [23, 33, 42, 47]; the Hopkins Symptom Checklist (HSCL) [20, 24, 28, 40, 42, 46]; the Center for Epidemiologic Studies Depression Scale (CES-D) [32, 41]; the Edinburgh Postnatal Depression Scale (EPDS) [37, 44]; the Patient Health Questionnaire (PHQ) [5, 38]; the World Mental Health Survey of WHO Composite International Diagnostic Interview (WMH-CIDI) [21] and the Composite International Diagnostic Interview (CIDI) [22]. There have been few attempts to validate these instruments in this culturally different setting [5, 28, 37, 40-42] and to adapt the instruments to specific cultures and terminologies [46]. While some may argue that instruments developed in one culture can be used in another, provided careful attention is paid to conceptual translation [48], this [conceptual translation] has not always been done when using these standard instruments. The most common practice is to do a direct translation of the instrument from its original language to the local language [22, 32]. This kind of translation does not always ensure correct conceptual translation and may even change the meaning of the concept under translation.

An important limitation of most standard instruments or diagnostic measures is that they may be culturally inappropriate [35]. Descriptions of health conditions like mental illness developed in one set of cultures may not be equally applicable in other cultures. Given the assumption that health conditions may be described differently in different cultures, if the nature of emotions, thoughts, and behaviors can be expected to vary by culture [49], uncritical application of standard instruments cross-culturally might yield misleading or erroneous results where such instruments are locally inappropriate [50]. Inconsistent findings in any research

effort may result from random processes and non-equivalent measures, procedures, or samples, but may also be explained by problems of low validity. Problems of validity are not new to epidemiology [51], but are more likely to occur in transcultural epidemiology, which is defined here as research in which the views, concepts or measures of the investigator extend beyond the scope of one cultural unit to another [52].

Context affects research validity. Evidence of measurement validity and reliability cannot be assumed to generalize across populations. This lack of generalizability may be especially problematic when the original measure is translated into another language, as is common in transcultural studies. Creating a culturally acceptable, comprehensible, relevant and semantically equivalent translation is difficult [53] making it essential to study the psychometric properties of translated measures that might have changed during imperfect translations. While we acknowledge the limitations of these 'foreign' generated instruments, we do not say that these instruments are irrelevant for studying health conditions in other contexts, but rather that their appropriateness will vary across cultures and by illness, and therefore needs to be explored. As a result, instruments may need to be adapted to the new situations or in other cases completely new instruments developed.

The BDI-II is a 21-Guttman item instrument presented in multiple choice format which purports to measure presence and degree of depression in adolescents and adults. The BDI was derived from clinical observations about the attitudes and symptoms displayed frequently by depressed psychiatric patients and infrequently by nondepressed psychiatric patients[54]. The clinical observations were consolidated systematically into 21 symptoms and attitudes which could be rated from 0 to 3 in terms of intensity. The items were chosen to assess the intensity of depression and were not selected to reflect a particular theory of depression. The 21 symptoms and attitudes are: (1) Mood, (2) Pessimism, (3) Sense of Failure, (4) Lack of Satisfaction, (5) Guilt Feelings, (6) Sense of Punishment, (7) Self-dislike, (8) Self-accusation, (9) Suicidal Wishes, (10) Crying, (11) Irritability, (12) Social Withdrawal, (13) Indecisiveness, (14) Distortion of Body Image, (15) Work Inhibition, (16) Sleep Disturbance, (17) Fatigability, (18) Loss of Appetite, (19) Weight Loss, (20) Somatic Preoccupation, and (21) Loss of Libido.

The BDI is one of the most widely used instruments for assessing depressive symptom severity. Not only is it used for assessing the intensity of depression in psychiatrically diagnosed patients[55], but also for detecting depression in nonpsychiatric patients with conditions like HIV/AIDS, diabetes and other chronic conditions[33, 42, 47, 56, 57]. It has also been used to detect depressive symptoms in normal populations. For instance, student populations have been examined[23, 58]. In a sample of students, the BDI had a sensitivity of 100% and a specificity of 93.2% at a cut off of 16 to identify major depressive episode [59]. A study of 104 students in Grades 8 and 11 at independent schools in Cape Town [60] found that the BDI had high internal consistency (Cronbach's $\alpha=0.86$) and adequate test-retest reliability.

The BDI has been used to assess depressive symptoms in sub-Saharan Africa [33, 42, 47, 61-63]. However, the psychometric properties of the BDI have not been well studied in SSA, especially among HIV infected patients.

Classical Test Theory (CTT) has been the underpinning for most instrument construction and theory. Item response theory (IRT) is a new approach to instrument development [64]. It is an improvement of the CTT approach. The main achievement of IRT is the ability to estimate parameters about the instrument items and the people that are comparable on the same scale. CTT focuses primarily on instrument-level information, whereas IRT focuses on item-level information as well as instrument-level information. .

Using IRT, we set out to characterize the BDI-II in a low income HIV-infected population taking antiretroviral medications. It is particularly important to characterize depression in this population because depression is associated with decreased adherence to antiretroviral medications and disease progression.

METHODS

Study Design

This analysis is based on data collected from a cross-sectional survey of 123 adults attending the Mother-To-Child-Transmission Plus (MTCT Plus) Program at Mulago Hospital in Kampala, Uganda.

Study Setting

The study setting has been described in detail elsewhere [33]. Briefly, Mulago Hospital is Uganda's largest teaching, referral, and research hospital. The MTCT Plus program is located at Mulago Hospital under the Makerere University-Johns Hopkins University Research Collaboration.

Study Procedures

Details of recruitment and data collection have been reported elsewhere [33]. Briefly, we recruited individuals who were newly initiating ART and individuals who were on chronic ART. Adherence to medication was the main outcome in this study. Adherence was assessed by three measures: 3-day self report, 30-day visual analogue scale and unannounced pill counts at the patients' homes. In addition to the other data collected, the research assistants administered the BDI-II at the beginning of the study to detect depressive symptoms. The instrument took about 10 minutes to administer.

Analyses

We conducted univariate analyses of the baseline characteristics of our study population, including socio-demographic and clinical variables. Categorical variables are presented as frequencies and percents, while continuous variables are presented as medians and interquartile ranges [IQRs]. Depression responses for each subject were scored by summing the ratings given to each of the 21 items. The depression scores were then categorized into four categories according to a recommended guideline: none or minimal depression, < 10; mild to moderate depression, 10-18; moderate to severe depression, 19-29; and severe depression, 30-63 (A. Beck et al., 1988).

Classical test theory (CTT)

Item and test characteristics were first evaluated using CTT analysis. Item indices included the item mean (item difficulty) and point biserial correlations. The internal consistency reliability of the test was calculated using Cronbach's alpha and adequate reliability was demonstrated with a reliability index of at least 0.70 [65]. The CTT analyses were performed using Conquest software [66].

Item Response Modeling

We used an item response modeling approach to calibrate the data [64]. The preference for IRT over CTT has been a result of several limitations of CTT [67, 68]. The primary limitation of CTT is that the item and scale statistics apply only to the specific group of subjects who took the test. That means that if the scale is to be administered to people who are different in some way, such as being a member of a minority group or patients rather than students, then it is necessary to re-establish its psychometric properties and perhaps develop new norms. Similarly, we would have to go through the same re-norming process if any of the items were altered or if items were deleted in order to develop a shorter version of the scale. The second problem is that it is impossible to separate the properties of a test from the attributes of the people taking it [69]. Thus, the instrument's characteristics change as we test different groups. A third problem is the assumption that each item contributes equally to the final score. While this is also true for certain IRT models, the important difference is that in IRT we test to see if this is true, that is we test for fit of the items. In CTT, the items are simply summed irrespective of how much each item correlates with the underlying construct. Fourth, in CTT it is also assumed that each item is measured on the same interval scale. This assumption often fails on two grounds: items are most often ordinal rather than interval, and the 'psychological distance' between response options differs from one item to the next [70]. IRT has been designed to overcome these limitations [71]. One advantage of IRT is that the scale that emerges from an IRT analysis truly has interval-level properties. A second advantage is that it provides a more precise estimate of measurement error. Third, when the model fits, it is invariant theoretically: that is, item characteristics are independent of the sample from which they are derived.

Analysis was done using Conquest [66] and ConstructMap [72] software. These two software packages are based on Rasch's family of logistic models. Two models for ordinal data were considered: the rating scale model (RSM) [73] and the partial credit model (PCM) [74]. The PCM does not assume that the distances between ordinal responses are the same for all items (i.e the distance between response options a and b, b and c, etc is not the same across all items). In contrast, the RSM assumes that the distances between ordinal responses are the same for all items (i.e the distance between response options a and b, and b and c, etc is the same for all items). The best fitting model was assessed by comparing the deviance estimates as well as the weighted fit indices for the items and the respondents (i.e infit statistics). Item misfit was established by infit mean square (MSQ) values outside the range of 0.75 to 1.33, with significant *t* values [64]. For the weighted *t* statistic, any value < -2.00 or > 2.00 was indicative of misfit. The likelihood ratio test (LRT) and MSQ were used to determine the best fitting set of items.

Using the estimated parameters, a Wright map was constructed. The Wright map is an empirical map based on respondents' self-reports [64]. The Wright map of person-item estimates displays the item locations and the extent to which the item locations reflected the full range of depressive symptoms. It is useful for identifying a set of items that span the range of

the construct. This map serves to assess qualitatively content representation by determining if the items assessed minimal, moderate or severe depression.

To investigate the reliability of the instrument, first the standard error of measurement for each person location was estimated. Using the standard error of measurement, the information for the whole instrument was calculated. The information for the whole instrument is the sum of the information for each item, where information for each item is the reciprocal of the square of the standard error of measurement. A quality-control index of consistency has been developed. Internal consistency was assessed by calculating the reliability coefficient using the expected a posteriori (EAP) procedure.

Validity of the instrument was investigated using evidence based on instrument content, internal structure and relations to other variables [75]. These are three of the five most recent standards for educational and psychological testing. To assess the presence of internal structure, we investigated consistency of the items with the instrument as a whole using mean locations of each group. In addition, differential item functioning (DIF) was investigated to determine if items functioned in a similar way for respondents across important subgroups. Finally, we explored evidence based on relations to other external variables that the construct (depression) should predict. In particular, we explored the relationship between depression and adherence to antiretroviral medications.

Ethical Considerations

All participants provided informed consent and all procedures were approved by the Faculty of Medicine Research and Ethics Committee and the Institutional Ethics Review Board of Makerere University, the Uganda National Council of Science and Technology, and the University of California, San Francisco Committee on Human Research.

RESULTS

One hundred twenty three patients were included in the analysis. The study population was predominantly low income women. Patient characteristics are summarized in Table 8. Fifty six percent (n=63) of those with complete data scored 10 or less on the whole instrument (Table 9). Those with incomplete data were generally comparable to those with complete data with respect to age and income but had a higher median CD4 cell count compared to those with complete data (332 [IQR 177-472] vs. 233 [IQR 159-381]).

Item Response Modeling

The Partial Credit Model was used for analysis. Compared to the rating scale model, the partial credit model fitted the data better, as one would expect given the nature of the alternative responses to the items. ($X^2 = 172.0582$; $df=24$, p value < 0.0001)

Item fit

Weighted fit statistics showed a good fit for most of the items. The weighted mean square range was 0.83 to 1.43. As an effect size, there is no absolute limit to what is a good mean weighted square value, but previous researchers have indicated that 0.75 is a reasonable lower bound and 1.33 is a reasonable upper bound [64]. Item 11 (agitation) showed the worst fit. *Infit* mean squares are given in Figure 3.

Wright Map

The right-hand side of the map (Figure 4) shows the calibrated item threshold locations. The k th Thurstone threshold is the point at which the probability of the scores below k is equal to the probability of the scores k and above. Because there are four response categories (0, 1, 2, 3), there can only be three possible threshold values: 1, 2 and 3. A threshold of 1 refers to the point at which response levels 1, 2, and 3 together become more likely than level 0; a threshold of 2 is the point at which levels 2 and 3 together become more likely than levels 0 and 1 and so on. From the Wright map we can see that there is generally a good spread of the items over the construct “feelings of depression”. The items cover the lower, middle and higher end of the construct. However, from the map it can be seen that there are some gaps in the construct that are not well covered by the items. Specifically, we notice a lack of enough items that cover severe depression. There is a wide gap between item thresholds 14.1 and 11.2. Likewise there is need for more items between thresholds 14.1 and 4.3. On the lower end of the scale there is a gap between 3.1 and 11.1. The consequence of these gaps is that the instrument may not adequately discriminate among respondents at the high end. However, given that the concern is not the exact level of depression but whether one is depressed or not given a cut off point, this may not be a problem and the current items may suffice.

The locations of the bars of the histogram (i.e the Xs) are the estimated locations of the respondents on the variable. The respondents tend to cluster in the lower half of the scale (below logit score 0) reflecting the fact that most patients had minimal to moderate depression (Table 9). There were few respondents at the extremes. The mean depression score was 8.86 with a standard error of 5.44.

Evidence for Reliability

Standard Error of Measurement (sem)

When using an instrument on an individual respondent, the *sem* is the most important tool for assessing the usefulness of that estimate of location (Figure 5). If the *sem* is too large, the measurer will not be able to make intelligible interpretation of the results. For this sample, the mean of the *sem* was 0.569 logits. This gives a 95% confidence interval width of 2.276 logits at the mean value. This is about 33% of the width of the entire Wright map from maximum to minimum locations. This implies that the instrument is not accurate for individual usage. To see this, let us consider the second threshold. The confidence interval spans (for the second threshold) the range from items 1 and 17 to items 8, 15 and 18 covering a wide range of depression states indeed. Thus, this instrument is probably not very useful for accurate clinical diagnosis, but it may be useful for initial screening or as a basis for group measures.

Test Information

The uncertainty in an estimate of person location is usually characterized for individuals using the standard error of measurement. Derived from the standard error of measurement, the information curve for the BDI-II instrument is given in Figure 6. Together with Figure 5, these graphs show that the most sensitive part of the instrument is from approximately -1.5 to +1.5 logits. Comparing with the Wright map, this range contains thirty six out of the forty seven item thresholds (77%) observed in these data. Thus, the instrument's range of maximum sensitivity makes general sense with respect to the items.

Internal Consistency Coefficients

Cronbach's alpha was 0.77 but when only complete cases were used the Cronbach's alpha coefficient was 0.79. The expected a posteriori (EAP) reliability was 0.86.

Evidence for validity

The structure of the discussion about validity below is based on the 1999 Standards for Educational and Psychological Testing [75].

Evidence based on instrument content

IRM is a new approach to instrument development that was not in existence at the time that Beck and colleagues developed the original BDI. [54] The first step in developing an instrument using IRM is to develop a construct map. [64] A construct map is a content structure in which the relative behaviors of a generic respondent having various amounts of the construct are arranged in order on one side, and potential items are arranged in order of endorsability on the other. Beck et al designed the instrument with the intent of building in strong content. The content was based on systematic observations and records of the characteristic attitudes and symptoms of depressed patients[54]. Beck selected a group of these attitudes and symptoms that appeared to be specific for these depressed patients and which were consistent with the descriptions of depression contained in the psychiatric literature. On the basis of this procedure, he constructed an inventory composed of 21 categories of symptoms and attitudes. He arranged responses in each category using a Guttman-like scale that endeavors to cover the entire spectrum of depressive symptoms. Based on these procedures, we say that there is evidence of validity based on instrument content.

Evidence for validity based on response processes

Evidence based on the response process consists of studies of how respondents react to the items and the instrument as a whole. These might consist of 'think alouds', 'exit interviews' or 'cognitive interviews' with samples of respondents. Such evidence was not available with our secondary analysis of the data, so this aspect of the framework is not available at this time.

Evidence for validity based on internal structure

One major criterion for internal or construct validity is an *a priori* theoretically based hypothesis about the order of item endorsability, the ease with which respondents rate items strongly. The Wright map is a very good tool for investigating such a hypothesis[64]. No such expectations were available for the BDI. The authors clearly stated that “the items were chosen on the basis of their relationship to the overt behavioral manifestations of depression and do not reflect any theory regarding the etiology or the underlying psychological processes in depression”[54]. This is also illustrated in the Wright map (Figure 4) where there is no discernible empirical order to the items of the BDI. Instead, the feature of the BDI-II items that maps out the BDI variable is the transitions between the response categories. One thing we would expect is a similar pattern of item locations at each threshold level but this is not the case. Hence this source of validity evidence is not available.

An important piece of evidence that an item is functioning as expected is that the increasing response levels of the item response options are operating consistently with the instrument as a whole. With the item response modeling approach, one way that this consistency can be made manifest is that respondents higher on the construct would, in general, also score higher on each item. In terms of the Wright map, one would consider the locations of the respondents within each score group for an item: if the mean location for each group tends to increase as the scores increase, it seems reasonable to say that this particular expectation that comes from the items design has been fulfilled. This analysis showed that for most items, the mean increases as the score rises (Table 11). The exception is item 6. This is probably due to the small number of respondents (4) in the second category (labeled 1). A second look at this evidence is provided by the point biserial correlation index. Item discrimination refers to the way an item differentiates respondents with a particular level of the construct [depression] from those with another level. Discrimination can be measured as a point biserial correlation. The point biserial correlation compares each respondent’s item performance with each respondent’s overall instrument performance. If a question discriminates well, the point biserial correlation will be highly positive for the correct answer and negative for the distractors. This indicates that the respondents who had a particular level of depression, say severe depression, endorsed the highest category that represents severe depression and those at lower levels endorsed the other lower categories, herewith referred to as distractors. Popham (1990) rates items with discrimination indices greater than 0.3 as “good” and those with discrimination indices greater than 0.4 as “very good”. [78] For this analysis, the point biserial correlations ranged from 0.03 to 0.53 (Table 10). The majority of the correlation scores had an absolute value above 0.30. The variation of the point biserial correlations indicates that there is variation in how well the items discriminate persons with different levels of depressive symptoms; however, on the whole, most item categories discriminate fairly well. The point biserial correlation for the lowest category for most of the items was negative. This implies that those who endorsed this category also generally scored low on the instrument. This is a good observation for this instrument because the lowest category represented the least depression, so if one endorsed this category on most items, then on the whole they lie in the mild depression category.

A standard requirement of the items design is that, across important subgroups, items function in a similar way for respondents who are at the same location--- that is they should exhibit no evidence of differential item functioning (DIF). DIF is, therefore, said to be present when respondents at the same location on the variable give different responses across different

subgroups. For this analysis, we investigated the effect of CD4 cell count on item functioning. Respondents were divided into two subgroups: those with CD4 cell count below 250 cells/uL and those with CD4 cell count above 250 cells/uL. Test results for items with DIF are shown in Table 12. In terms of DIF, a recommended standard of effect size is as follows: a logit difference value less than 0.426 is “negligible”, a value between 0.426 and 0.638 is “intermediate” and a value over 0.638 is “large” (Wilson, 2005). Overall, there was statistically significant DIF for the item set (Chi sq = 52.02, df=20, p-value <0.0001). Applying these criteria to the statistically significant logit differences in Table 12 shows that items 5, 11, 13, 14, 15, and 17 had large DIF. The rest of the items had no DIF.

Evidence based on relations to other variables

Where there are other external variables that the construct should (according to theory) predict, a strong relationship between the instrument under scrutiny and these external variables can be used as validity evidence. In another paper from the same study (Byakika-Tusiime et al., 2009), we demonstrated that depression was significantly associated with adherence to antiretroviral therapy [OR= 0.32, 95% CI 0.11-0.93]. Overall, respondents with CD4 cell count levels below 250 were 0.5 logits higher on the depression trait. This does not show DIF but evidence of differential impact. This result is not surprising, as being diagnosed with HIV would usually be associated with negative feelings.

Evidence based on consequences of using an instrument

The final form of validity evidence relates to consequences. We did not have any data on consequences of using this instrument hence our analysis did not evaluate this form of evidence.

DISCUSSION

There have been few data reported regarding the validity of the Beck Depression Inventory in an HIV-infected population, despite the fact that the BDI is commonly used to assess symptoms of depression in this population.

The findings of this analysis showed that the BDI-II has reasonably good psychometric properties when used in a low income HIV-infected population in Uganda. These findings corroborate those found in other populations [76, 77]. However, to our knowledge, this is the first attempt to validate the BDI-II in an HIV-infected population in sub-Saharan Africa. It is also the first attempt to use item response modeling to calibrate this instrument. Previous calibrations were based on classical test theory and now IRM further confirms the robustness of the BDI-II instrument.

The weighted mean square indicates that most of the items are fitting within reasonable bounds. Thus, the overall finding is that the BDI-II data fit the Partial Credit Model reasonably well. The Wright map generally revealed a relatively good spread of the items over the construct “feelings of depression”, although there were gaps identified in the instrument that may need bridging with more items or item responses. The biggest gap was observed at the top end of the instrument—that measures severe depression. The need to add more items or item responses for adequate discrimination of respondents at these levels would largely depend on the ultimate

purpose of this instrument. If the purpose is to detect respondents with severe depression then it should probably be augmented with more items/item responses at the upper end. On the other hand, if the purpose is to screen for minor to moderate or moderate to severe depression, then the current items seem to suffice.

The majority of respondents in this sample had mild to moderate depression and the distribution of the respondents shows that many respondents in this sample were below -1.5 logits. However, the test information curve tells us that the most sensitive part of this instrument is the region of moderate to severe depression (-1.5 to +1.5 logits). This implies that the instrument is not functioning optimally for quite a large proportion of this sample. However, because the ultimate purpose of this instrument is to detect levels of depression that would require intervention (moderate to severe depression) this is not a big problem. That is, the instrument captures respondents with moderate to severe depression, which is the main concern.

The findings of this analysis provide evidence for the validity of the instrument's usage. This evidence has been based on test content, internal structure, and relations to other variables. This is an alternative to the traditional way of assessing validity; that is, criterion, content and construct validity, which was superseded in 1999. ("American Educational Research Association, American Psychological Association, National Council for Measurement in Education. *Standards for educational and psychological testing*. Washington, DC: American Psychological Association," 1999) We have shown evidence of validity based on instrument content, evidence based on internal structure and evidence based on relations to other variables.

We found significant differential item functioning with CD4 cell count subgroups. Items 5, 11, 13, 14, 15, and 17 had large DIF. While DIF is an undesirable characteristic of any test, for this particular variable, DIF is expected. Disease stage as reflected by CD4 cell count would make patients with the same degree of the construct (depression) respond differently to some items. For example, it is expected that people with a lower CD4 cell count would have greater loss of energy than those with a higher CD4 cell count. Hence, somatic symptoms may have more to do with advanced HIV infection than depression. Therefore, DIF in this instrument, in this population, may not be a major problem. In addition, looking at all the items exhibiting DIF, those exhibiting positive DIF tend to cancel out those with negative DIF. Hence, overall, we can say that there is no significant DIF effect.

Internal consistency coefficients in this sample were good. We found a Cronbach's alpha of 0.79 and an EAP reliability coefficient of 0.86. This demonstrates low measurement error. The reliability coefficients obtained from this analysis are similar to what has been found elsewhere (A. Beck et al., 1988). Within nonpsychiatric populations, coefficient alphas of the range from 0.73 to 0.92 have been observed.

There were a few limitations to this analysis. First, the sample size was rather small. We would require a bigger sample size to confirm these findings. Second, the study sample was mainly female (75%). This may limit generalizability of our findings.

In conclusion, this analysis demonstrated good psychometric properties of the BDI-II when used to screen for depression in a low income predominantly female HIV-infected population in Uganda. These findings therefore, support the use of the BDI-II in assessing depressive symptoms for HIV-infected patients especially women in sub-Saharan Africa. The quality of item calibration must be supervised continuously. We cannot expect the items in an instrument to retain their calibrations indefinitely or to work equally well for every person with whom they may be used.

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Table 8 Patient Characteristics

Characteristic	n (%) N=123
Sex	
Female	92 (75)
Median age (years, IQR)	32 (28-36)
Education attainment	
Primary	62 (50)
Median income/mo (USD, IQR)	75 (25-100)
CD4 cell count <250 cells/ul	63 (51)

Table 9 Distribution of Depression Scores

Category	Frequency N=112* (%)
None to mild (<10)	63 (56)
Moderate (10-18)	45 (40)
Moderate to severe (19-29)	4 (4)

* includes only participants with complete data

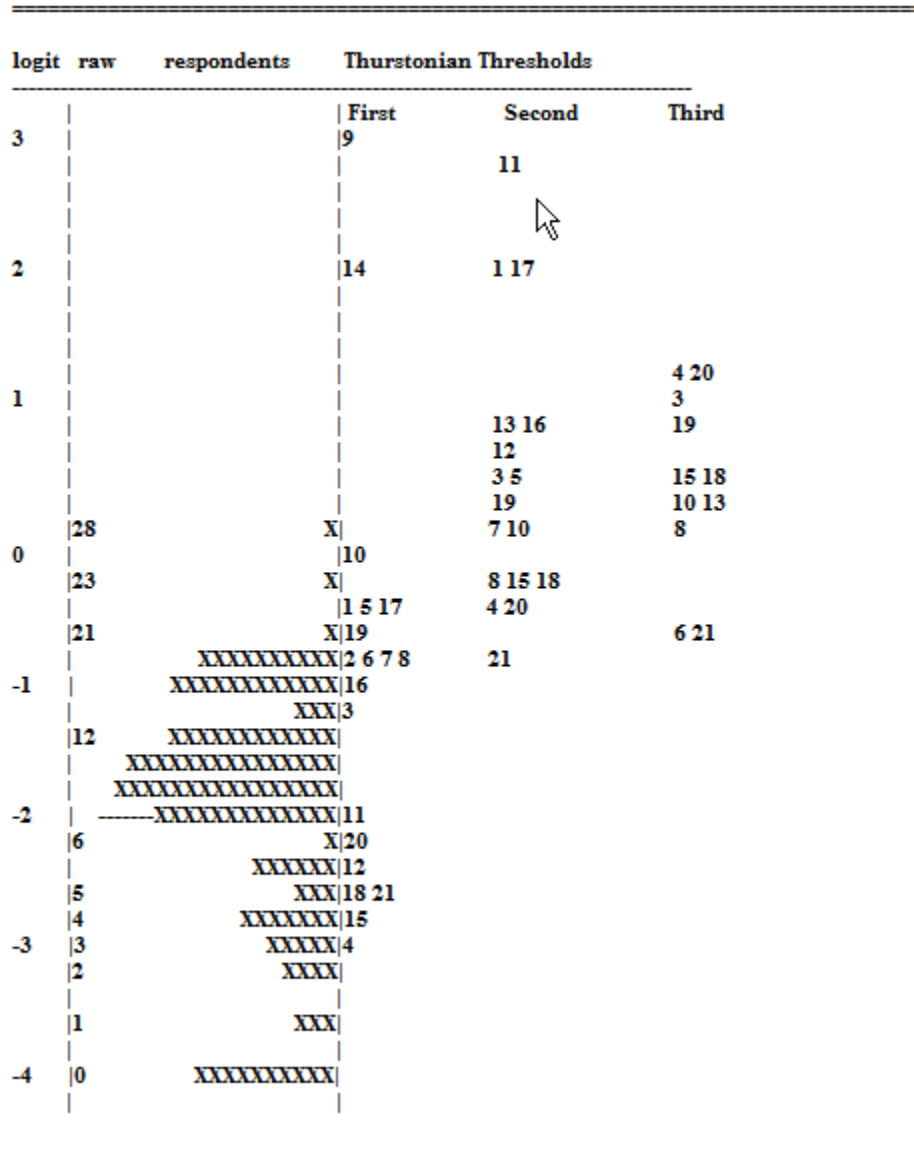
Table 10 **Item point-biserial correlations for the BDI-II**

Item #	Point-biserial correlation for each category			
	0	1	2	3
1	-0.36	0.35	0.05	-
2	-0.53	0.53	-	-
3	-0.43	0.40	0.11	0.05
4	-0.55	0.22	0.25	0.34
5	-0.50	0.40	0.26	-
6	-0.50	0.32	0.38	-
7	-0.53	0.39	0.31	-
8	-0.46	0.20	0.20	0.41
9	0.03	-0.03	-	-
10	-0.37	0.12	0.20	0.35
11	-0.45	0.39	0.32	-
12	-0.53	0.38	0.28	-
13	-0.22	0.22	0.05	-
14	-0.17	0.17	-	-
15	-0.37	0.14	0.13	0.33
16	-0.24	0.21	0.09	-
17	-0.49	0.48	0.09	-
18	-0.44	0.21	0.15	0.33
19	-0.41	0.32	0.09	0.32
20	-0.44	0.19	0.24	0.29
21	-0.49	0.17	0.13	0.35

Figure 3 **Infit Mean Squares**

	0.58	0.67	0.75	0.83	0.92	1.00	1.08	1.17	1.25	1.33	1.42
Q1			.						*	.	
Q2			.						*	.	
Q3			.							*	
Q4			.				*			.	
Q5			.							.	*
Q6			.						*	.	
Q7			.					*		.	
Q8			.						*	.	
Q9			.						*	.	
Q10			.		*					.	
Q11			.							.	*
Q12			.				*			.	
Q13			.				*			.	
Q14			.					*		.	
Q15			.	*						.	
Q16			.				*			.	
Q17			.				*			.	
Q18			.	*						.	
Q19			.				*			.	
Q20		*	.							.	
Q21			.	*						.	

Figure 4 Wright Map



Each X represents 1 patient, each row is 0.191 logits

Figure 5 The Standard Error of Measurement for the BDI-II Instrument

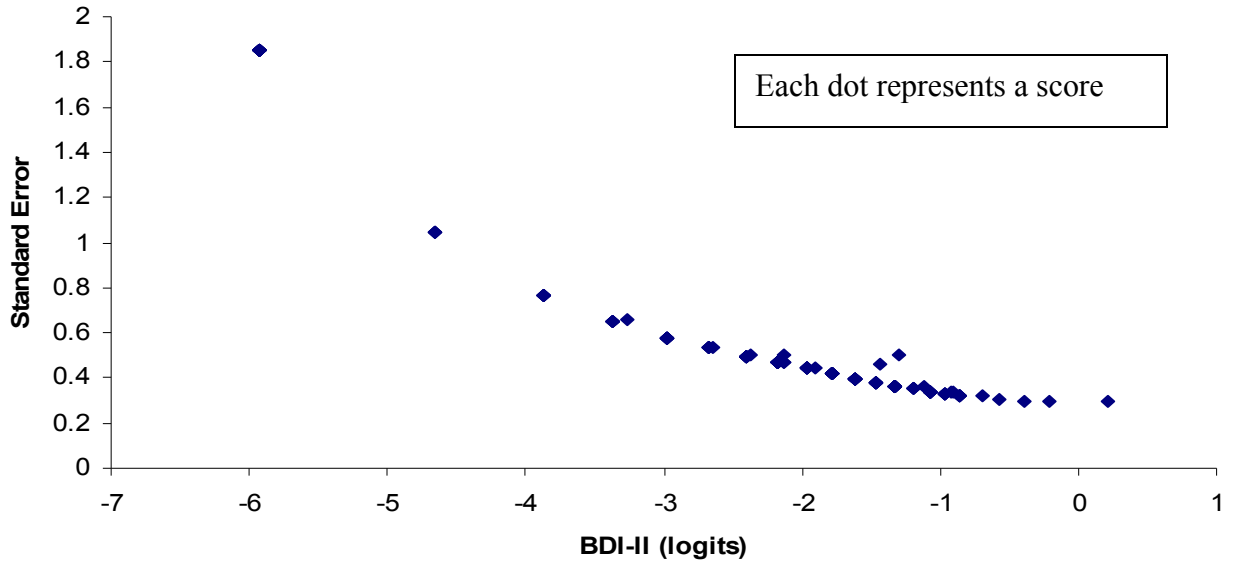


Figure 6 Test Information Curve

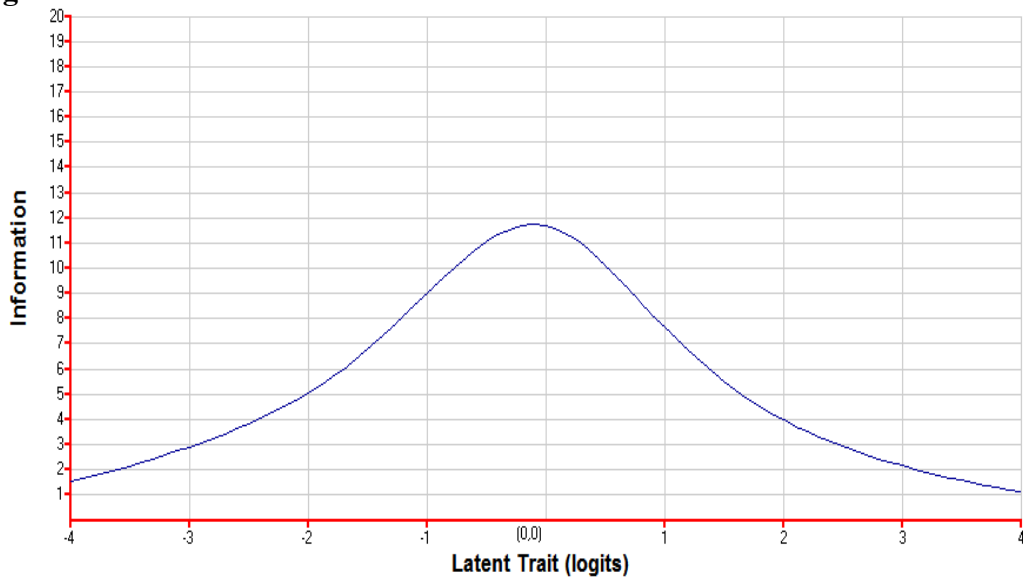


Table 11 Mean locations of respondents within each score group

Item #	Response Categories			
	0	1	2	3
1	-2.17	-1.39	-1.43	-
2	-2.28	-1.28	-	-
3	-2.29	-1.44	-1.52	-1.43
4	-2.91	-1.73	-1.44	-0.41
5	-2.20	-1.23	-0.93	-
6	-2.19	-0.85	-1.23	-
7	-2.27	-1.27	-1.13	-
8	-2.20	-1.49	-1.19	-0.67
9	-1.99	-2.09	-	-
10	-2.05	-1.45	-0.86	-0.40
11	-2.44	-1.59	0.00	-
12	-2.63	-1.58	-1.16	-
13	-2.06	-1.34	-1.56	-
14	-2.01	-1.38	-	-
15	-2.57	-1.83	-1.58	-0.74
16	-2.16	-1.66	-1.52	-
17	-2.22	-1.22	-1.08	-
18	-2.60	-1.77	-1.54	-0.74
19	-2.20	-1.38	-1.42	0.00
20	-2.53	-1.72	-1.45	-0.55

Table 12 Testing for DIF

Item #	Item Description	Logit Difference	DIF (Y/N)
5	Guilty feelings	-0.670	Y
11	Agitation	-1.742	Y
13	Indecisiveness	-0.738	Y
14	Worthlessness	+1.024	Y
15	Loss of energy	+0.790	Y
17	Irritability	+0.672	Y

CHAPTER V

Steady State Bioequivalence of Generic and Innovator Formulations of Stavudine, Lamivudine, and Nevirapine in HIV-Infected Ugandan Adults

ABSTRACT

Background: Generic antiretroviral therapy is the mainstay of HIV treatment in resource-limited settings, yet there is little evidence confirming the bioequivalence of generic and brand name formulations. We compared the steady-state pharmacokinetics of lamivudine, stavudine and nevirapine in HIV-infected subjects who were receiving a generic formulation (Triomune[®]) or the corresponding brand formulations (Epivir[®], Zerit[®], and Viramune[®]).

Methodology/Principal Findings: An open-label, randomized, crossover study was carried out in 18 HIV-infected Ugandan subjects stabilized on Triomune-40. Subjects received lamivudine (150 mg), stavudine (40 mg), and nevirapine (200 mg) in either the generic or brand formulation twice a day for 30 days, before switching to the other formulation. At the end of each treatment period, blood samples were collected over 12 h for pharmacokinetic analysis. The main outcome measures were the mean AUC_{0-12h} and C_{max} . Bioequivalence was defined as a geometric mean ratio between the generic and brand name within the 90% confidence interval of 0.8-1.25. The geometric mean ratios and the 90% confidence intervals were: stavudine C_{max} , 1.3 (0.99-1.71) and AUC_{0-12h} , 1.1 (0.87-1.38); lamivudine C_{max} , 0.8 (0.63-0.98) and AUC_{0-12h} , 0.8 (0.65-0.99); and nevirapine C_{max} , 1.1 (0.95-1.23) and AUC_{0-12h} , 1.1 (0.95-1.31). The generic formulation was not statistically bioequivalent to the brand formulations during steady state, although exposures were comparable. A mixed random effects model identified about 50% intersubject variability in the pharmacokinetic parameters.

Conclusions/Significant Findings: These findings provide support for the use of Triomune in resource-limited settings, although identification of the sources of intersubject variability in these populations is critical.

INTRODUCTION

Generic drugs provide patients with lower-cost alternatives to the more costly brand name drugs. Production of more affordable generic antiretroviral medications (ARVs) has greatly boosted global efforts to scale up access to these life-saving medications. Today, more than half of all ARV prescriptions in sub-Saharan Africa are filled with generic drugs. The use of generics has resulted in substantial savings to consumers and governments. These drugs have dramatically reduced the morbidity and mortality due to HIV/AIDS, and drug quality is a key factor in attaining the long term goals of sustained viral suppression with minimal drug resistance. Triomune[®], a fixed-dose generic combination of lamivudine, stavudine and nevirapine, has been shown to be bioequivalent to its innovator counterparts (Epivir[®], Zerit[®] and Viramune[®]) in a single dose study in healthy Indian volunteers [1]. However, drug pharmacokinetics and pharmacodynamics may vary in ethnically distinct populations of HIV-infected patients. Furthermore, since this is chronic therapy, there is a need to evaluate the steady-state pharmacokinetics of these drugs in the target population. A study conducted in Malawian HIV-infected adults showed that Triomune was not strictly bioequivalent to its innovator cousins at steady state [2], but this has not been replicated in other populations. Assurance of continual exposure to optimal plasma concentrations of these medications is essential to avoid the development of drug resistance resulting from sub therapeutic plasma concentrations. The goal of this study was to compare the pharmacokinetics of Triomune and its innovator counterparts in HIV-infected Ugandans with advanced disease. In addition, we sought to investigate the source of variation in pharmacokinetic parameters.

MATERIALS AND METHODS

Ethics committee review and informed consent

The study protocol and informed consent forms were reviewed and approved by the Makerere University Faculty of Medicine Ethics Committee and University of California San Francisco Committee on Human Research. All subjects provided written informed consent prior to participation in the study.

Study setting and subject selection

Subjects were recruited from an ongoing cohort study (Adherence Monitoring Uganda (AMU)) in Kampala, Uganda. AMU was an observational study of adherence and treatment response among individuals on self-pay HIV generic antiretroviral therapy conducted from 2002-2007 [3, 4]. AMU cohort members with a body weight of 60kg or above were approached by a member of the research team and invited to participate in the bioequivalence study and those who were interested and fulfilled other selection criteria were enrolled into the study. Subjects received quarterly adherence assessments using unannounced home pill count, Medication Event Monitoring System (MEMS) and laboratory monitoring of CD4 T cells, HIV RNA, and hematological function. All subjects had been taking Triomune-40 (stavudine 40 mg, lamivudine 150 mg, nevirapine 200 mg) twice daily for at least three years. Most of the subjects attended

the Infectious Diseases Clinic in Mulago, Uganda. Mulago Hospital is the main teaching and referral hospital in Uganda and the Infectious Diseases Clinic is a specialized HIV/AIDS clinic that treats over 13,000 patients, ~5000 of whom receive antiretroviral therapy.

Participants received a medical and laboratory examination no less than seven days prior to enrollment in this pharmacokinetic sub-study to exclude active opportunistic infections. A urine pregnancy test was performed on all women. Subjects were excluded if they had active tuberculosis or were taking rifampicin or other enzyme inducers, had gastrointestinal problems, hepatitis, hemoglobin less than 7 mmol/L for men and 6.5 mmol/L for women, liver and renal function test results 5 or 1.5 times the upper limit of normal, respectively, or were pregnant. We also excluded those patients who expected to change their regimen or move out of the study area within two months. Subjects on daily cotrimoxazole (trimethoprim/sulfamethoxazole) prophylaxis were included.

Study design

This study utilized an open-label, randomized, crossover design comparing the pharmacokinetics of generic and trade formulations of stavudine, lamivudine and nevirapine under fed conditions. Subjects were randomized to one of two formulations. Formulation 1 (generic) was a single tablet containing lamivudine (150 mg), stavudine (40 mg) and nevirapine (200 mg) (Triomune-40). Formulation 2 (brand) was a single tablet of lamivudine (150 mg, Epivir) plus one 40 mg tablet of stavudine (Zerit) and one 200 mg tablet of nevirapine (Viramune). Each formulation was taken twice daily. Seven subjects were randomized to the generic-to-brand arm and 11 were randomized to the brand-to-generic arm. Subjects took each formulation for 30 days prior to pharmacokinetic sampling. The generic formulation (Triomune-40) was manufactured by Cipla (Mumbai, India) and the brand formulations by: Zerit, Bristol-Myers Squibb, Princeton, NJ, USA; Epivir, GlaxoSmithKline, Middlesex, United Kingdom and Viramune, Boehringer Ingelheim, Ingelheim am Rhein, Germany. The study was conducted between March and May, 2006.

Study procedures

To monitor adherence, study drugs were dispensed to the participants in a bottle with an electronic cap that recorded each pill bottle opening using the Medication Event Monitoring System (MEMS) (Aardex. (2004), Union City, CA). In addition, the pills were counted and the number recorded manually at each visit. Adherence was also assessed by a 3-day self report and 30-day visual analogue scale [3].

Participants were admitted to the hospital ward the night before the pharmacokinetic sampling and administration of the 8 p.m. evening dose was witnessed by research staff. Participants were fasted overnight and were instructed not to take their morning dose(s) or eat until the first blood sample had been drawn the next day.

Each subject had an indwelling catheter inserted into an arm vein for drawing serial blood samples. A 6 ml blood sample was obtained before antiretroviral medications were administered ($t = 0$ hr) during a witnessed dose at 8 a.m. Additional 6 ml blood samples were obtained at 0.5, 1, 1.5, 2, 3, 4, 6, 10 and 12 hr post-dosing. Food and water intake were controlled during the study. After the 12 hr sample collection participants were given the alternate formulation in a

MEMS bottle and asked to report back to the clinic after 30 days. At the next visit, identical procedures were repeated.

Blood samples were collected in vacutainer tubes containing EDTA as the anticoagulant. Blood samples were immediately delivered to the Makerere University Johns Hopkins University Collaboration (MUJHU) laboratory where plasma was separated by centrifugation at 900g for 10 min and then stored at -70°C until analysis. Samples were transported in a single batch on dry ice to the Department of Biopharmaceutical Sciences at the University of California San Francisco for analysis.

Analysis of plasma samples

Prior to drug extraction, plasma samples (including controls) were heated at 56°C for 90 min to inactivate virus. Heat treatment was determined to have no quantifiable effect on drug stability or concentration. Drug concentrations were analyzed using a method reported previously [5]. Briefly, stavudine, lamivudine, and nevirapine were extracted simultaneously from 0.5 ml of plasma using solid-phase extraction columns (Oasis HLB Extraction Cartridges, Waters Corporation, Milford, Massachusetts, USA) and eluted with 1 ml of mobile phase, consisting of 0.1% glacial acetic acid in acetonitrile:water (80:20, v/v). Metaxalone was used as an internal standard. Samples were subjected to LC/MS/MS on an API 4000 system (Applied Biosystems, Foster City, California, USA) using a Waters 717plus autosampler and a Symmetry C18 (150 mm x 3.9 mm i.d., 5 μ m particle size, Waters Corporation) analytical column. The flow rate was 0.4 ml/min. All peaks were quantified using multiple reaction monitoring (MRM) mode to study the conversions from parent to product ion (m/z), and data were collected using Analyst version 1.4 (Applied Biosystems). The precision of the assay, as measured by the interassay coefficient of variation of control samples, was <13.2%, <15.2%, and <14.8% for stavudine, lamivudine, and nevirapine, respectively.

Pharmacokinetic analysis

Pharmacokinetic parameters were determined from plasma concentrations based on a non-compartmental model with extravascular input (Model 200) using WinNonLin software, version 5.2 (Pharsight Corporation, Mountain View, CA, USA). The AUC was calculated using the log-linear trapezoidal rule, in which the linear trapezoidal rule was used up to C_{max} , and thereafter the logarithmic trapezoidal rule was used. C_{max} and t_{max} were directly observed from the concentration-time data. Geometric mean ratios (GMR) and 90% confidence intervals (CI) for AUC_{0-12h} and C_{max} were used in the determination of bioequivalence, as defined by a 90% CI range of 0.80-1.25.

Statistical Analyses

A sample size of 18 subjects was estimated using a formula by Zhang *et al* [6] to provide 80% power to detect approximately a 20% difference on a log scale in AUC_{0-12h} and C_{max} between the brand formulation and the generic formulation. Power was based on findings from an earlier study [1] in which the coefficients of variability (CV) for stavudine, lamivudine and nevirapine AUC were 16%, 18% and 21%, respectively.

Primary parameters for statistical analyses were AUC_{0-12h} and C_{max} of lamivudine, stavudine and nevirapine and their intra- and intersubject variances. Differences between treatments with respect to AUC_{0-12h} , C_{max} and intra- and intersubject variances were assessed using the mixed random effects model. In particular, we used the simple random intercept model with bootstrapped standard errors because the drug concentrations, even after log transformation, were not normally distributed. The model employed for this study included sequence, period, and treatment as fixed effects and subjects (nested within sequence) as the random effects. The primary analysis was conducted on log-transformed (base e) AUC_{0-12h} and C_{max} . Measured values of C_{0h} and C_{12h} were compared to assess the presence of steady-state conditions and to evaluate adherence.

RESULTS

Subjects

Twenty HIV-positive adults (8 males, 12 females) were enrolled in the study. Two subjects were excluded from the analysis because their plasma samples were not sufficient for pharmacokinetic analysis. The analysis therefore includes 18 participants. Participants ranged from 28 to 50 years of age (mean \pm SD; 37.4 ± 6.0), weighed 68.3 ± 6.6 kg, averaged 165 ± 9.4 cm in height, and had a mean body mass index of 25.1 ± 3.4 kg/m². Patient characteristics did not differ between randomization arms (Table 13). All participants had been on the generic lamivudine/stavudine/nevirapine formulation for at least 36 months. Ten participants were taking prophylactic cotrimoxazole during the study. All subjects were physically healthy based on their medical examination and results from clinical laboratory tests. No tuberculosis, malabsorption, nausea, emesis, abdominal discomfort, chronic diarrhea, or hepatitis was reported. All females had a negative pregnancy screen.

Adherence and steady-state drug concentration

Based on MEMS adherence records, mean adherence for the 30 days prior to the first and second pharmacokinetic study visits was 99.7% and 99.0%, respectively. Fifteen out of eighteen subjects had 100% adherence before the first pharmacokinetic sampling and sixteen out of eighteen subjects had 100% adherence before the second pharmacokinetic sampling. Each of the 3 with subjects with incomplete adherence before the first PK sampling had 98% adherence and of the 2 subjects with incomplete adherence before the second PK sampling, one had 95% while the other had 88% adherence. In addition to the MEMS and pill count we also did a 3-day self report asking about adherence over the previous 3 days on the morning of the PK sampling. For each of these participants 3-day self reported adherence prior to PK sampling was 100% and the MEMS report showed that all doses were taken in the week preceding PK sampling. Any cases of non-adherence could have occurred at some other time but not immediately prior to PK sampling.

Trough plasma concentrations in each subject prior to the study dose of medication for each period were determined to confirm steady state conditions. All patients had measurable levels of each drug prior to administration of both the generic and brand formulations. Mean

trough plasma concentrations were similar for generic and brand lamivudine and stavudine, but trough nevirapine concentrations were significantly higher with the generic formulation during period 2 (Table 14).

Bioequivalence Evaluation

The mean concentration-time profiles over 12 hours for each drug after administration of the generic and brand formulations are shown in Figure 7. There is little noticeable variation in plasma levels of stavudine or nevirapine between the brand and generic formulations. In contrast, lamivudine levels are much higher following the dosing of the brand name formulation compared to the generic. As expected based on its long half-life, nevirapine levels were relatively constant during the 12 hr study period.

Both AUC_{0-12h} and C_{max} were analyzed as bioequivalence markers. The geometric mean ratios for AUC_{0-12h} were close to unity for all three compounds, suggesting similar exposure with the two formulations. The largest variation was seen for lamivudine, with a 20% reduction in exposure with the generic formulation. The FDA definition for bioequivalence of a 90% confidence interval of the geometric mean ratio between 0.8 and 1.25 is not met for AUC_{0-12h} for any of these drugs. Similar results were found for C_{max} , with nevirapine being the only drug that meets the bioequivalence criteria (GMR =1.1, 90% CI 0.95-1.23) (Table 15). For lamivudine, the C_{max} was 20% lower with the generic compared to the brand formulation while for stavudine the C_{max} was 30% higher with the generic compared to the brand formulation. Because the concentration-time profiles (Fig 7) are drawn on an ordinary scale the difference in C_{max} for stavudine is not very noticeable given that the arithmetic means for the brand and generic were very close.

A mixed random effects model demonstrated significant sequence effects for both nevirapine log transformed C_{max} ($p<0.0001$) and AUC ($p<0.0001$) as outcomes. Intersubject variability for log transformed C_{max} ranged from 0.24-0.40 and that for AUC ranged from 0.23-0.42. Intrasubject variability for C_{max} ranged from 0.21-0.44 and AUC ranged from 0.25-0.36 (Table 16). Intersubject variability accounts for approximately half of the variability in the C_{max} and AUC estimates.

DISCUSSION

We found generic stavudine, lamivudine and nevirapine in the form of Triomune fails to meet strict bioequivalence criteria in patients with objectively confirmed adherence on stable therapy. While Triomune failed to meet strict bioequivalence, the differences were relatively minor and are unlikely to be clinically significant. Our results are similar to a recently reported bioequivalence study carried out in Malawian HIV-infected patients [2]. One exception is that in the former study, the generic Triomune formulation resulted in a significant increase in stavudine C_{max} compared to the brand name. In both the present study and in the Malawian report, plasma levels of nevirapine following administration of either the generic or brand formulations were higher than those previously reported in Caucasian HIV patients [2, 7]. A large fraction of nevirapine is metabolized by the polymorphic CYP2B6 enzyme [8, 9]. Whether the elevated levels of nevirapine in these African populations is related to the higher allele frequency of the reduced function *CYP2B6* 516G>T polymorphism in these populations should be investigated in larger samples.

There are several reasons why the differences in drug exposure between brand and generic medication are unlikely to be clinically significant. Over 70% achieved undetectable viral load levels (<400 copies/ml) and had significantly improved their CD4 count at 12 and 24 weeks [3, 4]. Even in the case of lamivudine, for which C_{\max} and AUC were decreased 20-30% with the generic formulation compared to brand name, the plasma concentrations were similar to those reported following a single dose of Triomune or a second combination drug containing abacavir, lamivudine and zidovudine to healthy subjects [1, 10]. Furthermore, the mixed random effects model found no statistically significant difference in C_{\max} or AUC between the formulation types for any of the three drugs (results not shown).

We found a high degree of variability between study subjects. The random effects model produced correlation coefficients of about 50% for both log transformed C_{\max} and AUC values (Table 16). This implies that about 50% of all variability in these parameters was due to differences between study subjects. The interindividual variability in the pharmacokinetics of stavudine, lamivudine, and nevirapine may be attributable to various sources, such as environmental or genetic factors, that were not addressed in this study. In particular, genetic polymorphisms in transporters or drug-metabolizing enzymes for which these drugs are substrates may affect the pharmacokinetics of these drugs. For example, the cytochrome P450 2B6 (CYP2B6) enzyme, which is involved in nevirapine metabolism, contains a genetic polymorphism (516G>T) that has been shown to substantially decrease hepatic protein expression and function. [11] In patients with HIV, this polymorphism has been significantly associated with increased nevirapine plasma levels. [12] While the nucleoside reverse transcriptase inhibitors lamivudine and stavudine are not extensively metabolized in the liver, it is possible that polymorphisms in membrane transporters could influence the bioavailability of lamivudine and stavudine, thereby modulating plasma drug levels. Recently, a polymorphism in the ABCC4 transporter was associated with intralymphocytic lamivudine levels in HIV patients [13]; conceivably, this polymorphism may similarly affect ABCC4 activity in enterocytes, where drug absorption occurs. Due to the minor allele frequencies of these polymorphisms, a larger study population is needed to address these pharmacogenetic questions.

We observed a significant difference in the mean nevirapine trough concentrations between the brand and the generic formulation. However, these [steady-state] plasma nevirapine concentrations are far above the concentration required to inhibit 50% viral replication in vitro (the IC₅₀ for nevirapine is 10.6 ng/ml) [14] so this difference may not have clinical relevance. The first round of pharmacokinetic sampling does not show a significant difference in the mean nevirapine trough concentrations between the brand and generic formulation. This implies that the observed difference is not a systematic difference in the trough concentrations of the two formulations and may be due to the observed sequence effects. A limitation of our study was that we did not conduct drug content assays and in vitro dissolution tests. A drug content assay for each formulation would rule out formulation problems. In vitro dissolution testing would reveal variations in drug degradation between brand and generic formulations that could lead to in vivo differences in the rate of nevirapine absorption.

Our analysis model identified significant sequence effects for nevirapine C_{\max} and AUC. Although the exact causes of the sequence effects are not known, 'real' differences between the groups could contribute to the observed effects. While our study groups were similar in most baseline characteristics, the brand to generic group (B-G) was generally healthier than the generic to brand group (G-B). Ninety one percent (10/11) of the B-G group was virologically suppressed (<400 copies/ml) compared to 71% (5/7) in the G-B group. (Table 13) In addition,

mean CD4 cell count in the B-G group was about 80 units more than the mean CD4 count in the G-B group (402 cells/ μ l vs. 319 cells/ μ l). To control for this empirical confounding, baseline CD4 cell count and viral load were added to the model. We found a significant association between baseline CD4 cell count and nevirapine C_{max} (p=0.007) and AUC (p=0.02). This difference in health status may explain the observed sequence effects.

Triomune-40 ® contains a higher dose of stavudine than is currently recommended in treatment guidelines. Current WHO guidelines now recommend a 12 hourly dose of 30mg for all patients irrespective of weight. [18] This limits applicability of our results to patients currently on antiretroviral therapy. Studies exploring bioequivalence of fixed dose combination formulations containing 30mg of stavudine are needed.

In summary, the steady-state pharmacokinetics of the generic formulation (Triomune) in HIV-infected patients did not meet the strict bioequivalence requirement set by the FDA when compared to the brand name formulations of lamivudine, stavudine, and nevirapine. However, based on the measured plasma levels, the generic formulation is expected to produce a similar therapeutic response as the brand name formulations. There was a large degree of interindividual variability in antiretroviral exposure. Variability could have been due to individual disease state and progression, or the genetic variation within the subjects. Understanding the exact sources of this variability will be important for optimization of therapy. These results suggest that bioequivalence and pharmacokinetic studies are needed in specific populations in which these medications are used, to account for unique characteristics that may influence drug disposition. Drug regulatory bodies in countries in which generic antiretroviral medications are used should endeavor to test all ARVs imported into the country to ensure drug quality.

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Table 13 Baseline characteristics of participants according to randomization arm

	Generic→Brand	Brand→Generic
Number	7	11
Mean age [years(SD)]	35.3 (5.3)	38.8 (6.3)
Sex (M/F)	3/4	4/7
Mean weight [kg (SD)]	68.4 (10.2)	68.2 (3.8)
Mean height [cm (SD)]	163 (8.7)	167 (9.8)
Mean body mass index [kg/m ² (SD)]	26.0 (4.4)	24.5 (2.8)
Mean HIV RNA [log ₁₀ copies/ml (SD)]	2.8 (0.6)	2.8 (0.5)
HIV RNA < 400 copies/ml [n (%)]	5 (71.4)	10 (90.9)
Mean CD4 T cell count [cells/μl (SD)]	319 (116)	402 (267)
Mean Hemoglobin [mmol/l (SD)]	14.7 (1.5)	13.8 (1.9)
Mean alanine aminotransferase [U/L (SD)]	26.7 (7.34)	30.8 (18.2)
Mean aspartate aminotransferase [U/L (SD)]	27.7 (8.9)	29.9 (8.54)
Mean serum creatinine [mg/dL (SD)]	1.1 (0.1)	0.9 (0.2)
On prophylactic cotrimoxazole [n (%)]	5 (71.4)	5 (45.5)

Table 14 Trough plasma concentrations prior to initiation of pharmacokinetic study

	Plasma Concentration (ng/ml)^a		P-value
	Brand	Generic	
Period 1			
3TC	303 ± 270	212 ± 160	0.43
D4T	195 ± 342	161 ± 245	0.86
NVP	8230 ± 4270	6160 ± 1890	0.25
Period 2			
3TC	468 ± 486	391 ± 405	0.72
D4T	283 ± 331	511 ± 547	0.44
NVP	4770 ± 952	9300 ± 3640	0.01

^aPlasma concentrations are expressed as mean ± SD.

Table 15 Pharmacokinetic parameters of generic and brand name nevirapine, stavudine and lamivudine

Pharmacokinetic Parameter ^a	Arithmetic Mean ^b		Geometric Mean ^b		GMR ^c
	Generic	Brand	Generic	Brand	
Nevirapine					
C _{max} (mg/L)	9.2 ± 3.1	9.6 ± 5.5	8.8 ± 3.1	8.4 ± 5.5	1.1 (0.95-1.23)
AUC _{0-12h} (h*mg/L)	91.5 ± 35.2	88.2 ± 45.7	85.8 ± 35.2	79.2 ± 45.7	1.1 (0.95-1.31)
t _{max} (h)	1.8 ± 1.8	2.1 ± 2.6			
Lamivudine					
C _{max} (mg/L)	1.1 ± 0.5	1.7 ± 1.4	1.0 ± 0.5	1.3 ± 1.4	0.8 (0.63-0.98)
AUC _{0-12h} (h*mg/L)	5.6 ± 2.5	7.5 ± 4.9	5.2 ± 2.5	6.4 ± 4.9	0.8 (0.65-0.99)
t _{max} (h)	1.1 ± 0.8	1.2 ± 0.6			
Stavudine					
C _{max} (mg/L)	1.9 ± 1.1	1.8 ± 2.0	1.6 ± 1.1	1.3 ± 2.0	1.3 (0.99-1.71)
AUC _{0-12h} (h*mg/L)	4.1 ± 2.4	4.2 ± 3.9	3.6 ± 2.4	3.4 ± 3.9	1.1 (0.87-1.38)
t _{max} (h)	08 ± 0.5	1.1 ± 0.8			

^aAbbreviations used are C_{max}, maximum plasma concentration, AUC, area under the concentration-time curve, and t_{max}, time to maximum plasma concentration.

^bPharmacokinetic parameters are given as the mean ± SD.

^cGMR, geometric mean ratio; the 90% confidence interval is given in parentheses.

Table 16 Inter- and intrasubject variability in log transformed pharmacokinetic parameters

	Intersubject Variability^a	Intrasubject Variability^a	Correlation Coefficient (ρ)^b
logC_{max}			
Lamivudine	0.37	0.34	0.53
Stavudine	0.40	0.44	0.46
Nevirapine	0.24	0.21	0.57
logAUC₀₋₁₂			
Lamivudine	0.33	0.33	0.50
Stavudine	0.42	0.36	0.57
Nevirapine	0.23	0.25	0.46

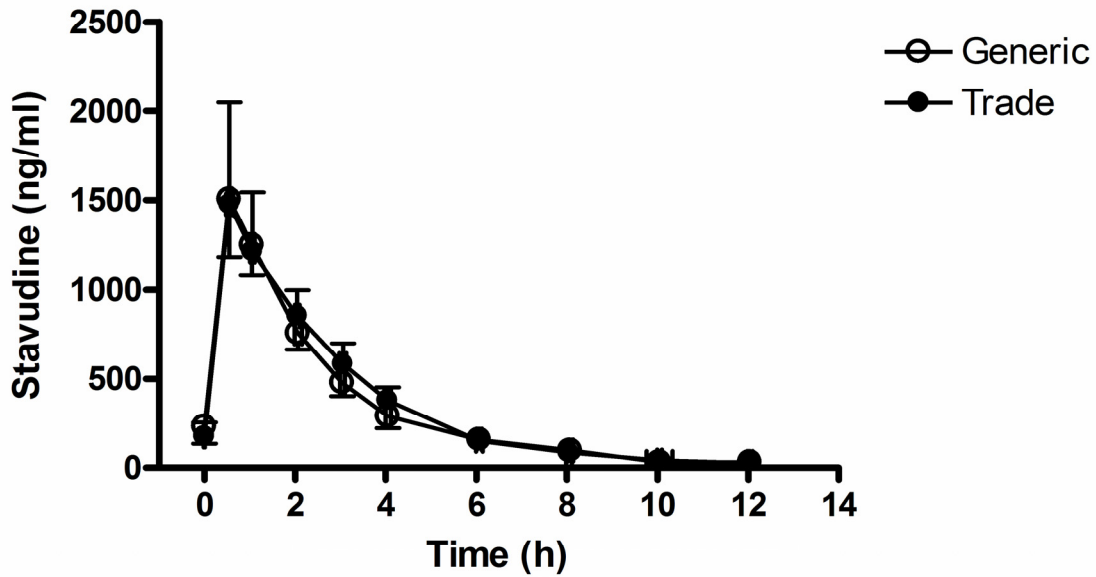
^aInter- and intrasubject variability indicate the standard deviation of the pharmacokinetic parameters between or within subjects, respectively.

^b ρ is the proportion of the total variability that is due to the variability between subjects

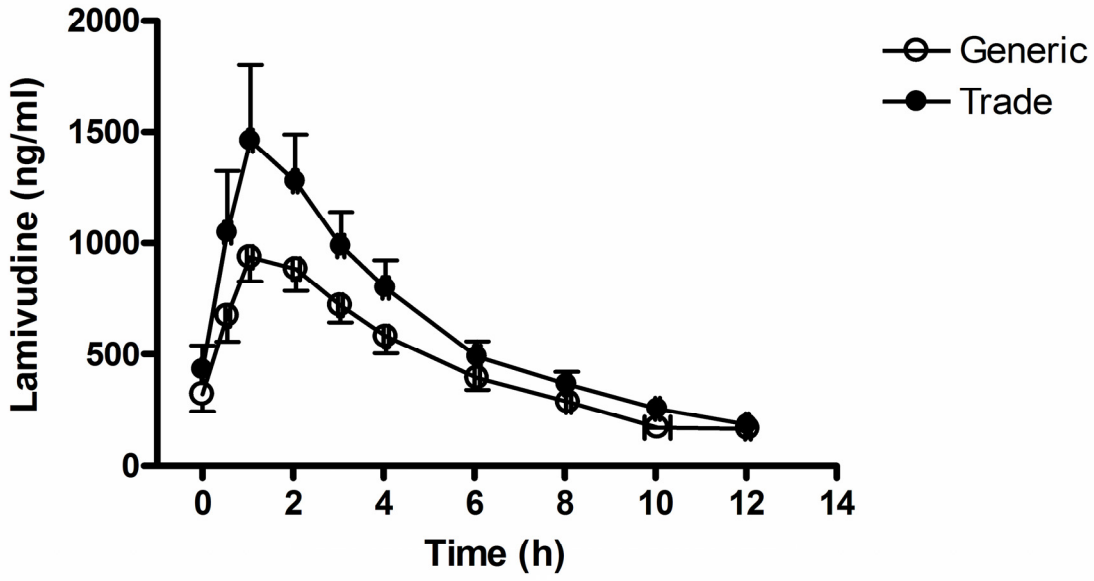
Figure Legends

Figure 7 Mean plasma concentration-time profiles of (A) stavudine, (B) lamivudine and (C) nevirapine in 18 subjects after oral administration of brand (closed symbol) or generic (open symbol) formulation. Each value represents the mean \pm SE for 18 subjects in each arm.

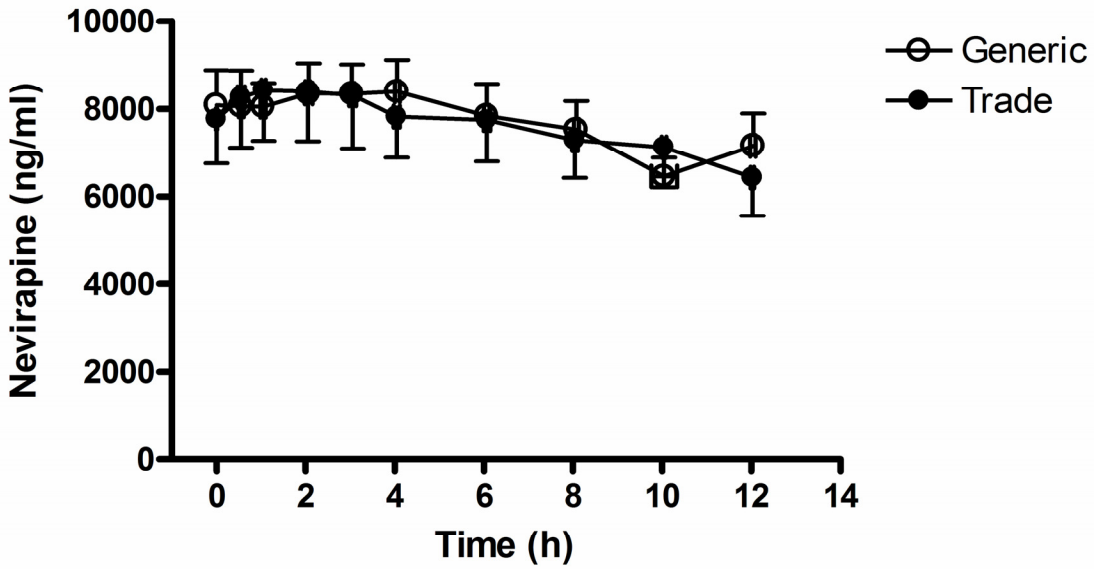
A)



B)



c)



CHAPTER VI

Summary
Implications for Research and Practice
Conclusions and Recommendations

SUMMARY

Compared to ten years ago, when use of ART in the developing world was just starting, many more HIV-infected individuals in resource-limited settings are now receiving ART [1]. The global community has prioritized increasing access to ART in the developing world. This gesture has contributed to less morbidity and mortality due to HIV/AIDS and better quality of life[1].

While donors and governments have concentrated on scaling up treatment, little emphasis has been put on other salient issues that may have an impact on the success of such treatment. Successful antiretroviral therapy requires the interplay of several factors; drug availability, drug quality, adherence, infrastructure for monitoring treatment outcomes, and human resources. This dissertation has looked at two of these factors: adherence and quality of ARVs. Adherence is the Achilles' heel of successful antiretroviral therapy and the benefits of good adherence only come when patients take good quality ARVs. Specifically, this dissertation examined the following issues:

a) Many studies have shown that the cost of ARVs is the major hindrance to complete adherence[2-5]. This challenge has been addressed by providing these drugs at no cost to the patients. This has, however, created other concerns, of patients not valuing these medications and consequently not adhering to them. One of the aims of this dissertation is to evaluate this concern with the hope that the findings will guide policy makers in drafting policies that will promote adherence.

b) Because HIV/AIDS is a family disease, it is imperative that all HIV-infected family members be treated. Only the MTCT-Plus program provides treatment to all HIV-infected members in a household. Even in this program, the impact of treating all HIV-infected family members has not been well studied. This study explored the impact of treating all HIV-infected family members on adherence.

c) Depression is a significant predictor of poor adherence [6-15]. Accurate measurement of depression is important to identify those patients who need intervention. The Beck Depression Inventory II is a commonly used instrument to measure depression in sub-Saharan Africa among HIV-infected individuals, yet it has not been validated in this population. This study examined the psychometric properties of the BDI-II in a low income predominantly female HIV-infected population in Uganda.

d) Most of the ARVs used in the developing world are of the generic type. Most of these generic drugs are not qualified but it is assumed that they will perform as well as the brands they copy[16, 17]. The great need for ARVs in the developing world has also promoted the supply of unqualified drugs to these countries. As a result, the quality of ARVs used in these countries is not guaranteed. Furthermore, the high demand coupled with high unit cost of these drugs attracts counterfeit products. Most of the patients in Uganda use generic ARVs. This study was conducted to assess the quality of ARVs used in Uganda.

SUMMARY OF STUDY FINDINGS

a) Using marginal structural models, a statistical procedure that allows making causal inference, patients receiving subsidized treatment had better adherence than those who paid for

their treatment. The adherence of patients on subsidized treatment was about four percent units higher than that of patients on self-pay treatment. These findings corroborate those from many studies that showed that cost was a predictor of adherence.

b) There was excellent adherence among adults attending the MTCT-Plus program (>95%) but less than optimal adherence among the children (~87%). Findings among the adults support the notion that treating all HIV-infected adults in a household promotes adherence. Less than optimal results among the children suggest that their caregivers are not doing a good job at administering the drugs as prescribed. This could be due to demands on their time by work or to children having several caregivers, none of whom takes full responsibility for the child's medication. Nabukera et al found that children whose primary caregivers had not disclosed the children's HIV serostatus were more likely to have poor adherence compared to those who had disclosed [18]. Depression was a significant predictor of poor adherence among the adults. This finding corroborates findings from other studies that have shown depression to be a significant predictor of non-adherence.

c) Using item response modeling, the BDI-II had good psychometric properties, with Cronbach's alpha of 0.79 and the expected a posteriori (EAP) reliability coefficient of 0.86. Most of the items fit the analysis model reasonably well. There was evidence for validity based on instrument content, internal structure and relations to other external variables.

d) The generic brand of stavudine/lamivudine/nevirapine did not meet the requirement for bioequivalence with the brand counterparts as per FDA guidelines[19]. However, according to the plasma concentration-time curves, drug exposures were very similar for both formulations. In addition, statistical analysis showed no significant difference between the two formulations. Based on these latter findings, the two formulations appear to be similar and are likely to perform equally well in this HIV-infected population.

IMPLICATIONS FOR FURTHER RESEARCH

This dissertation examined the impact of the direct cost of treatment on adherence. Further research is needed to examine the impact of indirect costs of treatment on adherence. These indirect costs include transport to the treatment centers and money to buy food, among others. This study was conducted among very sick individuals initiating ART. Further research should be conducted among patients who have been on ART for a reasonable amount of time to assess the impact of time on therapy on adherence. Given that the majority of HIV-infected individuals in the developing world are currently receiving no cost treatment, why is it that adherence is steadily dropping, despite the fact that cost [determined to be the greatest barrier to good adherence] was removed? What other interventions are necessary to promote good adherence?

The study among MTCT-Plus members did not have a control group; hence, the true effect of the intervention-[providing ART to all HIV infected household members] could not be evaluated. Further research with a control group is therefore needed to test the impact of providing HIV treatment to all HIV-infected household members on adherence.

The BDI-II appears to be a good screening tool but not a good diagnostic tool for depression in HIV-infected populations. The instrument does not have sufficient items that measure severe depression. Further research is needed to fill the entire spectrum of depressive symptoms (mild to severe) to increase the utility of this instrument.

In examining the quality of ARVs in Uganda, we looked at only two batches of ARVs. This is not representative of all ARVs being used in Uganda. There is need to conduct a survey that looks at different batches of drugs from different outlets in the country to assess the prevalence of counterfeits/substandard drugs in the country.

IMPLICATIONS FOR CLINICAL AND PUBLIC HEALTH PRACTICE

These findings suggest that no cost treatment should be provided to HIV-infected patients in resource-limited settings to promote adherence. However, other interventions should be designed to promote and maintain good adherence. Providing no cost treatment is not enough to maintain good adherence.

These findings suggest that treating all HIV-infected household members enhances good adherence. So, efforts should be made to treat the entire family instead of only a few individuals in a family. Good adherence among the adults does not necessarily imply that the HIV-infected children of those adults also have good adherence. Health care providers should pay careful attention to children and support the caregivers who administer the medicines to the children. Healthcare providers should always screen for depressive symptoms among HIV-infected patients and treat them.

The BDI-II can be used as a screening tool but not a diagnostic tool for depression.

The results of this study suggest that Triomune, a generic ARV, is comparable to the brand name pharmaceutical equivalents and may be used to treat HIV-infected patients.

CONCLUSIONS

Providing antiretroviral treatment at no cost to patients is necessary but not sufficient to protect against poor adherence.

Treating all HIV-infected members in a household may promote good adherence. Depression is a barrier to adherence and needs to be actively screened for and treated to minimize non-adherence.

Findings from this dissertation support the use of the BDI-II in assessing depressive symptoms among HIV-infected patients in sub-Saharan Africa, especially among women.

Though not strictly bioequivalent to its brand name equivalents, Triomune provides similar drug exposure and can be used interchangeably with the brand-name types.

RECOMMENDATIONS

1. National governments should provide ART to patients at no cost.
2. Together with providing no cost treatment, other interventions should be designed to promote and maintain good adherence.
3. All HIV-infected members of a household should be given ART, to enhance adherence.

4. Screening for depression among HIV-infected individuals on ART should be part of the routine management of these patients.
5. In its current form, the BDI-II should be used as a screening tool for depressive symptoms but not a diagnostic tool for clinical diagnosis.
6. Ongoing monitoring of the quality of antiretroviral medications is necessary to avoid treating patients with counterfeit/substandard drugs.

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