Research Assignment

A COMPARISON OF TREATMENT RESPONSE IN TWO COHORTS OF ONCE DAILY HAART AND TWICE DAILY HAART IN A SAMPLE POPULATION IN GABORONE, BOTSWANA

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Declaration of Originality.

I, **Dr. Rachel Seleke**, hereby declare that this dissertation is my own idea and the result of my own work; that it has not been submitted for any degree or examination at any other University, and that all the sources I have used or quoted have been indicated and acknowledged by complete references.

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Signed

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Date

ABSTRACT

Title A comparison of treatment response in two cohorts of once daily HAART and twice daily HAART in a sample population in Gaborone, Botswana.

Background Sub-Saharan Africa has been hard hit by the HIV/AIDS epidemic with an estimated 22.9 million adults infected in 2010. The advent of antiretroviral therapy (ART) has seen significant reduction in mortality from AIDS related illnesses. With the reduction of mortality and the indisputable positive results seen from the use of Anti-retroviral Treatment (ART), the demand both from people living with HIV and health care providers to phase in less toxic ARVs while maintaining simplified fixed-dose combinations has increased considerably. Botswana like most low-resource countries has adapted the WHO recommendation of daily ART as opposed to the previous twice daily HAART. No evidence from resource limited settings has been found that clearly indicates the superiority of regimens based on AZT, d4T or TDF.

Aim The primary aim was to compare treatment response between two cohorts. The secondary aim was to compare any association of regimen to age or gender.

Objectives To comparatively determine treatment response at 3 months based on immunological response (shown by an increase in CD4 above pre-therapy levels) and viral load response.

Methods The study is a retrospective comparative cohort study. Three ART sites were selected from a total of 6 sites. A sample size of 263 was required to achieve a 90% effect power. An equal number of patient records were reviewed per site and each arm had an equal number of reviewed records. A total of 286 patient record files which fit the inclusion criteria were retrospectively analysed and data entered in Excel before being analysed using Statistica Version 10. A $p < 0.05$ represents statistical significance whilst a 95% confidence interval was used for estimation of unknown variables.

Results n=263. The overall sample was predominantly male (75.19%). An overwhelming majority (95.88%) of patients in both arms had undetectable viral loads (VL<400). A significant association was found between the regimen and viral load (p=0.0315-Pearson Chi Test). The difference in CD4 between the two arms was not statistically significant (p=0.655890-ANOVA). A positive association was found between the regimen and gender (p=0.03190-Pearson Chi Test). This was possibly owing to the high numbers of males and no statistical adjustment to gender made. No association was found in the difference in CD4 cell counts for regimen and gender (p=0.612191-Anova).

Conclusion Treatment response at 3 months post initiation between once daily and twice daily HAART in Gaborone Botswana by use of virologic and immunologic response has been shown to be comparable. The use of one regimen over the other as first line as recommended by WHO and the subsequent adoption of the current first line regimen by the Botswana Ministry of Health may be justified. This study has therefore reinforced the applicability of previous findings in other settings of this recommendation. As part of the targeted audience and indeed as a partner in the care and management of HIV, the responsibility to ensure applicability of the recommendations set out for resource limited areas has been achieved through this study. However, bigger randomized trials in resource limited settings are needed to justify and accredit these findings as well as add to the evidence obtained in developed countries.

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Seleke Rachel; MD

Introduction

In 2010, an estimated 22.9 million adults in sub-Saharan Africa were said to be infected with the Human immune deficiency virus (HIV) with an estimated 1.2 million deaths in Africa attributed to HIV and AIDS (UNAIDS Report on the global AIDS epidemic-2010).[1] Botswana has a high adult prevalence of HIV/AIDS, estimated at 24.8% in 2010; the second highest in the world. The national response to HIV/AIDS which involved freely availing ART (Antiretroviral therapy) has seen a significant reduction in deaths related to HIV and AIDS in Botswana. Antiretroviral therapy (ART) has also evolved through the years with more tolerable and easier to take formulations coming into the market replacing previous therapies in the national guidelines. As these improved treatments become available to clinicians in the third world, efforts towards proven efficacy in the developing countries need to be set in place. Like many countries across the world, Botswana HIV/AIDS guidelines now recommend fixed combination therapy as first line in eligible adults.[2] Most studies looking into these new once daily ART were set to establish adherence but currently no published study in Botswana has been performed to describe effect of adherence on treatment outcomes in light of these 'new' fixed daily antiretroviral therapies,[3] which until 2008 were not dispensed in government ART clinics.

Some international studies have proved that frequency and ease of dosing for chronic medication use is associated with increased adherence patterns.[4,5] Fixed combination ART therefore appears to be the current best practice and these have been shown to be effective in managing progression of HIV infection as well as simplifying therapy.[6] On the basis of efficacy measures reflecting lowered viral load (percentage of patients with HIV RNA levels <400 copies/ml or <50 copies/ml) once daily administration regimens were consistently found to be at least as effective as twice-daily regimens, and sometimes more effective.[7] Although these fixed dose combinations of ART have been used in developed countries since approval by the US Food and Drug administration (FDA) some 6 (Europe) or 7 (United States) years ago, there is currently no published data in Botswana as to the rate of progression of HIV on this 'new' once daily dosing ART compared to the conventional 'older' twice daily ART dosing.[3]

The goal for highly active antiretroviral therapy (HAART) initiation in HIV is to achieve full virologic suppression in the shortest possible time to decrease opportunistic infections and decrease morbidity and mortality associated with persistent viraemia.[8] The motivation for this study therefore stems from the recent introduction of once daily HAART in our setting and the lack of available clinical data on treatment outcomes in our population on this fixed dose regimen compared to the 'older' combinations.[3] There is an absolute necessity for this determination for a country with such high HIV/AIDS prevalence to prescribe the best available ART with good expected outcomes. This is not withstanding the fact that this study may not fully address all confounding factors and other factors affecting treatment outcomes. The results of this study will add to the scarce available data on this topic and will assist clinicians managing HIV/AIDS to make informed decisions related to first line therapies for their patients considering ease of dosing as well as cost of medications. This in particular is of paramount interest to the family physician who in our setting is the primary physician managing and constantly deciding on the first line ART to initiate patients based on their lifestyle and possible adherence constraints as well as

clinical background and therapy cost, to mention a few. This is especially important as the age group often initiated on Atripla ® (once daily regimen) is younger than 30 years old and this age group has been proven to be a significant risk factor for virologic failure.[9] Although fixed dose combinations (FDC) have been proven to improve adherence and possibly treatment outcomes,[4] this has not been proven in the ART Botswana setting,[3] hence probing the research question: " How do patients on once daily fixed antiretroviral therapy compare to patients on conventional twice daily therapy in terms of treatment outcomes in Gaborone, Botswana?" In its systematic review for the 'Recommendations for a public health approach' revised in 2010, the WHO found no evidence from randomized controlled trials, non-randomised trials or observational studies from resource limited settings that clearly indicated the superiority of either regimens based on Zidovudine (AZT), Stavudine (d4T) or Tenofovir disoproxil fumarate (TDF) or the superiority of either Efavirenz (EFV) or Nevirapine (NVP) in triple-drug antiretroviral regimens for treatment-naïve patients.[10]

Central question: How does treatment response of once daily HAART regimen compare to twice daily HAART regimen in ART naïve patients?

Background

Atripla is a complete regimen in a single, fixed-dose combination tablet that contains: efavirenz (EFV) 600mg, Emtricitabine 200mg and tenofovir disoproxil fumarate (TDF) 300mg. Current treatment guidelines recommend this triple combination for initial therapy because of its excellent potency, tolerability and favorable safety profile.[11]The co-formulation of EFV/Emtricitabine (FTC)/TDF is bioequivalent to administration of its individual components.[12] In the treatment of many diseases, once-daily or twice daily doses are preferred.[4] Switch of initial HAART regimens to an efavirenz based regimen with good treatment outcomes as well as patient satisfaction have been documented.[13]

Experiences with HAART suggest that adherence is arguably the most important factor in successfully managing HIV/AIDS.[2,14] A review of studies looking at adherence to ART is therefore imperative as is a review of studies comparing efficacy/treatment outcomes in single versus multiple doses.

Eldred and colleagues found that patients on twice or daily doses reported better adherence (>80%) than patients on multiple doses per day and were more likely to take their medications when away from home.[5] Paterson and colleagues also found that twice-daily dose was associated with better adherence than a three times daily dose.[15] Other studies however have failed to confirm this association including the large Health Care Services Utilization Study with more than 1900 participants.[15] It is therefore part of this gap in the literature that this study seeks to fill especially in HIV as a chronic disease in a developing country with a high prevalence of the disease.

In study group 934, antiretroviral naïve patients were randomized to receive once daily tenofovir DF 300mg and emtricitabine 200mg as separate components (n=258) versus a twice-daily, FDC tablet of zidovudine 300mg/lamivudine 150mg (n=259) in combination with efavirenz for 48 weeks.[16] The objective of this particular study was to assess the non-inferiority of the tenofovir DF/emtricitabine-containing regimen as measured by the proportion of subjects maintaining HIV RNA levels <400 copies/ml through 48 weeks.[16] At week 48, a greater proportion of patients treated with tenofovir DF/emtricitabine (206/244; 84%) versus zidovudine/lamivudine (177/243; 73%) maintained RNA levels <400 copies/ml (p=0.002). This study was conducted in various European states including Germany, France, Italy, Spain and the UK as well as the USA.

However, no similar study has been conducted in the sub-Saharan region, thereby probing this question in these parts of the world.[3]

Some studies have compared other HAART fixed dose combinations for efficacy.[17] Although some of the fixed dose combinations used in some of the studies are not necessarily the same as the drugs under study, these studies have been included to show patterns of adherence and relevance to treatment outcomes with HIV progression as well as safety profiles and tolerability.

Elion et al conducted a single-arm, open label, multicenter 48-week study to evaluate the efficacy, safety, tolerability, pharmacokinetics, adherence and treatment satisfaction of atazanavir/ritonavir(ATV/r) 300mg/100mg and tenofovir DF/emtricitabine (TDF/FTC) 300mg/200mg once daily in antiretroviral-naïve HIV infected patients. This study concluded that ATV/r + TDF/FTC were safe, well tolerated and convenient for patients.[18] Although the study drugs used in this study are not the same as the drugs in to be studied, it does compare once daily HAART and proves efficacy of tenofovir/emticitabine combination as well as patient satisfaction on the regimen leading to improved adherence.

Several studies have been conducted to evaluate impact or comparing switching stable patients on AZT/3TC combinations to TDF/FTC. Fisher et al who conducted a randomized, 48 week, open-label, comparative study of twice daily AZT/3TC or replacement with once-daily TDF-FTC-EFV in individuals on successful EFV-based antiretroviral therapy. This study concluded that switching AZT/3TC to TDF/FTC in persons on EFV therapy maintains virological control, establishes a once-daily regimen, results in improvements in hemoglobin and key lipid parameters and preserves and restores limb fat relative to continuation AZT/3TC.[19] This study illustrates the continued efficacy of this regimen in patients previously on AZT/3TC. This therefore offers an alternative where such patients require switch from this 'older' regimen to the once daily TDF/FTC combination tablet. The following two studies mentioned also attest to this. DeJesus et al conducted a prospective, multicenter, single-arm 24 week trial to investigate the impact of switching virologically suppressed, HIV-1 infected patients from twice-daily fixed dose zidovudine/lamivudine to once daily fixed dose tenofovir disoproxil fumarate/emtricitabine. This study also concluded that patients switched to EFV +TDF/FTC maintained virologic suppression and tolerated the regimen with increased patient satisfaction and fewer side effects.[20]
Arasteh et al also conducted a prospective, 48 week, non-randomised, single group, open-label study to assess the efficacy and safety of a treatment switch from a twice-daily regimen containing AZT and 3TC plus a third agent to a once daily regimen containing the fixed dose combination of TDF-FTC plus a divergent third once daily agent in HIV-1 infected patients. The study concluded that virologic and immunologic control are maintained, with apparent benefits in haemoglobin.[13,21]

Although this study deals with treatment outcomes in patients initially started on the once daily ART, the studies cited above attest to the efficacy of this drug combination where patients may need antiretroviral switch. However it is noted that none of the mentioned studies were conducted in resource limited settings.

Overview of previous and current ART treatment protocols in Botswana[2]

The practice as it stands currently in Botswana per the 2012 ART treatment guidelines as regards HAART initiation in adolescents/adults (both pregnant and non-pregnant) is to initiate HAART if:
1.WHO clinical stage 3 or 4 disease or
2. CD4 <350 cells/µL (previously <250 cells/µL)

The first line and second line regimens for new, non-pregnant adolescents and adults are:
First line: TDF/FTC/EFV (previously Zidovudine (AZT)/Lamivudine (3TC)/Efavirenz (EFV) or Nevirapine (NVP) The choice for NVP usage was dependent on the woman's reproductive potential.

Second line: Zidovusine (AZT)/ Lamivudine (3TC)/ Lopinavir (Lop/r) (previously Stavudine (d4T)/Didanosine (ddI)/Nelfinavir and later Lamivudine (Lop/r)

Laboratory monitoring of patients on HAART:
After obtaining baseline, pre-initiation laboratory tests, routine laboratory monitoring should be carried out as follows:

1. **CD4 cell count /%:** 3 and 6 months post initiation, then every 6 months thereafter
2. **Viral load** : 3 and 6 months post initiation, then as follows:
   -every 6 months for adults
   -every 3 months for pediatric patients and adolescents
3. **FBC** : AZT-based HAART : at 4 and 12 weeks post-initiation, then annually only and as clinically indicated. If not on AZT-based HAART, annually only and as clinically indicated.

4. **AST/ALT** : NVP-based HAART: 2, 4 and 12 weeks post-initiation, thereafter only as clinically indicated
5. EFV-based HAART: 4 and 12 weeks post-initiation, thereafter only as clinically indicated
6. **Glucose and total cholesterol**: annually only if on PI-based HAART
7. **Creatinine and creatinine clearance (Ccreat):** 3 and 6 months post-initiation and then, if stable every 6 months (TDF only)

Aim

The aim of this study is to compare treatment response between the 'newer' once daily dosing anti-retroviral therapy regimen (Atripla- Tenofovir disoproxil fumarate/Emtricitabine/ Efavirenz) and the 'older' multiple dosing ART regimen (Zidovudine/lamivudine/Efavirenz) in a sample adult population in Gaborone, Botswana.

Objectives

1. To comparatively determine treatment response at 3 months based on immunological response (shown by an increased CD4 above pre-therapy baseline) with the use of 'newer' once daily ART as opposed to the 'older' twice daily therapy
2. To comparatively determine the treatment response at 3 months based on viral load response (shown by attaining viral suppression at 3 months) on the 'newer' once daily ART as opposed to the 'older' multiple dosing therapy.
3. To describe the frequency of opportunistic infections (AIDS defining illnesses) in the two treatment arms. (Quantitative study)

Definitions of terms

Treatment response: Treatment response to HIV is measured by clinical response, viral load response as well as immunologic response. Treatment response in this study was based on immunologic response, viral response as well as the clinical response as defined below.

Viral response: The use of HAART combinations is expected to suppress HIV within 24 weeks of initiating treatment. Viral suppression varies depending on the sensitivity of the test used. In this study, a good viral load response is defined at 12 weeks (3months) as having a VL<400copies/ml. An undetectable viral load is a VL of <400 copies/ml and that shows a good viral load response. A detectable VL is any VL reading above 400 copies and this is not adequate response to treatment even at 3 months post initiation.

Good immunologic response: According to WHO, a good immunological response is to maintain a CD4 count above the baseline after 24 weeks of initiating HAART. Immunological failure is defined as a drop in CD4 below baseline or a drop of 50% after at least 24 weeks of treatment. In this study a good immunological response is defined as having a CD4 count above pre-therapy baseline at 3 months after initiating HAART. A poor immunologic response is defined as failure to have a CD4 above pre-therapy baseline.

Opportunistic infections: as depicted by the WHO clinical stages III and IV i.e. severe and advanced HIV related illnesses. For purposes of this study, these would be defined as 'improving clinical state' or 'worsening' clinical state.

'Newer' once daily dosing ART regimen: Currently according to the Botswana 2008/10/12 guidelines, this involves the use of daily Atripla ® regimen.[2]

'Older' multiple dosing ART regimen: The 2006 guidelines which involved the use of either Combivir (AZT 300mg plus 3TC 150mg) /Efavirenz combination or Combivir/Nevarapine combination as first line depending on gender/age. The use of which ceased in 2008.

ART naïve: For purposes of this study, this refers to patients who have never been exposed to any HAART regimen. The patients whose data is analysed in this study would have been initiated on either regimen from the start.

Methods

Study design
This was a retrospective comparative cohort study comparing treatment response in two HIV treatment groups.

Setting
There were 6 identified ART clinics in Gaborone, Botswana which also included 1 in the referral hospital, Princess Marina. All the ART sites were situated within existing local clinics at specific identified geographic locations in the city. The largest site amongst these has an adult population of more than 8000 patients. A chart review was done in selected clinic sites.

The study participants were treatment naïve at initiation but at the time of the study they were already on either HAART regimen and were not initiated on the different regimens concurrently. Patients who fit the inclusion criteria were conveniently selected from patient record files at the selected ART clinics in Gaborone.

Participants were already on either regimen at the time of the research and were not initiated on the different regimens concurrently. Patients who fit the inclusion criteria were conveniently selected from patient record files at the selected ART clinics in Gaborone.

Routine viral loads and CD4 measurements after the initial 3 months on therapy were compared between the two groups. A further comparison of the relationship of treatment response given gender was made. A comparison of clinical manifestations by use of WHO clinical staging of patients at start of therapy and new development of opportunistic (AIDS defining illnesses) infections at 3 months post therapy was to be carried out but failed as no such data was available from the patient records.

Study Population

The study population consisted of adults who had been on ART for at least 3 months in clinics in Gaborone, Botswana. Of the 6 identified ART sites in Gaborone Botswana, 3 representative ART sites (Phase II, Extension 15 and Broadhurst Traditional clinic) were conveniently selected. A sample population was conveniently selected from the representative ART sites.

Inclusion criteria

Adult patients (defined as being 18 years or older) were considered for inclusion in the study if:

1. they had been on HAART for a minimum of 3 months
2. had baseline CD4 <250 cells /μL at initiation or WHO stage 3/4 disease
3. those on Atripla had Creatinine Clearance >60ml/min at initiation (estimated using the Cockcroft-Gault equation). [22]
4. they were on either Atripla or Combivir + Efavirenz for a minimum of 3 months.
5. they had been HAART naïve at initiation.
6. those on AZT-based regimen had an Hb>6g/dL at baseline

[The 3 month (12 weeks) end point for virologic suppression was chosen on the basis that most patients on HAART will have suppressed by 6 months and therefore the time to suppression (treatment response) would be difficult to ascertain and yet many had suppressed by 3 months. However in patients with high baseline HIV-RNA levels, maximal suppression may not be for 6-8 months.[23] No baseline HIV-RNA levels are done in the Botswana program. Although all ART guidelines are quite clear and stringent on the creatinine clearance cut off, some facilities still initiate patients on Atripla without a baseline creatinine clearance, hence the inclusion of this parameter in the inclusion criteria].

Tanner staging does not form part of the routine assessment but is routinely assessed in adolescents who may be under age 18 years but considered for initiation on a Tenofovir based regimen. This is due to a lack of pediatric dosing information and possible bone toxicity which precludes the use of Tenofovir in prepubertal children (Tanner Stages 1-3) or those <18 years of age.[24]

Exclusion criteria

All patients who did not fit the inclusion criteria were excluded from the study.
Measurable treatment variables (CD4/VL) to be assessed in the study were evaluated and compared at HAART initiation and at 3 months in the two arms.

Sample size calculation

The sample size calculation was based on the primary objective. The primary objective was to assess treatment based on any two of the following criteria:
1. Virologic response at 12 weeks of ART initiation.

2. Immunologic response at 12 weeks of ART initiation.
3. A clinical response at 12 weeks of ART initiation based on development of a WHO stage III/IV opportunistic infection.

Thus, these are both dichotomous outcomes and since two treatment regimens are being compared the Pearson`s Chi-square test has been used in the analysis. A sample size of 263 achieved 90% power to detect an effect size (W) of 0.20 using a 1 degree of freedom Chi-Square Test with a significance level (alpha) of 0.05.

The sample size was 263 distributed 50:50 in each treatment arm.

Sampling

In the initial study proposal, patients who fit the inclusion criteria were to be identified in the ART clinics and these patients were to be randomly selected by use of a computer based randomization process. However, in the actual study, sampling did not follow this process due to several unforeseen factors:

- An assumption had been made that most ART sites were using the computer based consultation tool and that the necessary information for the randomization would be readily available. Instead, of all the selected sites, none used this computer based tool and were therefore manually consulting and recording patient data.

-None of the sites, including the Ministry of Health which is meant to have all the data pertaining to the ART sites in the country, had the actual list of patients and their respective regimen to enable the randomization process.

-The regimen under study was until the latest guidelines mostly prescribed to males and non-reproductive females. This therefore meant the population under study was already expected to be mostly male dominated.

-The total number of active patient files (obtained from MoH) at the 3 sites was 5909. To obtain a list for randomization would have therefore required one to manually go through each file, entering all the variables before the randomization process, then going back to extract the required information from the 263 sampled files. This process could not be carried out as the Botswana human research and development committee (HRDC) had only given the principal investigator the authority to access patient files and therefore no assistants could be used. Limited time was therefore a factor.

Based on all the described factors, a convenient sample was obtained from the sites through a manual extraction of the required number of files per arm from each site.

Data Collection

Information was collected manually from the patient records and entered into a Microsoft excel page (see Annexure 1). The information collected included participant identification code; age; sex; period on HAART; CD4 at baseline and at 3 months; VL at 3 months; initial WHO stage and at 3 months.

Analysis of Data

Once the data was collected in the word table format, it was captured using MS Excel. STATISTICA version 10 (StatSoft Inc. (2011) STATISTICA(data analysis software system), www.statsoft.com) was used to analyse the data.

Primary analysis included the comparing of the two different regimens with regards to significant response either in terms of CD4 counts or viral loads. A Chi-square test with one degree of freedom was used to analyse the situation. A secondary analysis was performed to adjust for site effects. A generalized estimating equation (GEE) was analysed in order to adjust for the site effect.

Secondary analyses included the relationship of treatment response given age and gender.

Summary statistics was used to describe the variables. Distributions of variables are presented with histograms and or frequency tables. Medians or means are used as the measures of central location for ordinal and continuous responses and standard deviations and quartiles as indicators of spread.

Relationships between two continuous variables was analysed with regression analysis and the strength of the relationship measured with the Pearson correlation or Spearman correlation, if the continuous variables are not normally distributed.

The relationships between continuous response variables and nominal input variables were analysed using appropriate analysis of variance (ANOVA). When ordinal response variables are compared versus a nominal input variable, non-parametric ANOVA methods are used.
The relation between two nominal variables was investigated with contingency tables and likelihood ratio chi-square tests.

A p-value of $p < 0.05$ represents statistical significance and 95% confidence intervals will be used to describe the estimation of unknown parameters.

Ethical Consideration

Ethics approval was sought from the University of Stellenbosch Ethics committee and also from the Botswana Human Resource Development Committee (HRDC). Permission letters were sent to the sites participating in the study. The permission letters clearly stated the name and position of the person conducting the study as well as the purpose and design of the study and also an outline of the ethical considerations in the study, clearly illustrating anonymity of participants. After approval from the local district management team patient files could be accessed for purposes of the study.

The patients' confidentiality was protected by use of codes entered in the database. The codes used were completely anonymous thereby further protecting participant confidentiality. Based on the anticipated large number of data to be collected on these patients, obtaining individualized informed consent was not deemed practically feasible. As guided by international standards and the Government of Botswana research permit guidelines,[24] a waiver of consent was sought for this study. Despite the waiver of consent, the basic principles of autonomy, beneficence, non-maleficence and justice were upheld at all times during the study. The Declaration of Helsinki[24] as observed by the World Medical Association was abided to at all times during the study period to protect patient integrity and confidentiality at all times.

The principal investigator in this study is not a stakeholder in any of the drug manufacturing companies of medications under-study, nor of any of the sites to be used in the study and therefore has no conflict of interest.

Results

TABLE I

Actual frequencies of reviewed data per regimen

Frequency table: Regimen				
Category	**Count**	**Cumulative - Count**	**Percent**	**Cumulative - Percent**
cbv/efv	135	135	50.37	50.37
Atripla	133	268	49.63	100.00
Missing	0	268	0.00	100.00

Table I above shows the actual count as well as the cumulative frequencies and percentages in each arm. The gender frequency is illustrated in Table II. As shown in the tables, the numbers between the two arms is almost identical. The gender inequality between the two arms as illustrated in Table II is mainly due to previous guidelines where an Efavirenz tail was not prescribed to females of child bearing potential.

TABLE II

Overall gender distribution in sample

Frequency table: Gender				
Category	**Count**	**Cumulative - Count**	**Percent**	**Cumulative - Percent**
m	197	197	73.51	73.51
f	65	262	24.25	97.76
Missing	6	268	2.24	100.00

TABLE III

3 month virologic response in sample population

Frequency table: 3m VL Cat				
Category	**Count**	**Cumulative - Count**	**Percent**	**Cumulative - Percent**
0	1	1	0.37	0.37
<400	256	257	95.52	95.90
>=400	11	268	4.10	100.00
Missing	0	268	0.00	100.00

Table III illustrates the overall frequencies of virologic response in both arms. As shown, more than 95 percent of all patients in both arms had attained virologic suppression at 3 months post ART initiation.

Figure 1.

Histogram illustrating sample distribution by Regimen

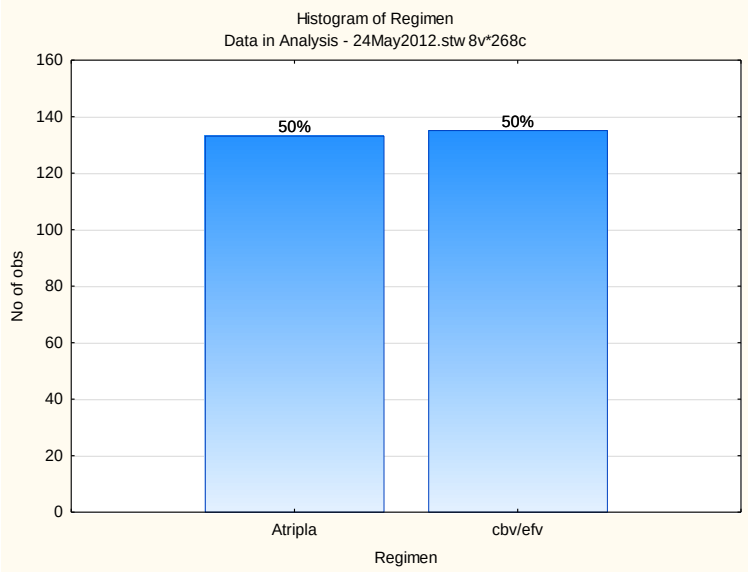
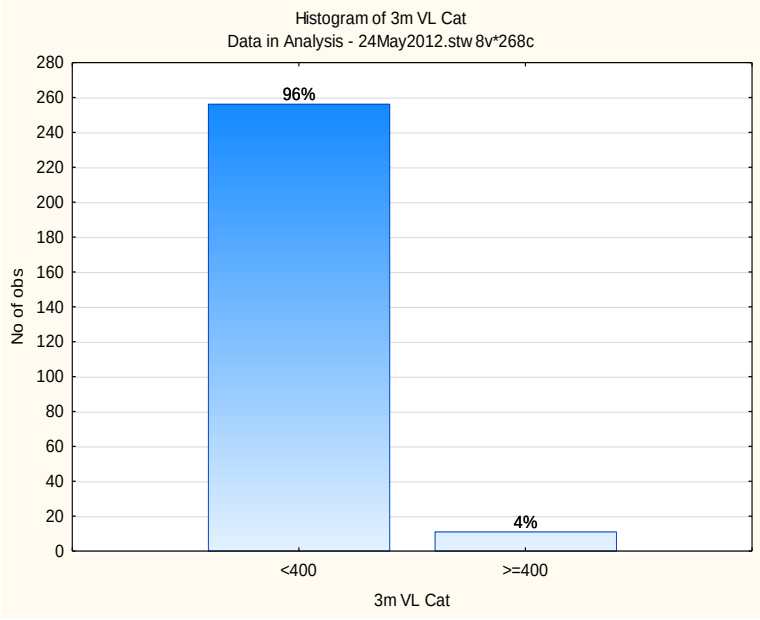


Figure 1 above is a histogram of frequencies illustrating the 'half-half' distribution between the two treatment arms.

Figure 2
Graphic illustration of 3 month virologic response on sample population



A clearer distinction of the proportion of virologically suppressed patients to those with detectable viral loads at 3 months in the sample group is illustrated in figure 2 above.

TABLE IV

2-Way Summary Table: Gender distribution against regimen

2-Way Summary Table: Observed Frequencies Marked cells have counts > 4			
Gender	**Regimen - cbv/efv**	**Regimen - Atripla**	**Row - Totals**
M	106	91	197
Column %	80.92%	69.47%	
Total %	40.46%	34.73%	75.19%
F	25	40	65
Column %	19.08%	30.53%	
Total %	9.54%	15.27%	24.81%
Totals	131	131	262
Total %	50.00%	50.00%	100.00%

Table IV above depicts observed frequencies of Regimen against Gender. In this table, the pattern of predominance of the male gender is clearly shown in the proportions shown (80.92% in the CBV/EFV arm and 69.47% in the Atripla arm).

TABLE V Cross tabulation: Gender (2) x Regimen (2)

Statistics: Gender(2) x Regimen(2)			
Statistic	**Chi-square**	**df**	**p**
Pearson Chi-square	4.603671	df=1	p=.03190
M-L Chi-square	4.636174	df=1	p=.03130
Yates Chi-square	4.010308	df=1	p=.04522
Fisher exact, one-tailed			p=.02237
two-tailed			p=.04474
McNemar Chi-square (A/D)	28.93836	df=1	p=.00000
(B/C)	36.42241	df=1	p=.00000

Table V above is a statistical analysis of the strength of the relationship between gender and regimen. For this purpose, the Pearson Chi-square test was used for analysis. From this analysis, an association between gender and regimen was shown (p=0.03190). This calculation was not adjusted for gender.

TABLE VI

Frequencies of 3 month viral load per regimen

2-Way Summary Table: Observed Frequencies Marked cells have counts > 4			
3m VL Cat	**Regimen - cbv/efv**	**Regimen - Atripla**	**Row - Totals**
<400	132	124	256
Column %	98.51%	93.23%	
Total %	49.44%	46.44%	95.88%
>=400	2	9	11
Column %	1.49%	6.77%	
Total %	0.75%	3.37%	4.12%
Totals	134	133	267
Total %	50.19%	49.81%	100.00%

Table VI above depicts observed frequencies of Regimen against viral load. In this table, the predominance of viral loads <400 is visible (total % 95.88) against viral loads >400 (total % 4.12) in both arms.

TABLE VII Cross tabulation 3m VL Cat(2) x Regimen(2)

Statistics: 3m VL Cat(2) x Regimen(2)			
Statistic	**Chi-square**	**df**	**p**
Pearson Chi-square	4.700866	df=1	p=.03015
M-L Chi-square	5.064468	df=1	p=.02442
Yates Chi-square	3.460437	df=1	p=.06285
Fisher exact, one-tailed			p=.02916
two-tailed			p=.03442
McNemar Chi-square (A/D)	105.5603	df=1	p=0.0000
(B/C)	116.1984	df=1	p=0.0000

Using the Pearson Chi-square test to test relation between Viral load (VL) and Regimen, an association between the regimen and viral load (p=0.0315) was found

Descriptive statistics dialog

TABLE VIII

Comprehensive descriptive data for CBV/EFV for CD4 and age

Regimen=cbv/efv Descriptive Statistics (Spreadsheet in Analysis - 24May2012.stw)												
Variable	**Valid N**	**Mean**	**Confidence - -95.000%**	**Confidence - 95.000%**	**Median**	**Minimum**	**Maximum**	**Lower - Quartile**	**Upper – Quartile**	**Percentile - 10.00000**	**Percentile - 90.00000**	**Std. Dev.**
Age	133	39.78	38.12	41.44	40.00	23.00	73.00	32.00	46.00	28.00	51.00	9.67
Baseline CD4	133	130.34	117.69	143.00	141.00	1.00	440.00	73.00	181.00	20.00	196.00	73.79
3mth CD4	135	226.26	205.3646	247.16	200.00	5.00	608.00	135.00	306.00	83.00	394.00	122.76
Difference in CD4: 3m – Baseline	135	97.85	80.57	115.14	84.00	-254.00	464.00	28.00	145.00	-2.00	202.00	101.54

Table VIII above illustrates the descriptive statistical data in the CBV/EFV arm. This table shows frequencies. From the results presented above, the mean age in this arm stood at 39.78 with a median difference in CD4 at 3 months of 84.00.

TABLE IX

Comprehensive descriptive data for Atripla for CD4 and age

Regimen=Atripla
Descriptive Statistics

Variable	Valid N	Mean	Confidence - - 95.000 %	Confidence - 95.000%	Median	Minimum	Maximum	Lower - Quartile	Upper – Quartile	Percentile - 10.00000	Percentile - 90.00000	Std. Dev.
Age	133	39.95	38.27	41.62	39.00	23.00	69.00	33.00	45.00	29.00	53.00	9.77
Baseline CD4	133	153.01	140.21	165.81	163.00	5.00	295.00	95.00	221.00	36.00	238.00	74.63
3mth CD4	133	263.15	243.01	283.29	256.00	38.00	624.00	174.00	341.00	119.00	401.00	117.43
Difference in CD4: 3m – Baseline	133	110.14	95.26	125.03	102.00	-58.00	438.00	49.00	155.00	11.00	206.00	86.77

In the Table IX above, a descriptive analysis for the Atripla arm is shown. This table illustrates frequencies in this group. The mean age in this group stood at 39.9474 with a median difference in CD4 at 3 months of 102.

Figure 3

Histogram of Difference in CD4: 3m - Baseline

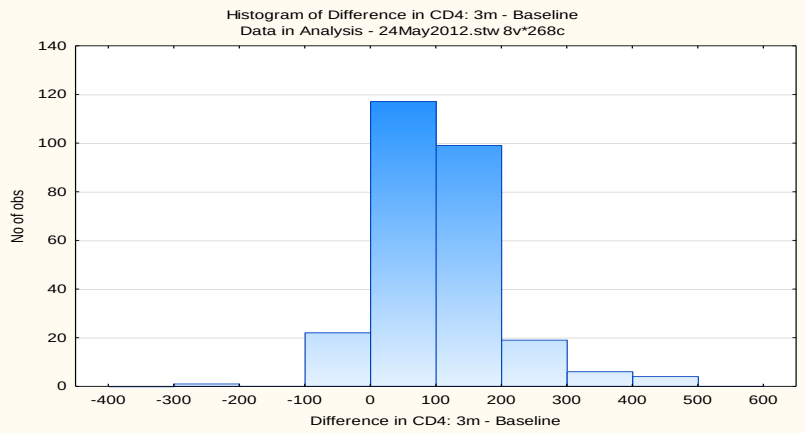


Figure 3 above illustrates the difference in CD4 at 3 months. This graph illustrates the spread of difference in CD4. From this data, the majority of patients had a difference in CD4 between 0 and 100.

The median baseline CD4 for the CBV/EFV arm was 141 and 163 for the Atripla arm. The same variable (median) for the 3 month CD4 was 200 for the CBV/EFV arm and 256 for the Atripla arm. The median difference between CD4 was 84 and 102 for the CBV/EFV and Atripla arms respectively. Figure 3 above shows the distribution of the difference seen in CD4 in the two study arms.

TABLE IX

Univariate Tests of Significance for Difference in CD4: 3m - Baseline

Univariate Tests of Significance for Difference in CD4: 3m - Baseline
Sigma-restricted parameterization
Effective hypothesis decomposition

Effect	SS	Degr. of - Freedom	MS	F	p
Intercept	2085160	1	2085160	241.1968	0.000000
Regimen	1721	1	1721	0.1990	0.655890
Gender	111	1	111	0.0128	0.910045
Regimen*Gender	2227	1	2227	0.2576	0.612191
Error	2230425	258	8645		

Table IX above is an appropriate analysis of variance (ANOVA) for the differences in CD 4 seen between the different regimens as well as across gender groups. No statistical difference in CD4 at 3 months between the two regimen arms (p=0.655890) and in the gender (p=0.910045) was found.

Results presentation and dissemination

Results from this study will be presented to the institution under which this study is conducted (Stellenbosch University) and will also be availed to the Botswana Health Research Development Committee (HRDC) under the hospice of the Ministry of Health(MoH), Botswana. If the study results are published, a recommendation will be made to the National HIV/AIDS guidelines committee and will also be presented to the Southern African HIV clinician's society- Botswana branch; for dissemination to HIV/AIDS managing clinicians in the country. Furthermore, these results will be presented at conferences in the region as well as internationally. The results will be published in HIV journals, through the media and local training institutions for HIV.

Discussion

The gender distribution in the study population was roughly 1:3 females against males. The proportion of males was 80.92% in the CBV/EFV arm and 69.47% in the Atripla arm. This proportion of gender in both arms was found to be significantly different across the two treatment groups; therefore an adjustment in analysis was carried out. The association between gender and regimen (Table V) (p=0.03190) was unadjusted for gender. The illustrated proportion of the sexes is an expected finding as previous guidelines discouraged the use of EFV in women of child bearing potential due to its purported teratogenic properties. This was based on previous animal studies and retrospective human case reports which linked the use of Efavirenz especially during the first trimester to an increase in central nervous system birth defect. The 2012 National guidelines however, based on meta-analyses of available evidence which have found no such increased risk when compared with exposure to other antiretrovirals, now recommends Efavirenz use in this population.[27]

Clinical Staging

The WHO clinical staging was one of the variables for HAART response but was not entered in the study record files and was left out of the analysis. As mentioned in the methods section, any 2

of the 3 endpoints could be used for analysis in the study, hence the use of CD4 and VL at 3 months.

Viral load (VL measurements)
In Botswana, baseline viral load does not form part of HIV management and care. This means the first viral load post initiation is carried out only at 3 months, to ensure viral suppression. This is in recognition of the vast majority of patients being virologically suppressed at 3 months although others take longer, suppressing at 6 months post initiation. For purposes of analysis in the study, this response was categorized as above 400 (>400) or below 400 (<400) at 3 months post initiation. Of particular note is an overwhelming proportion of patients with virologic suppression at 3 months post initiation from both arms (95.88%). An association between the viral load and regimen was shown (p=0.03015). This is an expectant outcome as HAART has been proven from multiple, previous studies to result in virologic suppression when appropriately adhered to.

CD4 measurements
In analyzing the changes in CD4 a variable of difference in CD4 was made by subtracting baseline CD4 from the CD4 at 3 months. From the data collected, the baseline pre-initiation CD4 cell counts between the two arms was not significant The appropriate analysis of variance (ANOVA) with the Univariate test of significance in CD4 cell counts between the two arms was not significant (p=0.655890) An analysis for difference in CD4 cell counts against regimen and gender was carried out with and without an adjustment for gender and showed no significant differences between the two arms (p=0.612191).

Comparison with other studies

Study group 934 sought to compare the non-inferiority of TDF/FTC/EFV as separate components to twice daily FDC AZT/3TC/EFV by measuring HIV RNA VL at 48 weeks. A significant proportion of patients responded better in the TDF/FTC/EFV than the AZT/3TC/EFV.[16]

A similar study conducted by Arribas et al comparing TDF/FTV/EFV and AZT/3TC/EFV, the TDF/FTC/EFV arm demonstrated superior virologic, immunologic and morphologic effects compared to the AZT/3TC/EFV regimen through 96 weeks in an open-label trial. A follow-up comparison through 144 weeks saw significantly more patients in the TDF/FTC arm reaching and maintaining HIV RNA level <400 copies/ml (71% receiving TDF/FTC EFV vs. 58% receiving AZT/3TC/EFV;p=0.004). The conclusion from the study suggests that a regimen of TDF/FTC/EFV demonstrates superior durability of viral load suppression and an improved safety and morphologic profile compared with AZT/3TC/EFV.[28]This study described above was a confirmatory study of the earlier extension phase results of the Study 934 Group that concluded that over 96 weeks, the combination of TDF/FTC/EFV was superior to fixed dose AZT/3TC/EFV for achieving and maintain an HIV RNA level <400 copies/mL and an increase in CD4 cells.[29]

Study Confounders
There are several confounders identified in this study which include: no control for adherence; pharmaceutical superiority and genetic make-up/natural course of viral infection

Adherence
This study compares treatment response but has no consideration or control on the adherence patterns of patients on both treatment arms. In a study comparing 234 patients randomized 1:1 on CBV/EFV and TDF/FTC/EFV where adherence was then checked at intervals of 4,12,24,48 weeks and beliefs about ART, (perceptions of necessity and concerns about adverse effects), treatment intrusiveness and quality of life were measured at the same intervals,[2] significantly higher adherence counts (p=0.049) were reported in the TDF/FTC/EFV arm compared to the

CBV/EFV arm at 48 weeks.[30] Concerns about ART and intrusiveness were also reported lower in those switched to the TDF/FTC/EFV arm. There were however no significant differences in necessity, beliefs, quality of life or viral loads between the randomized groups. A study of the psychosocial factors affecting medication adherence among HIV-1 infected adults receiving combination antiretroviral therapy in Botswana, though not describing the dosing types, found adults receiving HAART for the first 6 months to be least adherent.[31]

Pharmaceutical superiority
This is another confounding factor not catered for in this research. Some studies have comparatively shown superiority over the TDF/FTC/EFV arm against CBV/EFV in ART naïve patients through measurements of HIV RNA levels (VL) at 48 weeks.[16]

Natural course of HIV infection
The study results could be affected by several factors relating to the natural course of HIV infection. Several strains of HIV have been recorded in different parts of the world and although the most prevalent HIV strain in Botswana is HIV-1 subtype C, there may potentially be patients with different strains in Botswana. There are specific biological characteristics of HIV-1C including high genetic diversity which may potentiate the emergence of ARV drug resistant HIV strains.[32] Evidence of greater rates of disease progression in globally prevalent C and D subtypes highlight the importance of expanding early HIV detection, and determining subtype profile at baseline with CD4 staging to optimize the quality of ART delivery and care in global settings[33]. These facts have not been adjusted for in this study.

Study Limitations

The sampling method as already described was conveniently selected and not randomized as initially proposed. Although this was noted in the statistical analysis, it negatively impacts on the credibility of the results obtained as the likelihood of selection bias is introduced.

The study is limited to response to treatment in the initial 3 month period. The initial response does not necessarily translate to long term treatment outcome. Absolute CD4 cell counts were used as an endpoint but this variable has been found to fluctuate with individuals and with intercurrent illnesses.[10]

Conclusion

Treatment response at 3 months post initiation between once daily and twice daily HAART in Gaborone Botswana by use of virologic and immunologic response has been shown to be comparable. The use of one regimen over the other as first line as recommended by WHO and the subsequent adoption of the current first line regimen by the Botswana Ministry of Health may be justified. This study has therefore reinforced the applicability of previous findings in other settings of this recommendation. As part of the targeted audience and indeed as a partner in the care and management of HIV, the responsibility to ensure applicability of the recommendations set out for resource limited areas has been achieved through this study. However, bigger randomized trials in resource limited settings are needed to justify and accredit these findings as well as add to the evidence obtained in developed countries.

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$$eC_{Cr} = \frac{(140 - \text{Age}) \times \text{Mass (in kilograms)} \times Constant}{\text{Serum Creatinine (in } \mu\text{mol/L)}}$$

Where *Constant* is *1.23* for men and *1.04* for women.

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