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Nhiwatiwa, Melody

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COMPARISON OF DRUG- INDUCED HEPATO-TOXICITY IN FEMALE PATIENTS DURING ANTI-RETROVIRAL THERAPY

Melody Nhiwatiwa BPharm (UNIN), DCR (Upper Wimpole College of Radiography, London)

Student Number: 0707639H

Year started: 2007

Department of Pharmacy and Pharmacology

Supervisor: Prof Michael Danckwerts (PhD)

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of

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DECLARATION

I, Melody Nhiwatiwa, declare that this research is my own work. It is being submitted for the degree of Master of Science in Medicine in the branch of Pharmacotherapy, at the University of the Witwatersrand, Johannesburg.

It was not submitted before for any degree or examination at this or other any university.

Melody Nhiwatiwa

8th Day of September 2011

CHAPTER 1

ABSTRACT

Background: Long term antiretroviral therapy (ART) use is known to cause various toxic adverse effects in patients. Hepato-toxicity is one of the most significant adverse effects which have been associated with all antiretroviral therapy drugs in South Africa and worldwide.

The aim of the study was to make a comparison of drug –induced hepatotoxicity in HIV-infected adults on first line antiretroviral therapy (efavirenz based ART regime and nevirapine based regime) in a public health setting.

The objective was to determine the changes in the blood alanine aminotransferase (ALT) levels in patients on these first line ART regimes during the first six months of treatment as well as the effects of pregnancy BMI and age.

The study was carried out at an ART clinic at Bongani Regional hospital in Free State province of South Africa. The target population was adult female patients of ages between 18 to 45, who were on the 2 first line ART regimes for six months or longer. Participants were chosen as they presented to the ART clinic.

Results: Pre-treatment ALT levels were comparable between the two groups with a mean of 29.27IU/L (±21.56) in patients on regime 1A 22.57IU/L (±24.83) for patients on regime 1B.Most patients had normal pre-treatment ALT levels of 6-40IU/L (80% of those in group 1A and 94% of those 1B).

Significant changes in levels and distribution of ALT levels were documented after 6months of ART use. Patients on regime 1B demonstrated a significant rise in ALT levels (mean 13.76IU/L ±27.83) which was significantly higher than the rise in patients on regime 1A (mean 4.73IU/L ±27.23), (two sample t test, p=0.05).Most cases of ALT elevation were in the mild hepatotoxicity range of 41-80IU/L.

Factors taken into consideration that may have influenced results are pregnancy, alcohol intake, and age and body mass index. The mean increase in ALT levels for the two regimes were not compared by statistical testing due to the sample size of only one patient on regime 1A whose ALT level increased by75.00 IU/L. For the eight patients on regime 1B who had pregnancies the ALT change was a mean of 20.25IU/L (±28.40) which was not statistically significant (paired t test, p=0.084). The percentages of patients who were not pregnant in group1A (98.2%) and group 1B (92.5%) did not differ significantly (Fisher exact test, p=0.169). The patients who fell pregnant are 1.8% and 7.5% in 1A and 1B respectively did not differ significantly. The proportion of patients who consumed alcohol did not differ significantly in group 1A (3.6%) and 1B (4.7%) (Fisher exact test, p=1,000).The average decrease in ALT Levels for patients consuming alcohol

in the two regimes 1A and 1B (-25.50and -6.40respectively) did not differ significantly (two-sample t test, p=0.600). Significant differences of the ages of patients between the two groups were demonstrated. The mean age for patients on Regime 1A was 40.89(±3.60) years and for patients on Regime 1B mean age was 33.53(±5.44) years. This difference was statistically significant, (two- sample t test, p<0.001). Although patients on regime 1B were significantly younger than those on 1A, there was no significant correlation between age and ALT change (1A r=-00156 with p=0.910) and1B (r=-0.0883 with p=0.366) hence the age difference did not have an influence on ALT levels and changes. No significant differences in BMI values were demonstrated between the two groups of patients; mean BMI for regime 1A was 26.56 and for Regime 1B 25.20, (two-sample t-test p=0.200).There was no significant correlation between BMI and ALT change(1A r=-0.010 with p=0.941) and 1B(r=0.111 with p=0.257).Regression analysis showed that for both regime 1A and 1B, age as well as BMI, were not statistically significant predictors for the change in ALT level.

Conclusion:

The study demonstrates by using blood ALT levels that hepatotoxicity occurs more in patients on regime 1B (nevirapine based) than in patients on regime 1A (efavirenz based).No other factor was shown to play a role.

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My family members for their support;

ABBREVIATIONS

(1) ART -antiretroviral therapy.

(2) ARV – antiretroviral.

(3) BMI- body mass index.

(4) ALT- alanine aminotransferase.

(5) NNRTI- non nucleoside reverse transcriptase inhibitor.

(6) ARVLI- antiretroviral liver injury.

(7) bid- twice a day.

(8) Nocte- at night.

(9) NVP- nevirapine.

(10) ULN- Upper Limit of normal.

(11) DNA- deoxyribose nucleic acid.

(12) HIV-human immune deficiency virus.

(13) AST-Aspartate Aminotransferase.

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1.1 INTRODUCTION

About 583 000 adults and 69 000 children were estimated to receive antiretroviral therapy (ART) in the public health sector of South Africa by December 2008. There were more than 216 treatment sites nationwide making it the largest state –driven ART programme in the world. There was a 53% increase in the number of patients on ART between 2007 and 2008[1].

The national programme has expanded access to ART in the South African health sector which has resulted in thousands of South Africans being subjected to prolonged therapy with the risk of adverse drug effects. Among the common adverse effects is liver injury. The South African national guidelines offer an option of two first line ART regimens i.e. an efavirenz-based 1A and a nevirapine-based 1B both of which are potentially hepatotoxic. The national guidelines recommend regular blood Alanine Aminotransferase (ALT) assays as a means of monitoring for hepatotoxicity[2]. It is essential that a comparison of hepatotoxicity of the two regimens be made to help clinicians make the safer choice to apply in clinical practice.

Both regimes have a backbone of NRTI's consisting of lamivudine 150mg bid and stavudine 30mg bid such that the relative differences of hepatotoxicity will reflect the difference in hepatotoxicity between nevirapine and efavirenz.

Though many studies on hepatotoxicity using ALT assays have been conducted worldwide and in South Africa, there has been no such research work on the local population of Northern Free State in South Africa. The researcher has also made observation of many more prescription changes on patients on nevirapine due to hepatotoxicity. This observation is common in the first year of ART.

1.2 AIMS & OBJECTIVES

1.2.1 The aim of the study was to make a comparison of drug –induced hepatotoxicity which occurs in HIV-infected adults on the first line antiretroviral therapy (1A efavirenz based ART regimen and1B nevirapine based regimen) in a public health setting.

The main objective was to determine rise in blood alanine aminotransferase (ALT) levels and to make a comparison between patients on regimen 1A with patients on regimen 1B during the first six months of ART. The secondary objectives were to attempt to relate ALT rise to age, pregnancy, alcohol intake and BMI.

1.2.2 The study aims to answer the following questions;

(1) How does the magnitude of the rise in ALT in patients on regimen 1A compare with that in patients on regimen 1B?

(2) How do the pre-treatment ALT levels of patients on 1A compare with patients on 1B?

(3) What is the ALT level distribution pattern in patients on 2 regimens at the beginning of therapy?

(4) What is the ALT level distribution in patients on the 2 regimens at 6 months of therapy?

(5) Does the occurrence of pregnancy have an influence on the change in ALT levels between the 2 regimens?

(6) Does age have an influence on the extent of change in ALT levels between the 2 regimens?

(7) Does alcohol intake have an influence on the magnitude of change in ALT levels between patients in the 2 regimens?

(8) Does BMI have an effect on the magnitude of change in ALT levels in patients Between the 2 treatment groups?

1.3 METHOD

1.3.1 Setting;

The study was conducted at an ART clinic at a secondary level hospital (non teaching) in Welkom in the Free State province. The clinic forms part of the provincial ARV centers serving the municipalities of Matjhabeng, Virginia, Odendaalsrus, Bothaville, Theunissen and Wesselsbron with a total population of over 800 000. The main ART protocol is based on the national department of health's comprehensive care for HIV strategy which was introduced in 2003. About 11000 adult patients are receiving ART in the clinic. The ART clinic is a referral center for a group of primary care level clinics in the residential areas which are Matjhabeng clinic, Welkom city clinic, Theunissen clinic and Phomolong clinic.

The clinic's laboratory services are provided by the National Health Laboratory Services which is contracted to the Department of Health. Routine screening for hepatotoxicity forms part of the monitoring and evaluation process. ALT blood assays are part of the selected biochemical tests conducted.

1.3.2 Study design;

The study was designed as a comparative, retrospective analysis of changes in ALT values during the first six months of exposure to efavirenz based and nevirapine based ART.

1.3.3 Target Population;

The target study population was adult female patients aged 18-45 years in the first six months on regime 1A and regime 1B.

1.3.4 Drug composition;

Table 1: Drug composition of ARV regimens.

Regimen	**Drug**	**Strength**	**Dose**
1A	Stavudine	30mg	1 bid
	Lamivudine	150mg	1 bid
	Efavirenz	600mg	1 nocte
1B	Stavudine	30mg	1 bid
	Lamivudine	150mg	1 bid
	Nevirapine	200mg	1 bid

1.3.5Patient Sample selection;

The patient sample was selected from the target population as they presented themselves to Bongani Hospital's ARV clinic for the routine follow up.

1.3.6 Exclusion criteria;

Patients were excluded if their age was less than 18 or above 45years at the beginning of ART or if the following conditions were identified; viral hepatitis, blood ALT levels altering conditions such as diabetes mellitus and current use of medication which is known to cause hepatic biochemical changes; e.g. cimetidine, cocaine, methyldopa, carbimazole, ketoconazole, erythromycin salts, antiepileptic drugs, anti tuberculosis, diclofenac, aspirin and amiodarone.

1.3.7 Informed consent;

The first stage involved the researcher giving a verbal explanation to the patients. The information provided a clear explanation of the purpose of the study, the implication of the results on drug use, the source of funding, assurance of confidentiality and patient's right to say yes or no and to withdraw without retribution or compromise of

care. (Appendix A: The translated consent forms into Sesotho, IsiXhosa and Afrikaans were available.) This was followed by handing out of documents with written information of the study and invitation to participate. The consent was filled in by the patient without any interference from the researcher or the clinic nurse.

The researcher and one clinic nurse were responsible for obtaining consent from the participants. (Appendix B)

1.3.8 Questionnaire;

Consenting patients were given questionnaires in the language of their choice. The questionnaires were written in Sesotho, IsiXhosa, English and Afrikaans to cover language choices for the population of patients (appendix C). The researcher wrote the English version of the questionnaire which was then translated into 3 other languages with the assistance of a clinic nurse. Accuracy of comprehension of the questions was verified by trial questionnaires on some patients before the consenting patients answered the questions.

Question 1 Were you pregnant at any time during the first six months of starting your ART treatment? Y/N

Question 2 Do you drink alcohol? Y/N

Respondents were required to indicate their responses by encircling Y or N.

1.3.9 Measurements

Anthropometric measurements were performed by the researcher and the assisting clinic nurse. Patients' heights were measured using a Detecto physician's height measure (ProMed®, 1510 Gunbarrel Rd, #700, Chattanoga, TN 37421) with patients standing upright, bare foot and head positioned in the Frankfort horizontal plane. The body weights were measured using a Detecto physician's scale (entered in kilograms, estimated accuracy of 85.1% under standard environmental conditions).The patients were weighed without shoes and only wearing light clothing. Weights were recorded to nearest 0.1kg. BMI were calculated using the equation below.

$$BMI = \frac{weight\ (kg)}{height\ (m^2)}$$ Equation 1

The same equipment was used on all patients by the researcher and assistant clinic nurse.

1.3.10 Clinical and laboratory records;

The patients' clinical records were reviewed for information relating to age, gender, type of ART regimen and ALT levels at baseline and at

six months duration of treatment. Escalation in ALT levels is denoted as **+** and decline as **–.**

The Free State division of the National Health Laboratory Services, which is contracted to the hospital, measured the ALT blood levels.

The Abbot Diagnostic Architect Ci8200 integrated processor was used to process the specimens. The processor is a product of Abbott Laboratories Company. (100 Abbott Park Road, Abbott Park, Illinois 60064-5500, USA.) During the Ci8200 ALT assay procedure the ALT catalyses the reversible transamination of L alanine and α – ketoglutarate to pyruvate and glutamate.

Each patient's information was entered into the data capturing sheet a shown in appendix D. All information was entered onto the Microsoft Excel® spreadsheet.

1.3.11 Statistical method;

The Excel data sheet contained the following characteristics; the hospital file number, age in years, weight in kilograms, height in metres, alcohol intake, pregnancy pre-treatment ALT value, ALT value at six months and ALT change value. All the statistical analyses were performed on SAS, release 9, 1, 3 which was run under Microsoft Windows Vista Business for a personal computer. The data was both discreet (for age and duration) and continuous numerical (ALT levels and BMI).The main statistical measurements were mean and p-values using the Fisher exact test and the student t-tests. Correlations of variables were done by the Pearson's correlation coefficient. For statistical significance the following were used; paired t test, two-sample t test and Fischer exact test.

CHAPTER 2

LITERATURE REVIEW

2.1 BACKGROUND INFORMATION

The AIDS epidemic remains one of the greatest contributors to morbidity and mortality in the sub-Saharan African population. However, the advent of HAART has brought about improvement in survival rates and quality of lives in HIV infected patients in Southern Africa[3].

The goal of antiretroviral therapy is to restore host T-cell function by sustained maximum suppression of Human Immunodeficiency Virus (HIV) replication in infected patients[4].

Antiretroviral drug-related liver injury (ARLI) is a common cause of morbidity, mortality and can lead to discontinuation of treatment by patients[5].Prevention and management of ARLI has emerged as an important requirement for successful ART. Virtually every licensed antiretroviral medication has been associated with some degree of hepatotoxicity[6].

In the Viramune Hepatic Safety Project, Stern et al demonstrated that risk factors for asymptomatic ALT/AST elevation were elevated baseline

ALT/AST levels>2.5xUpper Limit of the Normal(ULN) as well as co-infection with hepatitis B and hepatitis C. Pre-existing viral hepatitis may predispose patients to ARLI[7, 8]. The NNRTI groups of drugs, particularly nevirapine and efavirenz have been noted to have a high incidence of drug induced hepatotoxicity. The NNRTI are a class of antiretroviral drugs which act as non-competitive inhibitors of Human Immunodeficiency Virus reverse transcriptase (HIV RT) [8].Their action disrupts HIV RT catalytic sites thereby blocking polymerase activity and preventing cDNA synthesis from the viral RNA genome[8].

Most ART programmes in sub-Sahara Africa are promoting the use of fixed dose first-line antiretroviral regimens containing stavudine, lamivudine with nevirapine or efavirenz[9].Although this is an effective combination, it carries the potential problem of nevirapine-induced hepatotoxicity[10].

2.2 Drug- induced hepatotoxicity;

Hepatotoxicity is one of the most relevant adverse effects of all antiretroviral therapy (ART).Possible pathogenic mechanisms are; direct drug toxicity, immune reconstitution in the presence of hepatitis B virus and or hepatitis c virus co-infections, hypersensitivity with liver involvement and mitochondrial toxicity [11]. Other pathogenic pathways involve induction of insulin resistance by some antiretroviral drugs which may progress to the development of steato- hepatitis. The approach to the management of liver toxicity depends on the clinical presentation of the

condition, the severity and possible pathogenic mechanisms. Severe cases require immediate stoppage of all ARV's [11, 12].

The drug nevirapine is metabolized by the hepatic cytochrome P450 enzyme system. Population pharmacokinetic studies have shown that indigenous populations in Thailand and South Africa have shown lower clearance rates of nevirapine than patients in the western countries[13]. This may lead to greater drug exposure in the former and may account in part for high rates of nevirapine induced hepatotoxicity particularly among women with lower body mass index as reported in a South African study[14].

However many studies which were reported in the early 2000s found no significant difference in the rates of grade 3 and 4 transaminase elevations in individuals receiving nevirapine compared to those receiving efavirenz – based regimes [15,16] . More recent studies using laboratory monitoring have shown higher prevalence of hepatotoxicity in patients on nevirapine (2-18%).Of these, only 2% presented with clinical hepatitis[17].

Studies using biochemical assays of blood ALT have demonstrated biochemical abnormalities in asymptomatic patients receiving nevirapine containing regimens [18]. Available data on the exact incidence of drug-induced liver disease are contradictory. This may be due to the failure to exclude other causes of increased liver enzymes (ILE), such as alcohol and other drugs like cocaine, aspirin and anti tuberculosis drugs. The syndrome of immune recovery may also play a part [18, 19].

Most clinical trials define severe liver toxicity (grade 3 and 4) as greater than fivefold increase in levels of alanine aminotransferase above the (ULN). ALT and AST are two aminotransferase used in the diagnosis of liver diseases.

Although many tissues produce these enzymes, the liver is the predominant source of ALT which makes the assays more specific for the liver diseases[20].A review of post marketing reports for nevirapine and efavirenz reveals two emerging patterns of toxicity; direct and hypersensitivity[20].

The nevirapine label and package insert has been revised several times to include more information on liver toxicity. Recent changes to the indication and usage of nevirapine label includes relative contraindication to use of the drug in women with CD4+ cell counts greater than 250cells/mm^3 unless perceived benefits clearly outweigh risk of hypersensitive hepatotoxicity[20].

Although symptomatic and asymptomatic liver toxicity (raised liver enzymes without clinical features) are observed with many ARV's, symptomatic liver toxicity is more common with nevirapine use compared to other antiretroviral drugs[21].

The Viramune Hepatic Safety Project found that 10% of the patients developed greater than fivefold ALT/AST elevations: the majority cases were asymptomatic [22, 10].

The Virgo study with a 36 –month follow up time, showed a good long term toxicity profile, while a small Swedish study did not find nevirapine-related side effects in 26 patients treated for a median duration of 31 months (range 8-40)[23].

The South African public health ART programme has adopted ALT assays as a routine testing for liver disease. My own observation in our population of patients is that prescription changes on the basis of hepatotoxicity are far more common in nevirapine regime than efavirenz, suggesting higher prevalence of adverse events in the patients on nevirapine.

2.3 Alanine Aminotransferase enzyme (ALT);

Chapter one introduced the topic of my research and therefore this chapter will briefly review the alanine transaminase enzyme as a biochemical marker used in this research project.

ALT is a transaminase enzyme which is found mainly in the liver but also in small amounts in the kidneys, heart, muscles and pancreas. The enzyme was formerly called serum glutamic pyruvic transaminase (SGPT). Low levels of ALT are normally found in the blood but in the presence of hepatic injury, liver tissue releases ALT into the bloodstream with consequent elevation of blood ALT levels[24].

ALT catalyses the reaction in which an amino group is transferred from alanine to α-ketoglutarate. The product of this reversible transamination reaction is pyruvate and glutamate[24].

Despite the association between greatly elevated ALT levels and hepato-cellular disease, the absolute height of ALT elevation does not correlate with the extent of liver cell damage. Accordingly, the absolute ALT elevation is of little prognostic value[25].

There are many other factors which cause ALT elevation. Antimicrobial agents were reported as the etiology in 45.5% of the patients and were the leading cause of drug induced liver injury in a recent prospective study in US as in most other countries[26].

A study from Spain showed that several antimicrobial agents including isoniazid, pyrazinamide, rifampicin, amoxicillin with clauvulanic acid, erythromycin, doxcycline, nitrofurantoin, ciprofloxacin, were among the most common agents associated with acute liver injury attributable to drugs[27, 28].

Anti-rheumatic agents are among commonly used drugs associated with hepatotoxic effects ranging from acute drug induced liver injury (DILI) to chronic drug –associated liver disease and drug induce autoimmune hepatitis. According to the results of a randomized study report by Paul B Watkins MD from the University of North Carolina, Chapel Hill,

acetaminophen (also known as paracetamol) at recommended doses can cause elevated levels of ALT. In the United States, a study on acute liver failure (ALF) demonstrated that unintentional acetaminophen use accounted for 48% of cases whereas 44% cases were due to intentional uses and in 8% of cases the intention was unknown. Studies on diclofenac hepatotoxicity have demonstrated that pathogenesis of acute idiosyncratic DILI is a multistep process involving interaction between metabolic and immunologic factors. Causes of drug-induced hepatotoxicity have been reported within the first month of treatment with diclofenac but can occur at anytime during treatment[28, 29, and 30].

Elevation of ALT is not exclusive to liver pathology. Common medical conditions such as heart disease and thyroid disease can cause liver transaminase elevations[31]. Genetic influences on the level of ALT are possible. A study of Danish twins showed that genetic factors accounted for 33 to 66 percent of the variation in ALT, gamma glutamyl transpeptidase, LHD and birilubin in patients 73 to 94 years of age[31].

ALT rise is the parameter chosen by the researcher, to determine and compare the frequency of hepatotoxicity in patients on efavirenz based (1A) and nevirapine based (1B) antiretroviral regimes. In this study hepatotoxicity was determined by measuring the ALT rise above the upper limit of the normal and were graded to determine the extent of rise as follows;

Class interval 1= 0-40IU/L, Class interval 2= 41-80 IU/L

Class interval 3= 81-120 IU/L, Class interval 4= 121-160 IU/L

Class interval 5=161-200 IU/L and Class interval 6= 201-249 IU/L

CHAPTER 3

RESULTS AND DISCUSSION OF RESULTS

3.1 The ALT rise between groups 1A and 1B;

The second chapter discussed the background information and literature review. In this section the results and data from this study are presented and interpreted. This section also illustrates the relevance of the elevation of ALT in relation to ARV induced hepatotoxicity.

Over a period of 8 weeks, 162 female patients were enrolled onto the study, 55 were on regimen 1A and 107 on regimen 1B.The changes in blood ALT levels of each patient was calculated by subtracting the baseline ALT value from the ALT value at 6 months of ART use.

The ALT results of all patients were then used to calculate the mean ALT change in each treatment regimen group.

For patients on regime 1B, statistical analysis showed a significant elevation of ALT levels as shown by mean ALT rise of +13.76IU/L (±27.83) following 6 months treatment (paired t test, $p<0.001$).

For patients on regime 1A statistical analysis did not show significant elevation of the ALT levels following 6 months of regimen 1A use. The mean ALT rise in 1A was +4.73IU/L (±27.23) (paired t test p=0.203) which was not statistically significant.

Therefore there is a significantly higher rise in ALT levels in patients on regime 1B (mean 13.76IU/L) compared to patients on regimen 1A (mean 4.73IU/L) (two-sample t test, p=0.05).

This is shown in the table 2 and figure 1.

Table 2. Comparison of magnitude of ALT changes between 1A and 1B treatment groups.

	Regimen 1A(n=55)	**Regimen1B(n=107)**
Minimum	-67	-98
Maximum	82	144
Mean change in ALT(0-6months)	+4.73	+13.76
Standard Deviation	+27.23	+27.83

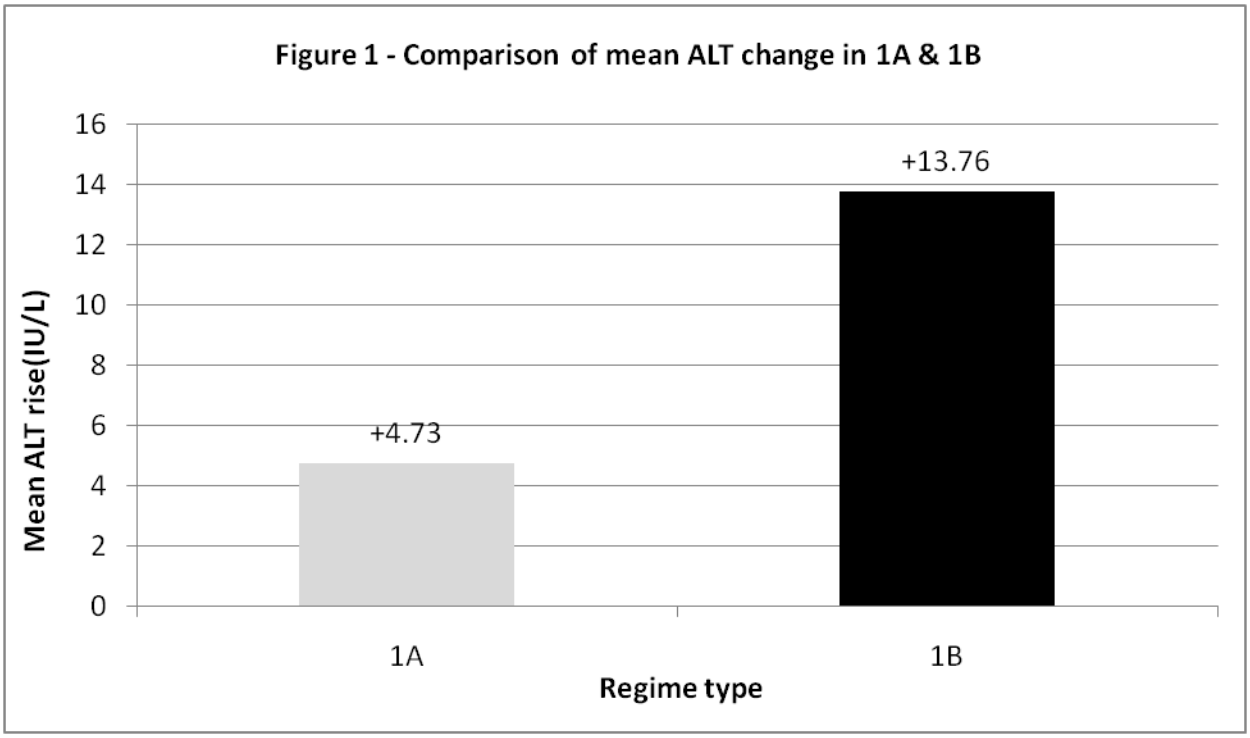


Figure 1: Comparison of mean ALT change between regimen 1A and 1B

The results show that by using blood ALT assays, significant but mild drug-induced hepatotoxicity occur in patients on regimen 1B within 6 months of treatment mean rise +13.76IU/L (±27.23) (paired t test $p<0.001$).Regime 1A does not show significant hepatotoxicity within the same period mean rise +4.73i.IU/L (±27.83)(paired t test $p=0.203$). The drug compositions of the treatment regimen differ regarding efavirenz in 1A and nevirapine in 1B. A deduction is made that the differences in ALT elevations indicate a higher hepatotoxicity due to nevirapine as compared to efavirenz.

The patients in group 1B showed a significant elevation of ALT values. The ALT rise was statistically significant as compared to the patients in group 1A where there ALT rise was not significant.

In addition to higher ALT values at 6 months of ART, a greater proportion of patients in 1B demonstrated elevation of ALT as shown in the figure 4.

The results are consistent with the study done by Roberto Manifred in which hepatotoxicity characterized by an at least 2 fold increase of serum transaminase occurred significantly in the nevirapine vs. efavirenz group throughout 18 months study period[33].

3.2 Comparison of drop in ALT

Drop in ALT values was defined as an ALT value at 6 months that is lower than the pre-treatment value. 43.6% of patients on regimen 1A demonstrated a drop in ALT's compared to 21.4% in 1B. This is shown in table 3.

Table 3. Comparison of the ALT drop between regimen 1A and1B.

Range of ALT Fall(IU/L)	% of patients with negative ALT change	
	1A	**1B**
0-40	41.8	19.6
41-81	1.8	0.9
82-122	0	0.9
123-163	0	0
Total	43.6	21.4

Comparison of the proportions of patients with negative ALT changes between the treatment groups.

Table 3 demonstrates that regimen 1A had more patients whose ALT levels decreased after commencing ART. Pretreatment values are influenced by many other factors like other drugs which are then stopped at the commencement of ARVs.

3.3 Pre-treatment ALT

All baseline ALT values in each group were analysed to calculate the mean, the standard deviation and the median for both groups. A comparison of ALT levels before treatment between 1A and 1B showed no significant difference, mean ALT 1A 29.56 IU/L (±21.56) and 1B 22.57 IU/L (±24.83) P>0.001(two-sample t-test) as shown in table 4 and figure 2;

Table 4. Comparison of pre-treatment ALT levels between the 1A and 1B groups

	Regimen 1A(n=55)	**Regimen 1B(n=107)**
Mean	29.27	22.57
Standard deviation	21.56	24.83
Median	23.00	18
Minimum	6	6
Maximum	135	245

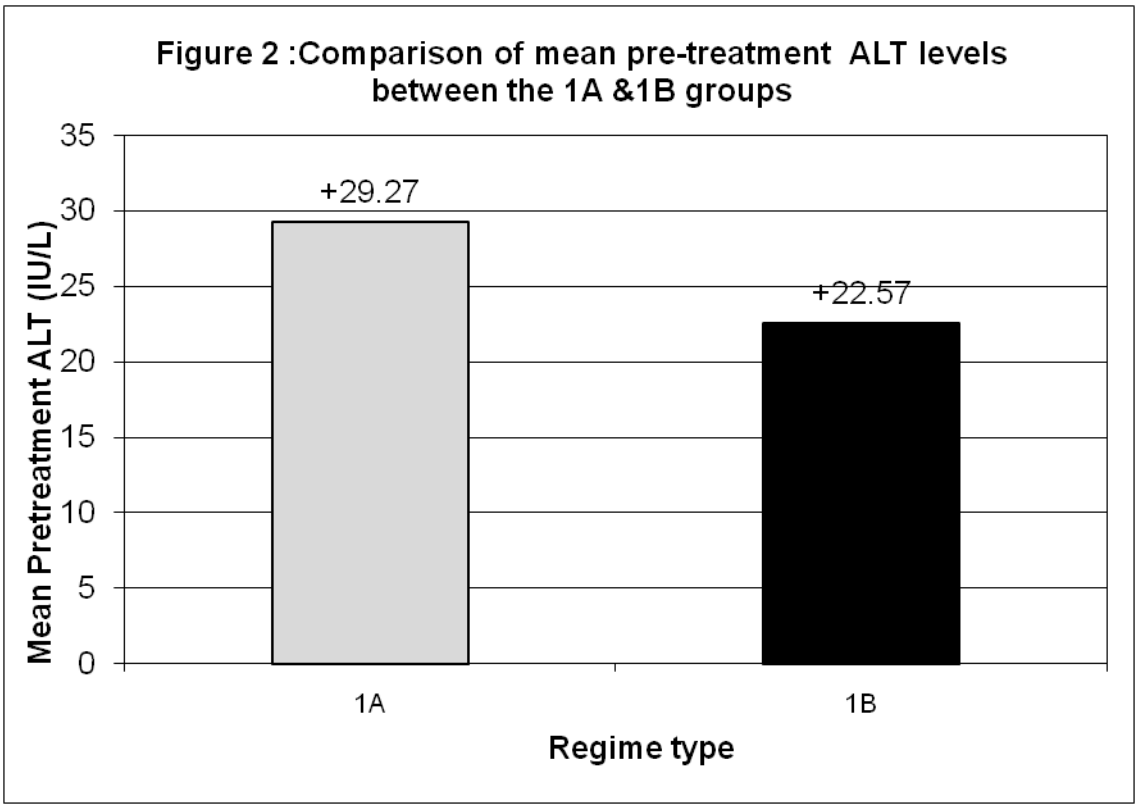


Figure 2: Comparison of mean ALT levels at baseline between the treatment groups.

Figure 2 shows higher mean pre-treatment ALT in 1A 29.27 IU/L (±21.56) than in 1B 22.57IU/L (±24.83). The difference is not statistically significant (p>0.001).The Pre-treatment ALT levels did not influence the ALT values at 6 months. This implies that the criterion of selection of treatment regimes does not introduce bias in the assessment of hepatotoxicity.

However extreme pretreatment ALT values were recorded in regimen 1B with maximum values of 245 IU/L compared to 135 IU/L in 1A. Genetic difference may play a role in some cases of ALT elevation in some patients. Extreme high baseline values can be as a result of patients being on other ALT elevating drugs e.g. antimicrobials and some non steroidal anti-inflammatory drugs.

3.3.1 Distribution of pre-treatment ALT values

The pre-treatment ALT values in each group were grouped into 6 class intervals and a comparison made as shown in table 5;

Table 5: Comparison of the distributions of Pre-treatment ALT values between patients on 1A and 1B regimens

Pre-treatment ALT values(IU/L)	**Number of Patients (%)**		
	1A	**1B**	**Total**
16-40	44(80)	101(94.4)	145(89.5)
41-80	9(16.4)	4(3.7)	13(8.0)
81-120	1(1.8)	1(0.9)	2(1.2)
121-160	1(1.8)	0(0)	1(0.6)
161-200	0	0(0)	0(0)
201-249	0	1(0.9)	1(0.6)
Total	**55(100)**	**107(100)**	**162(100)**

Table 5 and figure 3 shows that there are significantly higher number of patients with mild ALT elevation (41-80 IU/L) in regimen 1A (16.4%) as compared to regimen 1B (3.7%).The difference is statistically significant with p=0.011(Fisher exact test).

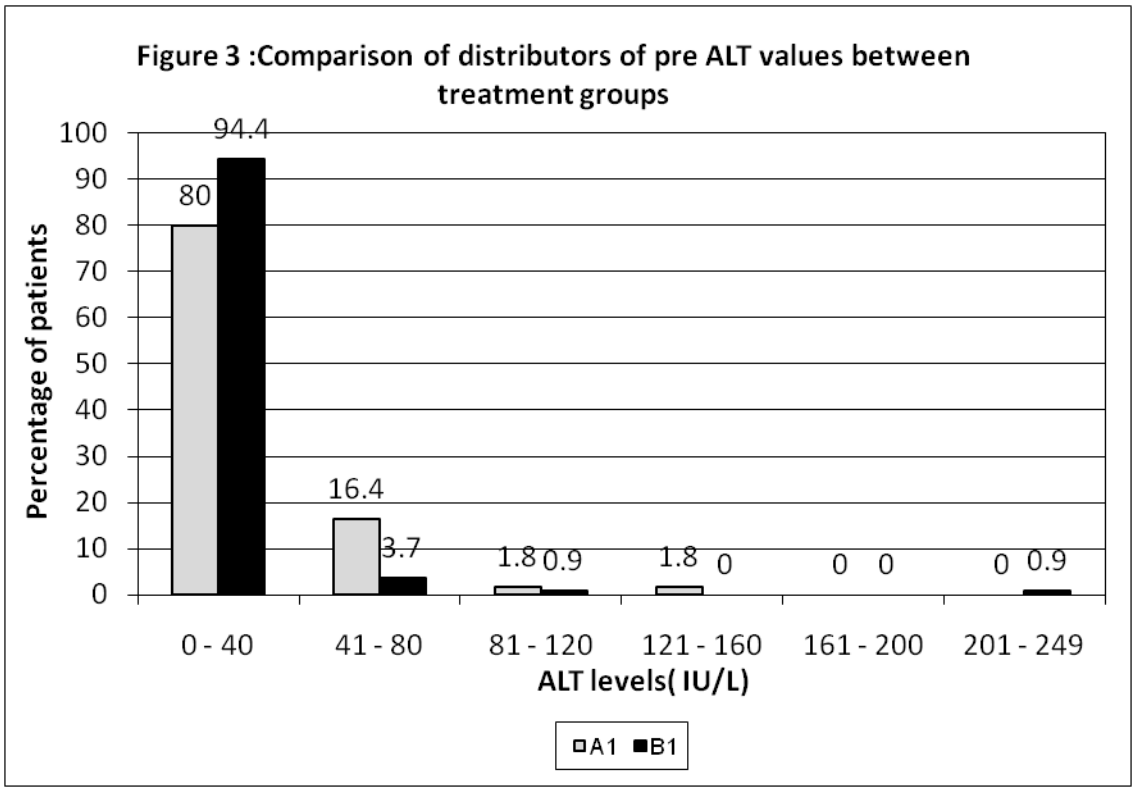


Figure 3: Distribution of pre-treatment ALT values between treatment groups.

Figure 3 above shows the percentage distribution of patients across the ALT group levels. The results indicate that most patients had normal baseline ALT values (1A 80%, 1B 94.4%) reflecting the possible absence of pre existing liver disease.

Overall, most baseline ALT values were normal with no significant differences between the 2 groups. However in the specific ALT range of 41-80 IU/L (mild hepatotoxicity) group 1A patients were proportionately higher. However, this difference does not affect the ALT levels at 6months.

3.4 ALT values at 6 months of ART

All the ALT values after 6 months were grouped into 6 categories (class intervals) to show the distribution of patients according to the ALT value as shown in table 6 and figure 4. The range of ALT values was 8-152IU/L. 76.4 % of patients in group1A and 73.8% of 1B fell in the first class interval (8-40) IU/L. In class interval (41-80) were 16.3% of 1A and 18.7% of group 1B patients. In the class interval (81-120) were 5.5% and 5.6% of patients in 1A and 1B respectively.

Table 6: ***Distributions of ALT levels in patients in both regimens at 6 months.***

ALT Values at 6 months ART(IU/L)	**Number of Patients (%)**		
	1A	**1B**	**Total**
6-40	42(76.4)	79(73.8)	121(74.7)
41-80	9(16.3)	20(18.7)	29(17.1)
81-120	3(5.5)	6(5.6)	9(5.6)
121-160	1(1.8)	2(1.9)	3(1.8)
161-200	0	0	0
201-244	0	0	0
Total	55(100)	107(100)	162(100)

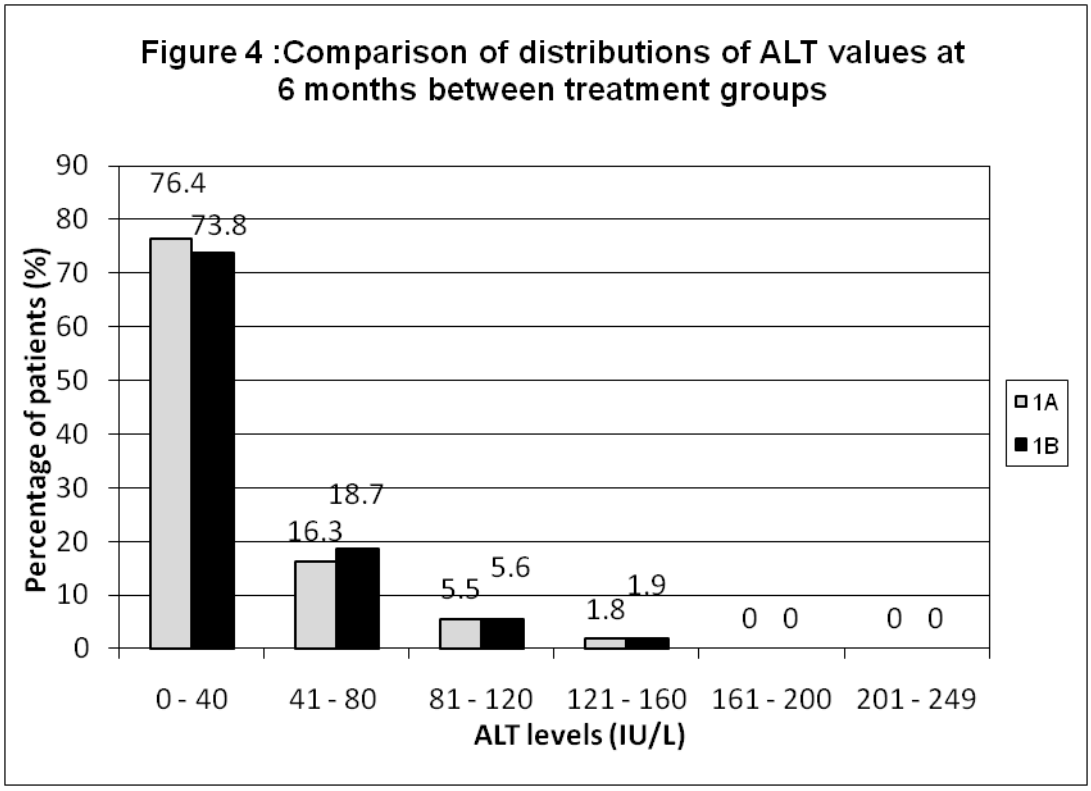


Figure 4: Percentage distribution pattern of ALT levels at 6 months in patients in 1A and 1B

The greatest increase in the number of patients with hepatotoxicity is observed in the class interval 41-80IU/L which represents mild hepatotoxicity. In group 1A there is a decrease from 16.4 to 16.3%. In group 1B there is an increase from 3.7 to 18.7%.

The implication is that even though more patients in 1B developed hepatotoxicity this is only pronounced in the mild range of hepatotoxicity (41-80IU/L).

There is also a lesser increase in the range 81-120 where 1A increased from 1.8 (figure 4) to 5.5% (figure 5) and in group 1Bfrom 0.9to 5.6%.

The patients in group 1B showed a significant elevation of ALT values. The ALT rise was statistically significant as compared to the patients in group 1A where ALT rise was not significant.

In addition to higher ALT values at 6 months of ART, a greater proportion of patients in 1B demonstrated elevation of ALT as shown in figure 4.

The results are consistent with the study done by Roberto Manifred in which a hepatotoxicity characterized by an at least 2 fold increase of serum tansaminase occurred significantly in the nevirapine vs. efavirenz group throughout 18 months study period[33].

Number of patients with increased serum liver enzymes dropped in the efavirenz group from 93 at baseline to 51 overall throughout the study period while it concurrently increased in the nevirapine treated patients from 80 cases observed at baseline to 134 patients during the 18 month observation ($p<0,0001$)[33].

A few cases had unexplained extreme ALT elevations. Genetic differences may play a role in such cases as demonstrated by Ciccacci *et al* in patients on nevirapine in Mozambique[34].

3.5 Effect of Pregnancy on ALT Change;

Only one patient on regimen 1A was pregnant and the ALT level increased by 75.00IU/L.

For the 8 patients on regimen 1B the ALT level increased on the average by 20.25IU/L (±28.40) which was not statistically significant (paired t test, p=0.084).

The mean increase in ALT levels for the two regimens were not compared by statistical testing due to the sample size of only one patient on regimen 1A.

Table 7: Pregnancy on ALT Change.

	Pregnancy = Yes		
	ALT Value		
	Pre	Post	Change
Regimen 1A(n=1)			
Mean	13.00	88.00	75.00
Std Dev	0	0	0
Regimen 1B(n=8)			
Mean	14.00	34.25	20.25
Std Dev	25.67	25.66	28.40

For non pregnant patients on regimen 1A the ALT levels increased on average by 3.43 IU/L (±25.70) which was statistically not significant (paired t test, p=0.332).

For patients on regimen 1B the ALT level increased on average by 13.23IU/L (±27.87). Which was statistically significant (paired t test, p=<0.001).

The average increase in ALT levels for the two regimens (3.43 and 13.23IU/L respectively) differ significantly (two-sample t test, p=0.034). See table 8 below.

Table 8: Non pregnancy and ALT change:

	Pregnancy = No		
	ALT Value		
	Pre	Post	Change
Regimen 1A(n=54)			
Mean	29.57	33.00	3.43
Std Dev	21.65	24.33	25.70
Regimen 1B(n=99)			
Mean	23.26	36.49	13.23
Std Dev	25.67	25.66	27.87

Nine patients were pregnant during the first 6 months of treatment. 1 patient from group 1A and 8 patients from the group 1B.The higher pregnancy rate in 1B may be explained by the younger ages in the group.

The percentages of the patients who were not pregnant in the two groups (98.2% and 92.5% respectively) do not differ significantly. (Fisher exact test, p=0.169) See the table 9.

The same conclusion holds for the complementary percentages of pregnant patients, i.e. the 1.8% and the 7.5% in1A and 1B respectively also do not differ significantly. (Fisher exact test, p=0.169).See table 9 below.

Table 9:Comparison of frequencies of pregnancies between the 2 groups.

Pregnant	**Regimen**	
	1A	**1B**
No	54(98.2%)	99(92.5%)
Yes	1(1.8%)	8(7.5%)
Total	**55(100%)**	**107(100%)**

The frequency of pregnancies in the 2 groups is low which reflects the effectiveness of the pre-treatment screening procedure. The higher pregnancies rate in patients in group 1B can be attributed to the

younger ages in that group of patients (see table 9).The percentages of 1.8% and 7.5% in 1A and 1B respectively do not differ significantly (Fischer exact test, p=0.169).

The same conclusion holds for the complementary percentages of patients who were not pregnant in the two groups (98.2% and 92.5% in 1A and 1B respectively. No significant difference (Fischer exact test, p=0.169)

The incidence of pregnancy in the study was very low. There were a total of nine pregnancies in the both regimens. Pregnancy state had no demonstrable influence on the ALT changes in this sample of patients. However, Daryl Schiller showed that women with CD4+ T-cell count of>250 cells/ml including pregnant women receiving long term antiretroviral therapy are at a considerably higher risk of developing hepatotoxicity than other women[35].Grade 3 ALT/AST elevations were demonstrated in only 1.5% of pregnant women on nevirapine in a study by Kondo *et al*[36].

Therefore, pregnancy rate is not an important factor in influencing the difference in ALT rise in these groups.

3.6 Effect of Alcohol use on ALT Change.

Alcohol consumption is low in both groups and it has no influence on ALT levels.

For the 2 patients on regimen 1A who consumed alcohol the ALT level decreased on average by 25.50 IU/L (±58.69) which was not statistically significant (paired t test, p=0.649).

For the 5 patients on regimen 1B who consumed alcohol the ALT level decreased on average by 6.40 IU/L (±34.99) which was not statistically significant (paired t test, p=0.704).

The average decrease in ALT levels for the two regimens (-25.50 and -6.40 respectively) do not differ significantly (two –sample t test, p=0.600).See Table 10.

Table 10: ALT changes in patients who drink alcohol:

	Alcohol = Yes		
	ALT Value		
	Pre	Post	Change
Regimen 1A(n=2)			
Mean	74.50	49.00	-25.50
Std Dev	85.56	26.87	58.69
Regimen 1B(n=5)			
Mean	29.80	23.40	-6.40
Std Dev	35.99	8.59	34.99

For the patients who do not drink alcohol on regimen 1A the ALT level increased on average by 5.87IU/L (±25.83) which was not statistically significant (paired t test, p=0.104).

For patients on regimen 1B the ALT level increased on the average by 14.75 IU/L (±27.26) which was statistically significant (paired t test, p=<0.001).

The average increases in ALT levels for the two regimens (5.87 and 14.75 respectively) do not differ significantly (two- sample t test, p=0.052) See Table 11.

Table 11:ALT Changes in patients who do not drink alcohol:

	Alcohol = No		
	ALT Value		
	Pre	Post	Change
Regimen 1A(n=53)			
Mean	27.57	33.43	5.87
Std Dev	16.14	25.26	25.83
Regimen 1B(n=104)			
Mean	22.22	36.96	14.75
Std dev	24.36	26.08	27.26

Only 2 patients in 1A reported alcohol use as compared to 5 patients in 1B. In group 1A 53(96.4%) patients did not use alcohol as compared to 102 (95.3%) patients in 1B.The proportions of patients who did not use alcohol in the two groups do not differ significantly (Fisher exact test, p=1. 000). The same conclusion holds for the complementary percentages of patients who use alcohol, i.e. the 3.6% and the 4.7% (Fisher exact test, p=1.000).See table 12 below.

Table 12:Theproportions of patients taking alcohol in each regimen.

Alcohol	**Regimen**	
	1A	**1B**
No	53(96.4%)	102(95.3%)
Yes	2(3.6%)	5(4.7%)
Total	**55(100%)**	**107(100%)**

Alcohol consumption was very low in each group making it an unlikely factor to influence ALT levels or comparative hepatotoxicity between the two treatment groups.

Fisher exact test also showed that the percentage of patients who used alcohol did not differ significantly, (p=1.000). Research reports in literature show that alcohol- induced liver disease can lead to higher nevirapine concentration (6.5µg/ml and lower c max (3.72µmol/L vs. 5.74µmol/L) for efavirenz[37].

3.7 The effect of Age on ALT Change.

The ages of all patients were first analysed per treatment group and then a comparison made between the 2 groups.Group1A range was 27 to 45 (18yrs) and group 1B 20 to 44 (24yrs) .The mean ages for Regimen 1A and Regimen 1B (40.89yrs and 33.53yrs respectively) differ significantly (two-sample t test, $p<0.001$). The patients on Regimen 1B were significantly younger than patients on Regimen 1A. See Table 13.

The criteria of regimen selection takes into account the potential teratogenicity of efavirenz and this tends to shift younger women with reproductive potential to regime 1B and older women to regimen 1A.

Table 13:Comparison of average age of patients in the 2 regimens.

	Regimen	
	1A(n=52)	**1B(n=107)**
Mean	40.89	33.53
Standard deviation	3.60	5.44
Median	42.00	34.00
Range	27 - 45	20 - 44

Following on the age differences, attempts to correlate age to ALT changes were made using the Pearson correlation factor.

For regimen 1A the correlation between age (mean value 40.89) and the change in ALT (mean value 4.73) is r= -0.0156 with p=0.910. Therefore there is no significant correlation.

For regimen 1B the correlation between age (mean value 33.53) and the change in ALT (mean value 13.76) is r= -0.0883 with p =0.366 as shown in the table 14 below.

Therefore there is no significant correlation. However, earlier work by Hernandez on 65 patients on HAART showed that an older age (>40yrs) and CD4 count of less than 310 cells/ml were significantly associated with ART –induced liver diseases[38].

Table 14: Correlation of Age and ALT Change.

Correlation Coefficients		
Change in ALT level and Age	**r**	**p**
Regimen 1A	-0.016	0.910
Regimen 1B	-0.088	0.366

3.8 Age distribution.

The frequency distribution of ages of all patients was demonstrated by classifying all ages into 6 class intervals as determined by the total number of group 1A and 1B patients which is 162(55+107).The age range was 20-45 years with intervals, number and percentages of patients as shown in table 15;

Table 15:Age distribution of patients between the 2 regimens.

Age	Number of Patients (%)		
	1A	**1B**	**Total**
20-23	0	5(4.7)	5(3.1)
24-28	1(1.8)	20(18.7)	21(13.0)
29-33	1(1.8)	25(23.4)	26(16.0)
34-38	8(14.6)	36(33.6)	44(27.2)
39-43	34(61.8)	20(18.7)	54(33.3)
44-45	11(20)	1(0.9)	12(7.4)
Total	55(100)	107(100)	162(100)

Table 15 demonstrates that 81.8% of patients on regimen 1A were aged above 38 years. 80.4% of patients on 1B were below 38 years.

Patients in group 1A were significantly older than patients in group 1B. However, attempts to correlate age to ALT values failed to show significant correlation in both groups. Hence age difference between the two treatment groups does not influence the ALT values at 6 months. In a study done by Vincent Soriano, age is listed as one of the risk factors associated with the antiretroviral liver injury[39].

3.9 Effect of BMI on ALT Change.

Each patient's BMI value was calculated using the formula weight divided by the square of height in meters (Equation 1, appendix E).The BMI values were used to calculate the mean, standard deviation and the median in each group as shown in table 16.

The mean BMI values for Regimen 1A and Regimen 1B (26.56 and 25.20 respectively) do not differ significantly (two-sample t test, p=0.200). The conclusion made is that the comparative differences in ALT rises between the two treatment groups are not influenced by the patients' BMIs'. See table 16.

Table 16:Comparison of BMI statistics in patients in the 2 regimens.

	Regimen	
	1A(n=52)	**1B(n=107)**
Mean	26.56	25.20
Standard deviation	6.69	5.16
Median	24.50	24.50
Range	14.68 – 50.60	15.01 - 42.17

* 3 missing values

BMI and ALT Change:

For both regimen 1A and 1B the change in ALT level did not correlate with BMI as shown in table 17 where correlation coefficients of regimen 1A and 1B is(r -0.010, 0.111 and p 0.910, 0.257)respectively .This study does not show correlation between BMI and ALT levels. This is in contrast to results of a study by Hadigan C in which he identified hepatic steaotosis(liver fat content> 5%) in 42% of his subjects and showed that there was a link between the BMI , liver fat content>5% and ALT levels[40].

This may be explained by generally low prevalence of obesity in the study population in the first 6 months of ART.

Comparison of mean BMI of the patients on nevirapine-based and efavirenz based regimens show no significant difference indicating that obesity is not playing a role in the prevalence of hepatotoxicity.

Table 17: Correlation between BMI on ALT Change.

Correlation Coefficients		
Change in ALT level and BMI	r	p
Regimen 1A	-0.010	0.941
Regimen 1B	0.111	0.257

3.10 Limitations.

The study design was such that an exhaustive exclusion of other causes of liver diseases in HIV patients such as hepatitis B and C was not practical. The study was limited by the range of tests which are done as part of the routine care of patients on ART in the public sector.

The study assumes patient compliance and adherence with ARVs. The ART programme has no reliable means of verifying compliance other than patient reports and pill counting.

One other limitation was that information on exposure to other drugs depended on patients' memory and recollection of use particularly drugs which could be purchased over the counter which are potentially hepatotoxic, for example diclofenac.

CHAPTER 4

CONCLUSION AND RECOMMENDATIONS

4.1 Conclusion.

In this study which used blood ALT levels as biochemical markers of liver injury, drug-induced hepato-toxicity was much more prevalent in patients on nevirapine –based regimen than patients on efavirenz-based first line ART regimen.

This difference was demonstrated statistically despite having fewer participants in the efavirenz based group. Whilst very high levels of ALT were recorded in some cases, hepato-toxicity in this population was generally mild with no morbidity.

Despite some patients having extreme ALT escalations, no alterations were made to their nevirapine-based treatment. This may be explained by the delays in getting the laboratory results or delays due to long intervals between patient visits. Age, pregnancy, obesity, and alcohol intake had no influence on hepato-toxicity in this study.

4.2 Recommendations.

A more regular and comprehensive system of monitoring for drug-induced liver diseases in patients on nevirapine-based regimens is to be considered.

A pre-treatment screening process that includes common liver disorders such as hepatitis B and C before initiation of nevirapine-based regimen is to be considered.

A system of alerting clinicians of very high ALTs and a more effective method of pharmacovigilance may help clinicians to effect timeous changes to the treatment regimens.

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Appendix A

Information sheet.

Hi, my name is Melody Nhiwatiwa. I am a pharmacist and currently studying for a Master's degree with Witwatersrand University.

I would like to invite you to take part in a study on the medications you are using at present. HIV medicines can bring about side effects one which is liver disease. The study will inform us more about antiretroviral therapy- induced liver diseases. The results may benefit our patients including you. The study is done by me and supervised by University of Witwatersrand faculty of Health Sciences and sponsored by conditional grant for HIV for the Free State department of health. Do not hesitate to contact me should you need more information.

Thank you,

Melody Nhiwatiwa: 057 357 1271, 0832613610

Appendix A in Sotho

Leqephe la lesedinyana.

Nna ke Melody Nhiwatiwa kopo ho wena ho nka lesedi ho dipatlisiso, e le ho thusa ka lesedi ka lefu lakebete le bakwang ke ditlare tsa ART, e leng enngwe eo e leng ditlamoraho tsa di ART.

Dipheto di ka thusa wena le ba bang ka kakaretso.

Dipatlisiso di etswa ke University ya Witwatersrand lefapha la bophelo le disaense (batsa mahle) Mme ba tsheheditswe ka ditjelete ke ba grant yaHIV ba lefapha la Freistata la bophelo bo botle.

Kea Leboa

Melody Nhiwatiwa 057 357 1271, 083 261 3610

Appendix A in Isixhosa

Sakhubona,

Ndingu Melody Nhiwatiwa. Ndingu Sokhemesti, ndenza izifundo zesidanga se Masters kwidyunivesi yase Witwatersrand.

Ndithanda ukumema ukuba uthabathe inxaxheba kwizifundo ezimalunga namachiza owasebenzisayo. Amachiza esandulela ntsholongwane (HIV) abangela emiphumela efana nesifo sesibindi.

Izifundo ezi zizakusinika ulwazi mayela nonyango lwezintinteli zifo (antiretroviral) ezihlasela isibindi. Iziphumo ziyakunceda izigulane zethu kwakunye nawe.

Izifundo ezi zenziwa ndim kwaye zonganyelwe yidyunivesi yase Witwatersrand kwicandelo lezempilo nenzululwazi ngenkxaso yecandelo lezempilo laseFreystata.

Wamkelekile ukunxibelelana nam ngemiba enoba yimfuneko kuwe.

Engkosi,

Melody Nhiwatiwa: 057 357 1271, 083 261 3610

Appendix A in Afrikaans

My naam is Melody Nhiwatiwa. Ek is `n gekwalifiseerde apteker en huidiglik besig met my meestersgraad by die Universiteit van die Witwatersrand.

Ek wil u graag uitnooi om deel te neem aan `n studie ten opsigte van die medikasie wat u huidiglik gebruik. MIV medikasie kan verskeie newe effekte veroorsaak waarvan een onder andere lewersiektes is. Die studie wat ek gaan doen gaan `n persoon meer inlig ten opsigte van antiretrovirale terapie en geïnduseerde lewer siektes wat daarmee gepaard gaan. Die eindresulte kan van groot waarde by ons pasiënte, asook uself wees. Die studie word deur myself, onder toesig van die Gesondheidsfakulteit van die Universiteit van die Witwatersrand, gedoen en geborg deur die Vrystaatse Departement van Gesondheid met `n voorwaardelike borg vir MIV. Moet asseblief nie huiwer om my te kontak indien daar enige verdere inligting benodig word nie.

Byvoorbaat dank,

Melody Nhiwatiwa: 057 357 1271, 0832613610

Appendix B:

Consent Form

Good day!

You are required to fill in a questionnaire and grant permission for your blood biochemical assays to be used for the study. Your name and personal information will not be attached to the results. If you agree to participate you will retain your right to withdraw anytime without compromise to care you receive in the clinic.

I agree:

*allow my blood biochemical assays to be used in the study

Signature of patient……

I do not agree……..

For further information contact Melody Nhiwatiwa @ 083 261 3610

Date…../…/….. GM number……..

Nurse

Name…….. Signature…….

Appendix B in Sotho

Leqephe la tumellano

O koptjwa ho tlasa dipotso mme o fane ka tumellano ya ho sebedisa lipalo tsa madi hao ho thusa dipatlisisong.

Lebitso la hao le keke la sebediswa le ditsohle tsa hao.

Ha o dumela hokena dipatlisisong o ka se qobellwe ha eba ose batla ho ikgula o sa tele tshwaro e ntle eo ntse o e fumana cliniking.

Ke dumela

Lebitso la mokuli…….

Hake dumele……..

Hafumana tlakisetso letsetsa Melody Nhiwatiwa 0832613610

Letsatsi……

Lebitso la mooki……..

Nomoro ya sepetlele……..

Appendix B in Afrikaans

Goeie dag!

Hiermee word u versoek om `n vraelys in te vul en daarmee toestemming te gee dat ek u bloedmonster vir die studie kan gebruik. U naam en persoonlike inligting sal nie by nie resultate aangeheg word nie. Indien u instem om deel te neem, behou u nogsteeds die reg om enige tyd te onttrek sonder dat die diens wat u by die kliniek ontvang, daardeur beïnvloed sal word.Hiemee gee ek toestemming dat my bloedmonster in die studie gebruik mag word.

Handtekening-----------

Hiermee gee ek nie toestemming dat my bloedmonster in die studie gebruik mag word nie.

Handtekening: ----------

Vir verder inligting skakel asseblief vir Melody Nhiwatiwa @ 0832613610

Datum: --------/------/---------- **GM nommer: ----------**

Suster

Naam: ---------- **Handtekening**: ----------

Appendix B in Isixhosa

Iphepha elinika imvume

Uyacelwa ugcwalise eliphepha elinika imvume yokuba sisebenzise amagazi akho kuphando elenziwayo. Amagama akho akaz'kubhalwa kulamagazi, akukhomntu azokwazi ukubangakabani.

Ukuba unika imvume yokusetyenziswa kwalamagazi ungarhoxa nokuba kunini xa uthewafuna, impatho yakho ekiliniki ayizokutshintsha.

Ndinika imvume:

*Amagazi angasetyenziswa kuphando

Umbhalo wesigulana.....

Andiyiniki imvume:.....

Xa uthe wafuna ukwazi ngoluphando, ungathetha no Melody Nhiwatiwa ku 083 261 3610

Umhla: ...../..../.....

GM Number:.....

Nurse Name:.....

Signature:.....

Appendix C

Questionnaire for the ALT study

Name _____ GM Number _____ Date _ _ / _ _ / _ _ _ _

English

1. Were you pregnant during the first six months of commencing treatment? Y/N

2. Do you drink alcohol? Y/N

Afrikaans Arae

1.Was u miskien swanger gedurende die eerste 6 maande nodor behandeling begin is? J/N

2.Gebruik u enige alkohol? J/N

Sotho

1. O ne O le Moimana na dikgwedi tse tshweletseng tse qalang ha o nwa meriana? ehe/tje

2. O nwa jwala na? ehe/the

Appendix D

Data Collecting sheet

Name	Number	Age	pregnant	Alcohol	weight kg	Height m	BMI	Regime	ALT-pre	ALT-6mnt	ALTchang

Appendix E

Equation 1

$$BMI = \frac{weight\ (kg)}{height\ (m^2)}$$

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Nhiwatiwa

CLEARANCE CERTIFICATE	**PROTOCOL NUMBER M081030**
PROJECT	Comparison of Drug Induced Hepato-Toxicity in Female Patients during antiretroviral Therapy
INVESTIGATORS	Ms M Nhiwatiwa
DEPARTMENT	Pharmacy & Pharmacology
DATE CONSIDERED	08.10.31
DECISION OF THE COMMITTEE*	Approved unconditionally

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE 08.11.26 **CHAIRPERSON** .....
(Professor P E Cleaton Jones)

*Guidelines for written 'informed consent' attached where applicable

cc: Supervisor : Prof P Dankewerts

DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10004, 10th Floor, Senate House, University.
I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. **I agree to a completion of a yearly progress report.**

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES...