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TITLE: HEPATOTOXICITY AMONG HIV-INFECTED PATIENTS RECEIVEING THE HIGHLY ACTIVE ANTI-RETROVIRAL THERAPY: IMPACT OF THE DURATION OF THE THERAPY AND COMORBID DISEASES.

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HEPATOTOXICITY AMONG HIV-INFECTED PATIENTS RECEIVEING THE HIGHLY ACTIVE ANTI-RETROVIRAL THERAPY: IMPACT OF THE DURATION OF THE THERAPY AND COMORBID DISEASES.

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Table of Contents

Acknowledgments i

Table of Contents ii

List of Tables v

List of figures vi

List of Abbreviations vii

Abstract x

1. Introduction 1

1.1. Back ground 1

1.1.1. Human Immunodeficiency Virus (HIV) 1

1.1.1.1. Morphology of HIV 1

1.1.1.2. Replication of cycle of HIV 2

1.1.1.3. Transmission of HIV 4

1.1.1.4. Diagnosis of HIV infection 4

1.1.1.5. Laboratory monitoring of patients with HIV infection 5

1.1.1.6. Clinical manifestations 7

1.1.2. Antiretroviral Therapy 8

1.1.3. Hepatotoxicity 13

1.1.4. Drug induced hepatotoxicity 14

1.1.4.1. Drug –induced hepatotoxicity by metabolic activation 15

1.1.4.2. Hypersensitivity reaction 16

1.1.4.3. Immunologically mediated liver injury 17

1.1.4.4. Drug-induced mitochondrial injury 18

1.1.5. Basis of laboratory diagnostic approaches 19

1.1.5.1. Bilirubin metabolism and abnormalities 19

1.1.5.2. Ammonia Metabolism 20

1.1.5.3. Liver enzymes and Serum proteins 20
1.1.5.4. Coagulation Factors 21
1.1.5.5. Other diagnostic approaches 21
1.2. Antiretroviral drug induced hepatotoxicity 21
1.2.1. NRTIs hepatotoxicity 24
1.2.2. NNRTIs hepatotoxicity 25
1.2.3. Protease inhibitors 26
1.3. Significance of the study 26
1.4. Hypothesis 27
1.5. Objective of the study 27
1.5.1. General objective 27
1.5.2. Specific objectives 27
2. Materials and Methods 28
2.1. Study Design 28
2.2. Study Subject 28
2.3. Study Area 28
2.4. Sample Size Determination 28
2.5. Data Collection 28
2.6. Selection and exclusion criteria 29
2.6.1. Inclusion criteria 29
2.6.2. Exclusion criteria 29
2.7. Specimen Collection and storage 29
2.8. Methods for biochemical investigations 29
2.8.1. Assessment of the Alanine Transaminase (ALT) 29
2.8.2. Assessment of the Aspartate Transaminase (AST) 31
2.8.3. Assessment of the Alkaline Phosphatase (ALP) 32

2.8.4. Assessment of the bilirubin level 34
2.9. Statistical analysis 36
2.10. Ethical Clearance 36
3. Results 37
3.1. Characteristics of patients in NVP and EFV groups before the start of HAART 37
3.2. Population distribution and LFT median values before the start of HAART 38
3.3. Population distribution and duration of therapy 38
3.4. Proportion of patients receiving NVP and EFV with in each hepatotoxicity grade 39
3.5. Proportion of patients at different duration of therapy receiving NVP and EFV 39
3.6. The change of LFTs with duration of therapy and HAART types 39
3.7. Correlation of pattern of change of the four LFTs with each other 48
3.8. Risk factor association of hepatotoxicity of NVP based regimen with sex and age 48
4. Discussion 49
4.1. Hepatotoxicity of NVP and EFV based regimens 49
4.2. Impact of the duration of therapy on hepatotoxicity of NVP and EFV 50
4.3. Risk factors for hepatotoxicity of NVP associated with sex and age 51
4.4. Correlation of pattern of changes of the four LFTs with each other 51
5. Limitations of the study 52
6. Conclusion 52
7. Recommendations 52
8. References 53
ANNEX 62

List of Tables

Table 1.1: Structure of antiretroviral medications……10

Table 1.2: ACTG Grading of Liver Toxicity……21

Table 3.1: Characteristics of variables for NVP and EFV groups before the start……37

Table 3.2: Population distribution and LFT characteristics before the start of HAART...38

Table 3.3: Population distribution and duration of therapy ……..38

Table 3.4: Correlation of the four LFTs with each other……..48

Table 3.5: Risk factors for hepatotoxicy……...48

List of figures

Figure 1.1: ***A***. Electron micrograph of HIV and ***B***. Structure of HIV-1……2

Figure 1.2: The life cycle of HIV virus……...……...3

Figure 1.3: Summary of Xenobiotic Transformations and Biohazards……14

Figure 1.4: Mechanism for the role of reactive metabolites in immunoallergic hepatitis..……15

Figure 1.5: Hypersensitivity (allergic) reaction ……..……..17

Figure 2.1: Reaction principle for the determination of alanine aminotransferase (ALT)…….30

Figure 2.2: Reaction principle for the determination of aspartate aminotransferase (AST)…….31

Figure 2.3: Reaction principle for the determination of Alkaline phosphatase (ALP)**...**……..32

Figure 2.4: Reaction principle for the determination of bilirubin……34

Figure 3.1: Proportion of patients taking NVP and EFV based regimen in the four hepatotoxicity grades: **A** for ALT, **B** for AST, **C** for ALP and **D** for TB……..41

Figure 3.3: Hepatotoxicity of NVP and duration of therapy for the four LFTs: **A-** ALT**, B-** AST**, C-** ALP and **D-** TB……....43

Figure 3.4: Hepatotoxicity EFV and duration of therapy for the four LFTs**, A-** ALT**, B-** AST**, C-** ALP and **D-** TB……**.....**45

Figure 3.5: Pattern LFTs change with time for the four drugs: **A** – ALT, **B** – AST**, C** – ALP and **D** – TB……..47

List of Abbreviations

ABC	Abacavir
ACTG	AIDS clinical trials group
AIDS	Acquired Immunodeficiency Syndrome
ALP	Alkaline Phosphatase
ALT	Alanine Transaminase
ANOVA	Analysis of Variance
APV	Amprenavir
ART	Anti-Retroviral Therapy
AST	Aspartate Transaminase
BCG	Bromocresol Green
BCP	Bromocresol Purple
CDC	Centers for Deasese control & Prevention
CD4	Cluster of Differentiation four.
ddI	didanosine
ddc	Zalcitabine
d4T	Stavudine
DLV	Delavirdine
DNA	Diriboneuclicacid
EFV	Efavirenz
EIA	Enzymes Immuno Assay
ELISA	Enzyme Linked Immunosorbent Assay
Emtriva	Emtricitabine
FAPV	Fosamprenavir
FDA	Food & Drug Adminstration
GGT	Gammaglutamyl Transpeptidase

HAART	Highly Active Anti-Retroviral Therapy
HIV	Human Immunodeficiency Viruses
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HTLV	The Human T Lymphotropic viruses
IDU_S	Injection Drug Users
IDV	Indinavir
IL	Interluikin
IgE	Immunoglobulin E
IRB	Institutional Research Board
LDH	Lactate Dehydrogenase
LFTS	Liver Function Tests
LPV	Lopinavir
MHC	Histocompatibility complex
MOH	Ministry of Health
NFV	Nelfinavir
NRTIs	Nucleoside Reverse Transcriptase inhibitors
NVP	Nevirapine
NNRTIs	Non-Nucleoside Reverse Transcriptase inhibitors
PIs	Protease Inhibitors
PLP	Pyridoxal Phosphate
RBC	Red Blood Cells
RTV	Ritonavir
ROS	Reactive oxidative species
SEM	Standard Error of Mean
SQV	Saquinavir

TASH	Tikur Anbesa Specialized Hospital
3TC	Lamivudine
TH2	T helper cell 2
TB	Total bilirubin
VCT	Voluntary Counseling and Testing
UNAIDS	United Nations AIDS
WBC	White Blood Cell
WHO	World Health Organization
ZDV	zidovudine

Abstract

The rapid death and morbidity due to opportunistic infections in Human immunodeficiency virus (HIV) patients has been reduced by the introduction of Highly Active Retroviral therapy (HAART). However, hepatotoxicity is now evident as a limiting factor of the benefit of the treatment. The over all incidence is estimated to be 3- 18% and it is up to 30% in patients on HAART. The objective of the study was to assess the liver function of HIV-infected patients receiving HAART so that clinicians will be more careful in prescribing the medications for their patients. In this retrospective cross-sectional study, 750 HIV infected patients who have already started HAART participated. 521 and 229 patients were taking NVP and EFV based regimen, respectively. Chi square and Mann-Whitney test were used to compare base line characteristics between the two groups, which showed no significant difference. Assessment of hepatotoxicity of the different HAART regimens, after initiation of therapy showed no hepatotoxicity with time, as can be seen from median values of the four LFTs (ALT, AST, ALP and TB). The proportion of patients in different hepatototoxcity grades were also analyzed for both NVP and EFV groups. The results showed that severe hepatotoxicity was more common in NVP users groups than EFV groups. While with time, both groups showed high proportion of hepatotoxicity grades before treatment which generally decreases after the initiation of therapy. With in three months of initiation therapy, more severe cases of hepatotoxicity were found when compared with other durations of therapy. Risk factor determination by multivariant logistic regression revealed that sex and age were not factors for hepatotoxicty while corrilation results by spearman rank correlation coefficient of LFTs with each other showed only correlation of TB with the other LFTs was not significant. The suggestion made from these findings is that, hepatotoxicity is more common in NVP users than EFV users and both the drugs are associated with elevation of LFTs just after the start of HAART thus frequent monitoring of liver enzymes after initiation antiretroviral therapy is recommended. However, because of NVP and EFV-associated severe hepatotoxicity cases were observed after the first 12 weeks of therapy, these data indicate that such assessments should continue throughout the treatment period.

1. Introduction

1.1. Back ground

It was in 1983 that human immunodeficiency virus (HIV) was isolated from a patient with lymphadenopathy, and by 1984 it was demonstrated clearly to be the causative agent of AIDS. In 1985, due to the development of a sensitive enzyme linked immunosorbent assay (ELISA), it was recognized that the HIV was epidemic at first in the United States and other developed nations and ultimately among developing nations throughout the world.

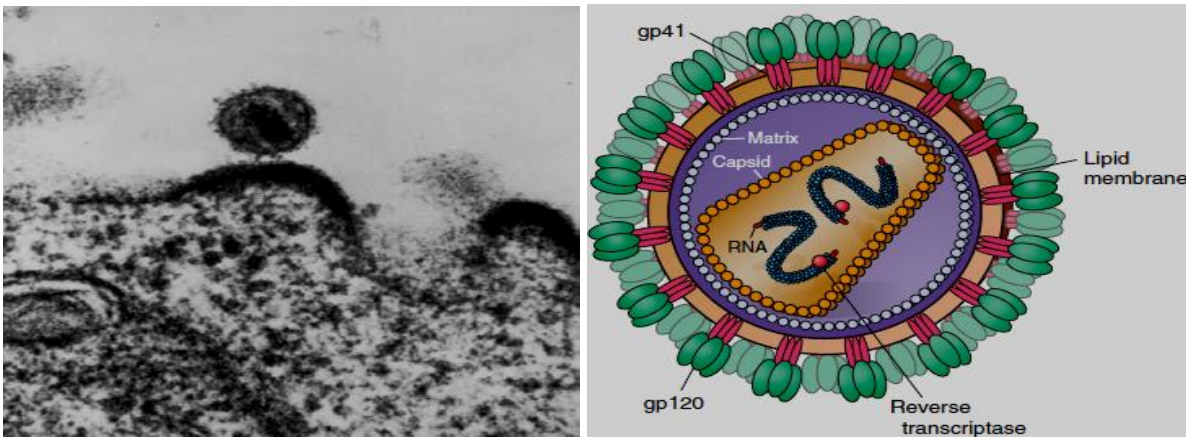
In 2008, an estimated 1.9 million people became newly infected with HIV in Sub Saharan Africa, bringing the total number of people living with HIV to 22.4 million and about 1.4 million AIDS-related deaths occurred in this region (UNAIDS, 2009). According to Ministry of Health (MOH), it was reported that 304,465 people enrolled in HIV health care in 2008, in Ethiopia. National adult HIV prevalence is 1.4%, with infection levels highest in the regions of Gambela 6% and Addis Ababa 4.7% (Demographic and Health Survey, 2005).

1.1.1. Human Immunodeficiency Virus (HIV)

The etiologic agent of AIDS is HIV, which belongs to the family of human retroviruses (Retroviridae) and the subfamily of lentiviruses. The four recognized human retroviruses belong to two distinct groups: the human T lymphotropic viruses (HTLV) I and HTLV-II, which are transforming retroviruses; and the HIV-1 and HIV-2, which are cytopathic viruses. HIV-1 was first identified in 1986 in West African patients and was originally confined to West Africa. However, a number of cases that can be traced to West Africa or to sexual contacts with West Africans have been identified throughout the world. HIV-2 likely originated from the *Pan troglodytes* species of chimpanzees in whom the virus had co-evolved over centuries.

1.1.1.1. Morphology of HIV

Electron microscopy shows that the HIV virion is an icosahedral structure. Fig. 1.1 *A* containing numerous external spikes formed by the two major envelope proteins, the external gp120 and the transmembrane gp41. The virion buds from the surface of the infected cell and incorporates a variety of host proteins, including major histocompatibility complex (MHC) class I and II antigens into its lipid bilayer. The structure of HIV-1 is schematically diagrammed in Fig 1.1 *B*.



A ***B***

Figure 1.1: ***A***. Electron micrograph of HIV. ***B***. Structure of HIV-1, including the gp120 outer membrane, gp41 transmembrane components of the envelope, genomic RNA, enzyme reverse transcriptase, p18 (17) inner membrane (matrix), and p24 core protein (capsid)

1.1.1.2. Replication of cycle of HIV

HIV is an RNA virus whose hallmark is the reverse transcription of its genomic RNA to DNA by the enzyme *reverse transcriptase*. The replication cycle is as follows

1. High affinity binding of the gp120 protein via a portion of its V1 region near the N-terminus to its receptor on the host cell surface, the CD4 molecule.
2. The gp120 undergoes a conformational change that facilitates binding to one of a group of co-receptors, CCR5 and CXCR4. The use of one or the other or both receptors by the virus for entry into the cell is an important determinant of the cellular tropism of the virus. Certain dendritic cells express a diversity of C-type lectin receptors on their surface, one of which is called *DC-SIGN*, that bind with high affinity to the HIV gp120 envelope protein, allowing the dendritic cell to facilitate the binding of virus to the CD4+ T cell upon engagement of dendritic cells with CD4+ T cells.
3. The viral envelope changes dramatically, and fusion with the host cell membrane occurs via the newly exposed gp41 molecule penetrating the plasma membrane of the target cell and then coiling upon itself to bring the virion and target cell together.
4. The HIV genomic RNA is uncoated and internalized into the target cell (figur 1.2).

5. The reverse transcriptase enzyme, which is contained in the infecting virion, catalyzes the reverse transcription of the genomic RNA into double-strand DNA.
6. The DNA translocates to the nucleus, where it is integrated in a somewhat, but not completely, random fashion into the host cell chromosomes through the action of another virally encoded enzyme, *integrase*. Sites of HIV integration into the nuclear DNA are preferential for active genes and regional hotspots. This provirus may remain transcriptionally inactive (latent) or it may manifest varying levels of gene expression, up to active production of virus.

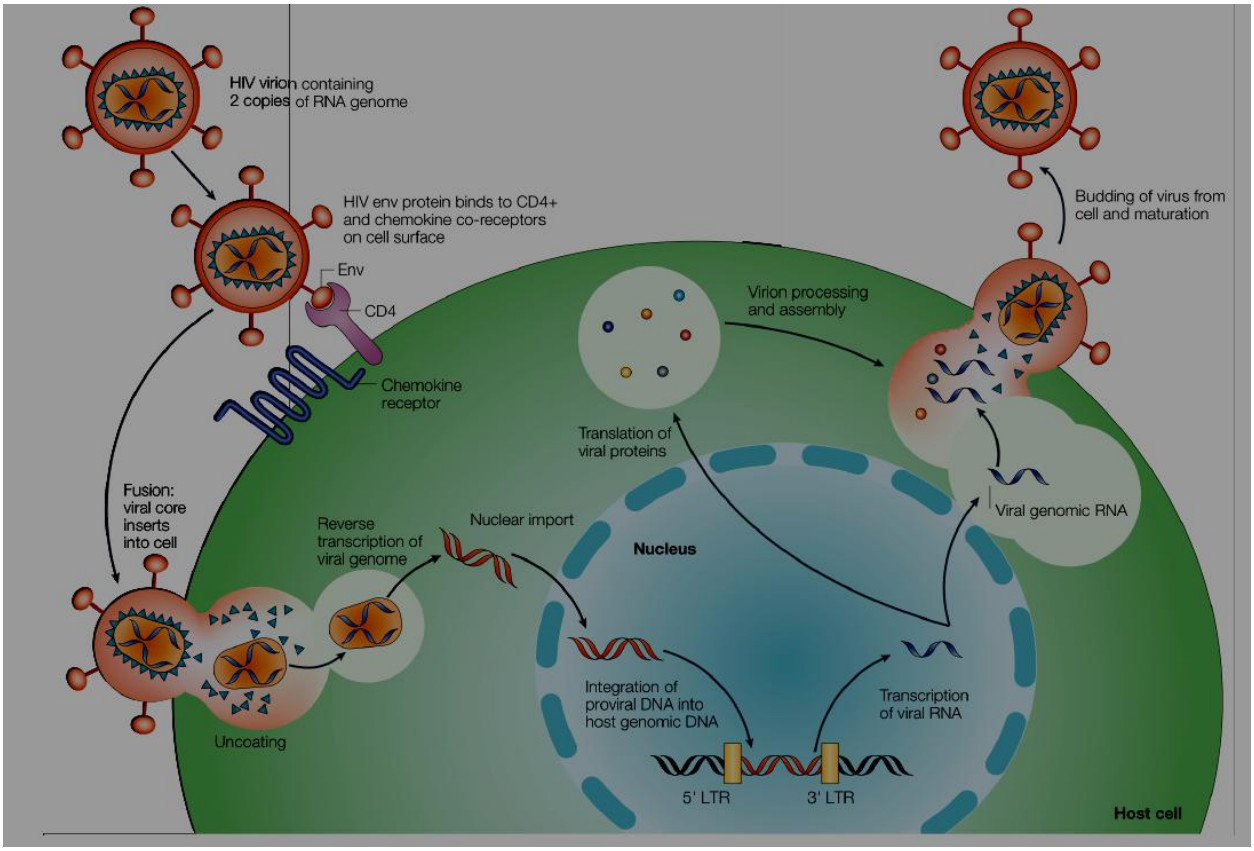


Figure 1.2: The life cycle of HIV virus (Andrew et al., 2004)

Cellular activation plays an important role in the life cycle of HIV and is critical to the pathogenesis of HIV disease. Following initial binding and internalization of virions into the target cell, incompletely reverse-transcribed DNA intermediates are labile in quiescent cells and will not integrate efficiently into the host cell genome unless cellular activation occurs shortly after infection. Furthermore, some degree of activation of the host cell is required for the initiation of transcription of the integrated proviral DNA into either genomic RNA or mRNA.

This latter process may not necessarily be associated with the obvious expression of the classic cell surface markers of activation. In this regard, activation of HIV expression from the latent state depends on the interaction of a number of cellular and viral factors. Following transcription, HIV mRNA is translated into proteins that undergo modification through glycosylation, myristylation, phosphorylation, and cleavage. The viral particle is formed by the assembly of HIV proteins, enzymes, and genomic RNA at the plasma membrane of the cells. Budding of the progeny virion occurs through specialized regions in the lipid bilayer of the host cell membrane known as *lipid rafts*, where the core acquires its external envelope. The virally encoded protease then catalyzes the cleavage of the gag-pol precursor to yield the mature virion. Progression through the virus replication cycle is profoundly influenced by a variety of viral regulatory gene products. Likewise, each point in the replication cycle of HIV is a real or potential target for therapeutic intervention (Anthony et al., 2005; Hoffmann, 2006).

1.1.1.3. Transmission of HIV

Sexual transmission: HIV has been demonstrated in seminal fluid both within infected mononuclear cells and in the cell-free state. The virus appears to concentrate in the seminal fluid, cervical smears and vaginal fluid, particularly in situations where there are increased numbers of lymphocytes and monocytes in the fluid, as in genital inflammatory states such as urethritis and epididymitis, conditions closely associated with other sexual transmitted diseases.

Blood and blood product transmission: HIV can be transmitted to individuals who receive HIV-tainted blood transfusions, blood products, or transplanted tissue as well as to IDUs who are exposed to HIV while sharing injection paraphernalia such as needles, syringes, the water in which drugs are mixed or the cotton through which drugs are filtered.

Maternal - fetal/infant transmission: HIV infection can be transmitted from an infected mother to her fetus during pregnancy, during delivery or by breast-feeding (Anthony et al., 2005).

1.1.1.4. Diagnosis of HIV infection

The diagnosis of HIV infection depends upon the demonstration of antibodies to HIV and/or the direct detection of HIV or one of its components. As noted above, antibodies to HIV generally appear in the circulation 2 to 12 weeks following infection (Anthony et al., 2005).

Enzyme immunoassay (EIA): is an extremely good screening test with a sensitivity of > 99.5%. The tests are generally scored as positive (highly reactive), negative (nonreactive), or indeterminate (partially reactive). While the EIA is an extremely sensitive test, it is not optimal with regard to specificity. For these reasons, anyone suspected of having HIV infection based upon a positive or indeterminante EIA result must have the result confirmed with a more specific assay.

Western bloting: is the most common conformation test. This assay takes advantage of the fact that multiple HIV antigens of different, well-characterized molecular weights elicit the production of specific antibodies. These antigens can be separated on the basis of molecular weight, and antibodies to each component can be detected as discrete bands on the western blot. In a patient with a positive or indeterminate EIA and a negative western blot, one can conclude with certainty that the EIA reactivity was a false positive. On the other hand, a western blot demonstrating antibodies to products of all three of the major genes of HIV (*gag*, *pol*, and *env*) is conclusive evidence of infection with HIV. Criteria established by the U.S. Food & Drug Administration (FDA) in 1993 for a positive western blot state that a result is considered positive if antibodies exist to two of the three HIV proteins: p24, gp41, and gp120/160. If the western blot for HIV-1 is indeterminate, it should be repeated in 4 to 6 weeks; in addition, one may proceed to HIV-1 RNA assay, or HIV-1 DNA PCR. These tests are extremely sensitive. One frequent consequence of a high degree of sensitivity is some loss of specificity and false positive results have been reported with each of these techniques. For this reason, a positive EIA with a confirmatory western blot remains the "gold standard" for a diagnosis of HIV infection, and the interpretation of other test results must be done with this in mind.

1.1.1.5. Laboratory monitoring of patients with HIV infection

The close relationship between clinical manifestations of HIV infection and CD4+ T cell count has made measurement of the latter a routine part of the evaluation of HIV-infected individuals. Determinations of CD4+ T cell counts and measurements of the levels of HIV RNA in serum or plasma provide a powerful set of tools for determining prognosis and monitoring response to therapy. While the CD4+ T cell count provides information on the current immunologic status of the patient, the HIV RNA level predicts what will happen to the CD4+ T cell count in the near future, and hence provides an important piece of prognostic information.

CD4+ T cell Counts

CD4+ T Cell Counts the laboratory test generally accepted as the best indicator of the immediate state of immunologic competence of the patient with HIV infection.

This measurement, which is the product of the percent of CD4+ T cells (determined by flow cytometry) and the total lymphocyte count (determined by the white blood cell count (WBC) and the differential percent) has been shown to correlate very well with the level of immunologic competence. Patients with HIV infection should have CD4+ T cell measurements performed at the time of diagnosis and every 3 to 6 months thereafter. More frequent measurements should be made if a declining trend is noted. According to most guidelines, a CD4+ T cell count <350/μL is an indication for consideration of initiating Antiretroviral Therapy (ART) , and a decline in CD4+ T cell count of <25% is an indication for considering a change in therapy. Once the CD4+ T cell count is <200/μL, patients should be placed on a regimen for *P. carinii* prophylaxis, and once the count is <50/μL, primary prophylaxis for *M. avium complex* infection is indicated.

HIV RNA Determination

HIV RNA determination has become an essential component in the monitoring of patients with HIV infection. The two most commonly used techniques are the RT-PCR assay and the bDNA assay. Both assays generate data in the form of number of (viral load or number of copies of HIV RNA per milliliter of serum or plasma) up to as few as 50 to 75 copies of HIV RNA per milliliter of plasma. Measurements of plasma HIV RNA levels should be made at the time of HIV diagnosis and every 3 to 4 months thereafter in the untreated patient. In general, most guidelines suggest that therapy be considered in patients with > 50,000 copies of HIV RNA per milliliter. Following the initiation of therapy or any change in therapy, plasma HIV RNA levels should be monitored approximately every 4 weeks until the effectiveness of the therapeutic regimen is determined by the development of a new steady-state level of HIV RNA. In most instances of effective therapy this will be < 50 copies per milliliter.

This level of virus is generally achieved within 6 months of the initiation of effective treatment. During therapy, levels of HIV RNA should be monitored every 3 to 4 months to evaluate the continuing effectiveness of therapy.

HIV Resistance Testing

HIV resistance testing can be done through either genotypic or phenotypic measurements. In the genotypic assays, sequence analyses of the HIV genomes obtained from patients are compared to sequences of viruses with known antiretroviral resistance profiles. In the phenotypic assays, the in vivo growth of viral isolates obtained from the patient are compared to the growth of reference strains of the virus in the presence or absence of different antiretroviral drugs or a comparison of the enzymatic activities of the reverse transcriptase or protease genes obtained by molecular cloning of patients' isolates to the enzymatic activities of genes obtained from reference strains of HIV in the presence or absence of different drugs targeted to these genes. These tests are quite good in identifying those antiretroviral agents that have been utilized in the past in a given patient. These tests are quite good in identifying those antiretroviral agents that have been utilized in the past in a given patient.

1.1.1.6. Clinical manifestations

Clinical manifestations encompass a spectrum ranging from an acute syndrome associated with primary infection to a prolonged asymptomatic state to advanced disease. The first stage is *Acute HIV syndrome,* in which individuals with HIV infection experience an acute clinical syndrome approximately 3 to 6 weeks after primary infection. This includes fever, lymphadenopathy, nausea, vomiting, diarrhea, neurologial and dermatologic manifestations. Symptoms usually persist for 1 to several weeks and gradually subside as an immune response to HIV develops and the levels of plasma viremia decrease. In most patients, primary infection with or without the acute syndrome is followed by a prolonged period of clinical latency, *Asymptomatic stage*. During this period of HIV infection, the average rate of CD4+ T cell decline is <50/μL per year. When the CD4+ T cell count falls to <200/μL, the resulting state of immunodeficiency is severe enough to place the patient at high risk for opportunistic infection and neoplasms, and hence for clinically *Symptomatic stage* which includes,

- Disease of the Respiratory System,
- Diseases of the Cardiovascular System,
- Hepatobiliary Disease,
- Diseases of the Kidney and Genitourinary Tract,
- Diseases of the Endocrine System and Metabolic Disorders,

- Rheumatologic Diseases,
- Diseases of the Hematopoietic System,
- Dermatologic Diseases,
- Neurologic Diseases,
- Ophthalmologic Disease,
- Additional Disseminated Infections and Wasting Syndrome, Neoplastic Diseases.

1.1.2. Antiretroviral Therapy

The first antiretroviral drug was introduced in 1987. The five classes include: Nucleoside reverse transcriptase inhibitors (NRTIs), Non-nucleoside reverse transcriptase inhibitors (NNRTIs), Protease inhibitors (PI), Entry inhibitors receptor 5 (Maraviroc) and Integrase inhibitors (raltegravir).

NRTIs include: Zidovudine (ZDT), Didanosine (ddI), Zalcitabine (ddC), Lamivudine (3TC), Stavudine (d4T), Abacavir (ABC), Emtricitabine (Emtriva), and Tenofovir (Viread). ZDT was the first antiretroviral drug to be discovered. To inhibit HIV replication they are first triphosphorylated intracellularly to nucleotides and then incorporated into the growing viral DNA chain by the viral reverse transcriptase enzyme. This creates a pool of dideoxynucleotide analogue triphosphates, which compete with endogenous deoxynucleotide triphosphates for substrate binding to reverse transcriptase. Unfortunately, these agents can also be substrates for other enzymes capable of DNA formation including human DNA polymerase in the mitochondria (Lewis and Dalakas, 1995; Van et al, 2004). As a result, anaerobic glycolysis in aerobic conditions occurs, with increased production of lactate due to dysfunctional mitochondria. In the liver, the disruption of mitochondrial function causes micro-vesicular and macro-vesicular steatosis with triglyceride accumulation (Lai et al, 1991; Freiman et al, 1993; Olano et al, 1995). Insulin resistance, cardiomyopathy, pancreatitis and peripheral neuropathy are among diseases related to mitochondrial toxicity.

The replication of mitochondrial DNA is variably inhibited by NRTIs in the following order; ddC › ddI › d4T › 3TC › ABC in vitro. It is not yet clear that *in vitro* NRTI inhibition of *mt*DNA is an accurate predictor of *in vivo* toxicity. (Kakuda, 2000; Boyle et al, 2003).

NNRTIs were first described in 1990. They include; Nevirapine (NVP), Efavirenz (EFV), and Delavirdine (DLV). NNRTIs are non-competitive inhibitors of the enzyme reverse transcriptase that impair the catalytic function of this enzyme by binding to specific tyrosine residues situated near the active site. These agents are usually used in combination with two or more NRTIs and/or PIs in Highly Active Antiretroviral Therapy (HAART) (Kontorinis and Dieterich, 2003; Dirk et al., 2007). In contrast to NRTIs, NNRTIs do not require activation within the cell. The two most common NNRTIs (NVP and EFV) are extremely effective when combined with nucleoside analogs (Podzamczer et al., 2002; Robbins et al., 2003).

PIs include Indinavir (IDV), Ritonavir (RTV), Saquinavir (SQV), hard and soft gel, Nelfinavir (NFV), Amprenavir (APV), Lopinavir/Ritonavir (LPV), and Fosamprenavir (fAPV). PIs are molecules that target the active site of HIV-1 aspartic protease, the enzyme responsible for cleaving of the precursor viral polyprotein, gag-pol, into its constituent proteins, which include the reverse transcriptase. Inhibition of the cleavage of the viral polyprotein results in production of immature non-infectious viral particles. All currently available PIs are extensively metabolized in the liver by the cytochrome P450 3A4 enzyme system (Sulkowski, 2003).

Entry inhibitors block the chemokine receptor CCR5 which HIV uses as a coreceptor to bind and enter a human macrophage. Intregrase inhibitors has structural motif that possesses metal-chelating functions, and it interact with divalent metals within the active site of HIV-1 integrase preventing it from working (Bailey and Fisher, 2008).

The introduction of potent combinations with three or more antiretroviral agents based on the administration of at least two NRTIs with a NNRTI commonly as first line regimens or a protease inhibitor as second line. It is referred to as HAART introduced in 1996. It has changed the HIV infection from a rapidly progressive fatal disease complicated by opportunistic infections to a chronic one (Palella et al., 1998).

When used properly, HAART leads to sustained virologic suppression and subsequent immunologic recovery and due to its reduced price it has become more available. According to the Ethiopian MOH, the total number of persons currently on ART throughout the country as of October 08, 2009 is 167,271 of whom, 165,263 (98.8%) are on the first line combinational ART regimens (MOH- FHAPCO, 2009). In Ethiopia the most widely used drugs are NRTIs and

NNRTIs. PIs are used as second line drugs. The fist line adult regimens are based on the administration of two NRTIs with a NNRTI.

Preferred first line regimen includes,

1. TDF+FTC+EFV
2. ZDV+ 3TC+ NVP (1c)
3. ZDV+3TC+EFV (1d)

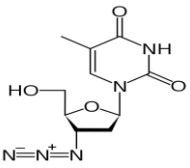
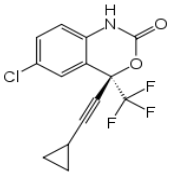
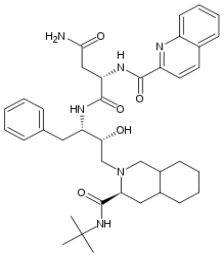
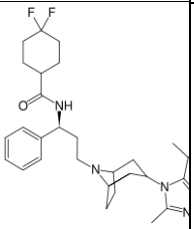
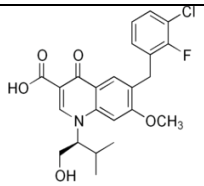
As an alternative,

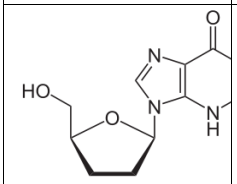
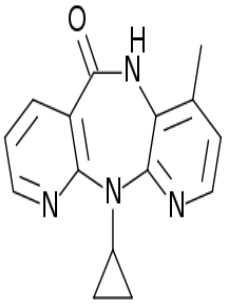
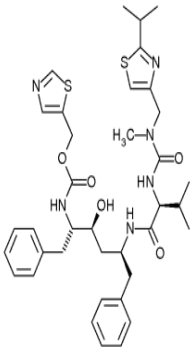
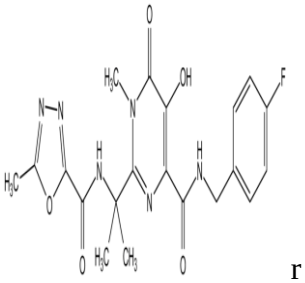
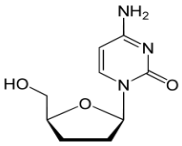
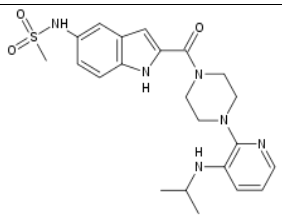
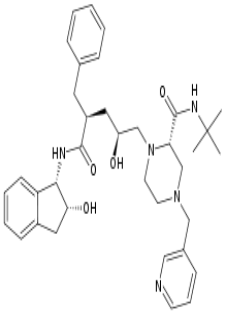
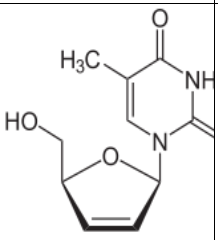
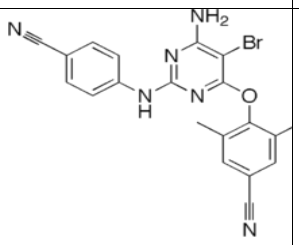
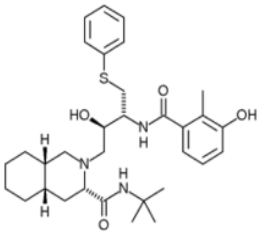
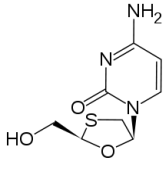
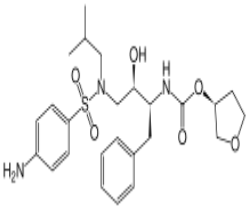
1. D4T+3TC+NVP (1a)
2. D4T+3TC+EFV (1b)
3. TDF+3TC+NVP
4. D4T+3TC+ 3TC+EFV
5. ABC+3TC+NVP
6. ABC+3TC+EFV
7. ABC+3TC+ZDV

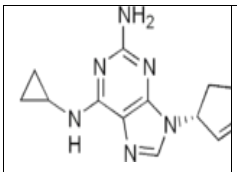
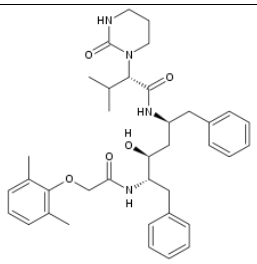
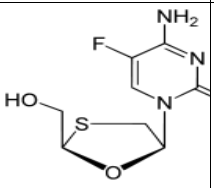
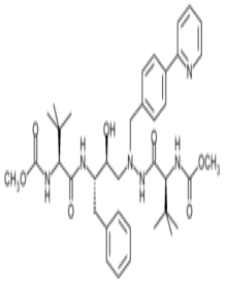
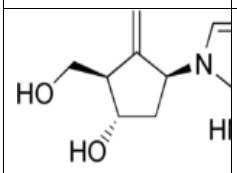
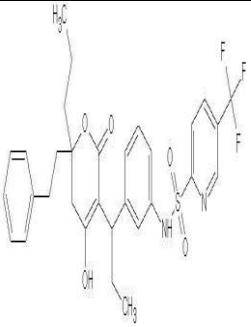
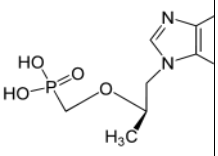
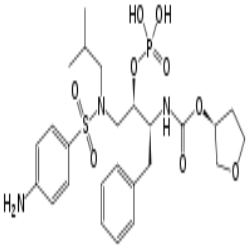
The second line regimens are based on the use of two nucleosides with a PI. The following combinations of drugs are in use: (MOH-FHAPCO, 2008).

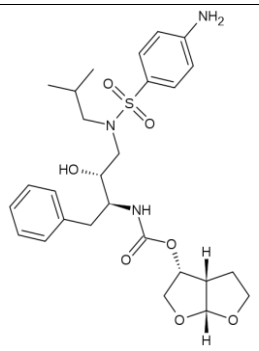
1. Abacavir/ Didanosine/ Lopinavir (ritonavir)
2. Abacavir/ Didanosine/ Nelfinavir
3. Tenofovir/ Didanosine/ Nelfinavir
4. Didanosine/ Lopinavir (ritonavir)

Table 1.1: Structure of antiretroviral medications

NRTI	NNRTI	PI	Entry inhibitors	Integrase inhibitors
 Zudovudine	 Efavirenz			 Elvitegravir

		Saquinavir	Maraviroc	
 Didanosine	 Nevirapine	 Ritonavir		 altegravir
 Zalcitabine	 Delavirdine	 Indinavir		
 Stavudine	 Etravirine	 Nelfinavir		
 Lamuvudine		 Amprenavir		

 Abacavir		 Lopinavir		
 Emtricitabine		 Atazanavir		
 Entecavir		 Tipranavir		
 Tenofovir		 Fosamprenavir		

		 Darunavir		

Even though HAART dramatically improved the clinical course, prognosis, and survival of HIV-1 infected patients, issues on adverse drug reactions, resistances and comorbidities have now become evident as a limiting cause of benefit in a substantial proportion of patients receiving HAART. Each antiretroviral medication is associated with its own specific adverse effects or may cause problems only in particular circumstances. Similarly, class-specific adverse effects may occur. However, the subtle and serious adverse effects are lactic acidosis, hepatic steatosis, hyperlactatemia, hepatotoxicity, hyperglycemia, fat maldistribution, hyperlipidemia, bleeding disorders, osteoporosis and skin rash.

Hepatotoxicity, as characterized by an increased rate of cytolysis and a significantly elevated serum transaminase levels, is recognized after the vailability in 1987 of the first NRTI, the ZDV (Barbaro et. al, 1999; Lai et.al, 1991; Melamed et. al, 2000) and the rate rose in 1996 even further with the use of PIs and HAART (Arribas et al, 1998; Braun et al, 1997).

1.1.3. Hepatotoxicity

Hepatotoxicity implies chemical-driven liver damage. The liver plays a central role in transforming and clearing chemicals and is susceptible to the toxicity from these agents. Certain medicinal agents, when taken in overdoses and sometimes even when introduced within therapeutic ranges, may injure the organ. Other chemical agents, such as those used in laboratories and industries, natural chemicals (e.g. microcystins) and herbal remedies can also induce hepatotoxicity. Chemicals that cause liver injury are called hepatotoxins.

While there are many causes of liver disease, they generally present clinically in a few distinct patterns, usually classified as hepatocellular, cholestatic (obstructive), or mixed. In

hepatocellular diseases such as viral hepatitis or alcoholic liver disease, features of liver injury, inflammation, and necrosis predominate. In cholestatic diseases such as gall stone or malignant obstruction, primary biliary cirrhosis, some drug-induced liver diseases, features of inhibition of bile flow predominate. In a mixed pattern, features of both hepatocellular and cholestatic injury are present (such as in cholestatic forms of viral hepatitis and many drug-induced liver diseases) (Dienstag and Isselbacher et al, 2005).

1.1.4. Drug induced hepatotoxicity

Most drugs, which are water-insoluble, undergo a series of metabolic transformation steps, culminating in a water-soluble form appropriate for renal or biliary excretion. This process begins with oxidation or methylation initially mediated by the mixed-function oxygenases cytochrome P450 (phase I reaction), followed by glucuronidation or sulfation (phase II reaction) or inactivating by conjugation with glutathione. Most drug hepatotoxicity is mediated by a phase I toxic metabolite, but glutathione depletion, precluding inactivation of harmful compounds by glutathione S-transferase, can contribute as well.

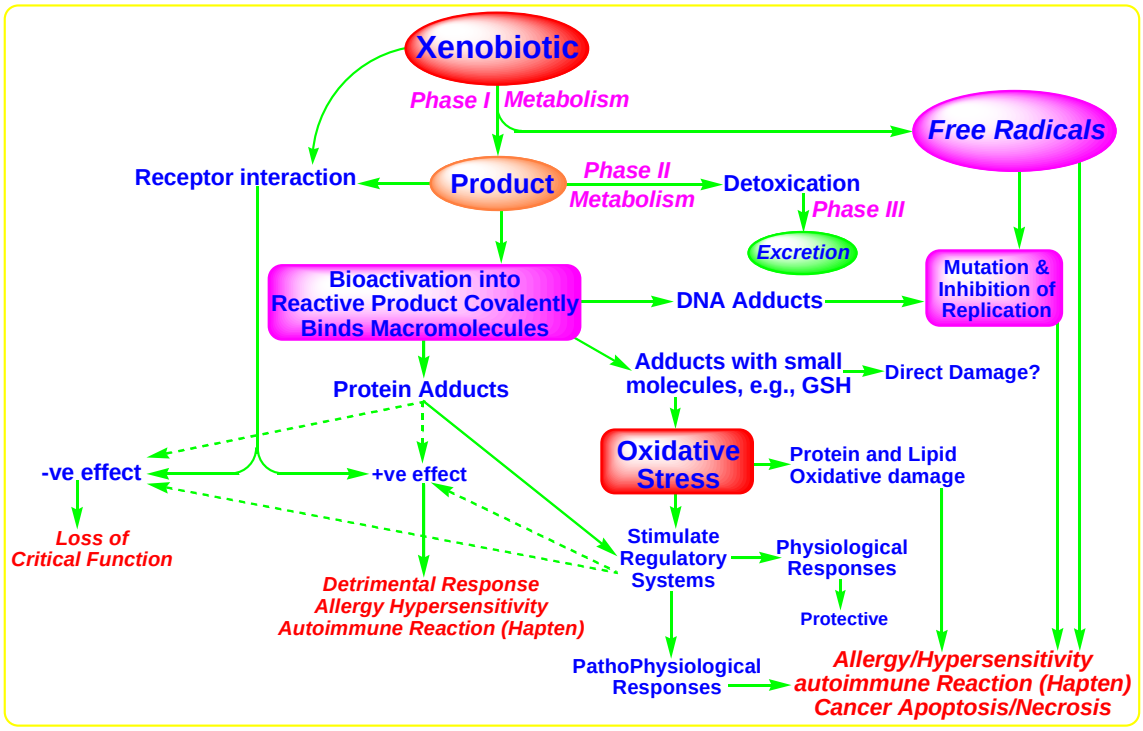


Figure 1.3: Summary of Xenobiotic Transformations and Biohazards.

Hepatotoxic drugs can cause liver disease in all the three patterns. It injure the hepatocyte directly, e.g., via a free-radical or an active metabolic intermediate that causes peroxidation of membrane lipids and that results in liver cell injury. Alternatively, the drug or its metabolite can distort cell membranes or other cellular molecules, activate apoptotic pathways, or block biochemical pathways or cellular integrity. Such injuries, in turn, may lead to necrosis of hepatocytes; injure bile ducts, producing cholestasis; or block pathways of lipid movement, inhibit protein synthesis, or impair mitochondrial oxidation of fatty acids, resulting in lactic acidosis and fat accumulation (steatosis). (Dienstag and Isselbacher et al, 2005*;* Dufour et al 2000a and b; Arneson and Brickell, 2007).

There are four known mechanisms involved in the development of hepatotoxicity. They are generally of two types, predictable or unpredictable (Kaplowitz, 2002). Liver injury may result from direct toxicity of the drug or its metabolites or may be an idiosyncratic response in persons with a characteristic genetic predisposition. The latency period between the initiation of therapy and the onset of liver disease provides clues to its etiology.

Predictable hepatotoxic reactions are dose dependent and host independent (Kaplowitz, 2002). Early-onset toxicity (within a few days) is strong evidence for direct drug toxicity, particularly if there has been no previous exposure. Unpredictable hepatotoxic reactions are host dependent and not dose related (Zimmerman, 1999). Unfortunately, the vast majority of drug reactions are unpredictable. They occur when the drug is transformed into an intermediate metabolite that is either toxic (host-mediated metabolism) or provokes an immunological response hypersensitivity reaction. We can also classify the mechanism of drug induced hepatotoxicity in four; *metabolic host-mediated hepatotoxicity*, *hypersensetivity reaction*, *mitochondrial toxicity* and *immune reconstitution phenomena.*

1.1.4.1. Drug –induced hepatotoxicity by metabolic activation

Halothane is the best studied drug with respect to immunoallergic hepatitis. Proposed mechanism is the drug undergoes bioactivation in the hepatocytes leading to drug-protein conjugate formation in the liver. The resulting modified protein is internalized by Kupffer cells and presented to cognate T cells that recognize modified peptide and native peptide. This in turn can lead to the generation of cytotoxic T cells and B lymphocytes producing antibody (Kenna, 1997).

Studies have already shown that patients with deranged liver function as a consequence of taking nevirapine have circulating T cells that recognize the drug.

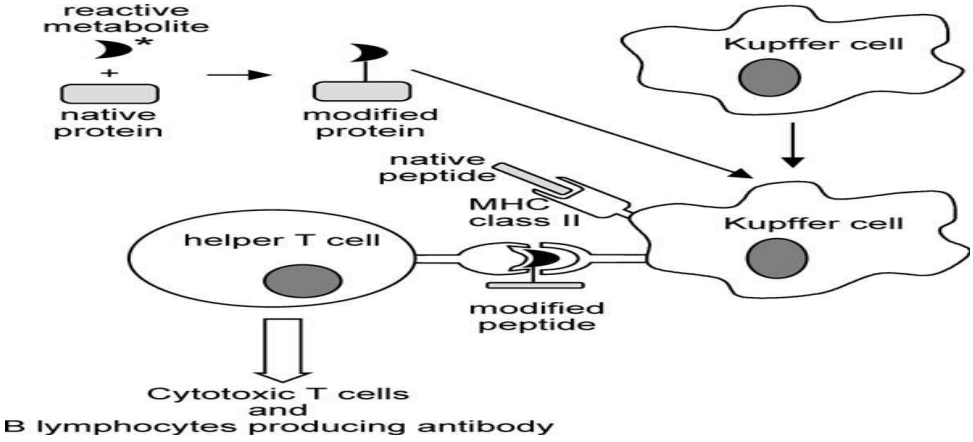


Figure 1.4: Mechanisms for the role of reactive metabolites in immunoallergic hepatitis.

1.1.4.2. Hypersensitivity reaction

In an allergic reaction, a macrophage phagocytoses the offending drug, and displays its fragments to a type 2 T helper (TH2) cell (Kuby et al., 2007). The TH2 cell identifies an allergen as pathogenic and releases the cytokine Interleukin-4 (Il-4), which stimulates a naive B cell to undergo cellular proliferation. These B cells differentiate into two types: IgE secreting plasma cells, and memory cells. Plasma cells secrete IgE into the blood stream which binds to an FcεRI receptor present on the surface of mast cells and basophiles, two types of white blood cells. The antibody remains bound to the surface of these cells for long periods of time; a mast cell or basophil that is coated with IgE is referred to as activated. Upon a subsequent exposure to the same allergen, the allergen binds to, and cross-links, the IgE molecules bound to the surface of mast cells and basophils. This stimulates the cells to release granules containing histamine, an inflammatory mediator. Other inflammatory mediators such as leukotrienes, prostaglandins and cytokines are also released. These chemicals stimulate the contraction of smooth muscle, increase vascular permeability and cause the blood vessels to dilate (vasodilation). These are the effects of inflammation which is characterized by swelling, redness, heat and pain.

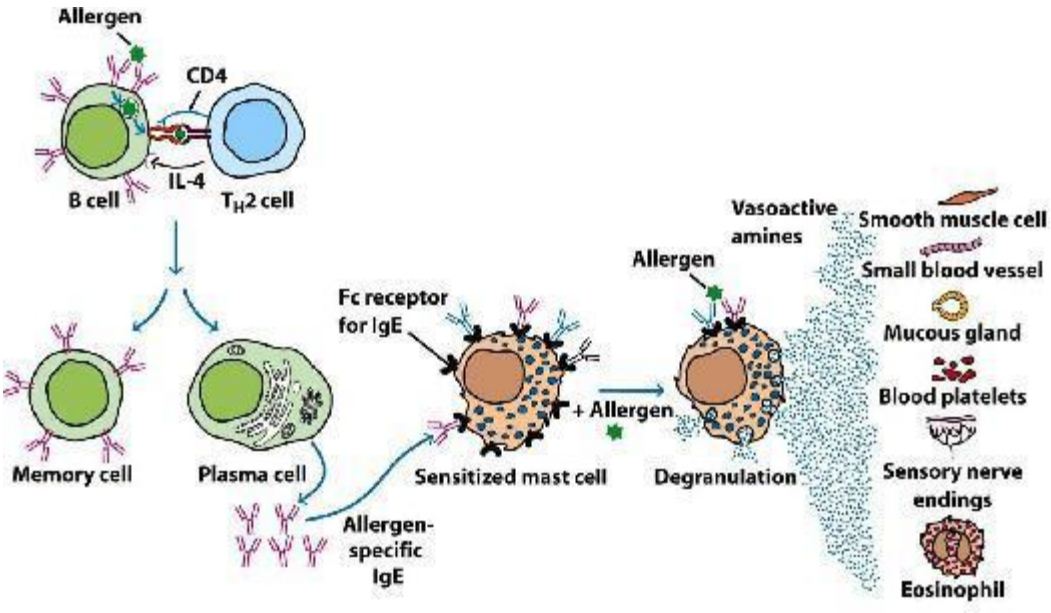


Figure 1.5: Hypersensitivity (allergic) reaction (Kuby *et al*, 2007).

1.1.4.3. Immunologically mediated liver injury

Immune-mediated drug reactions appear to involve the generation of neoantigens that are formed by the reaction of liver proteins with reactive drug metabolites. This is inferred in part from circulating specific antibodies in patient sera, which increase promptly upon rechallenge with the antigen. Additional evidence for an immune-mediated pathology is the finding that the response to the drug is delayed and that multiple exposures are required (Furst et al., 1997). An approach that has been explored for cutaneous drug reactions examines the activation of human peripheral mononuclear cells to a drug or drug metabolite *in vitro*; if a metabolite is the suspected toxin, the reaction must include an initial exposure of drug to hepatic microsomes and NADPH. These assays, which are host-dependent, are a promising approach to identifying susceptible individuals (Knowles et al., 2000). The incidence of immune-mediated hepatotoxicity is relatively low, possibly because the liver is tolerogenic, a property that may be attributable to the liver's production of anti-inflammatory cytokines (*e.g.*,IL-10, IL-6, IL-4, IL-13) and other inhibitory factors (*e.g.*, prostaglandins). The latter can down-regulate TH 1 reactions and thus reduce specific immune responses. These factors may function to prevent allergic hepatitis as well as acute hepatotoxicity through inhibition of CD81 T cell.

1.1.4.4. Drug-induced mitochondrial injury

A relatively uncommon but distinctive form of hepatic injury due to drugs is microvesicular fatty liver, which can be caused by alcohol, aspirin, tetracycline, amiodarone, valproic acid, and several antiviral nucleoside analogues, the most prominent of which is fialuridine (Mckenzle et al., 1995; Sokol and Treem, 1999). The hallmark of this injury is accumulation of microvesicular fat in hepatocytesand decreased numbers of mitochondria.

Fialuridine provides the most striking example of a drug causing microvesicular fatty liver and acute liver failure. This is a fluoridinated pyrimidine analogue. It inhibits DNA polymerase γ (the enzyme responsible for replication of mitochondrial DNA) but has little effect at physiologic concentrations on DNA polymerase α or β. Similar findings for ZDV suggest that differential inhibition of DNA polymerase activities may account for mitochondrial toxicity of nucleoside analogues. As a result, anaerobic glycolysis within the cytoplasm occurs, with increased production of lactate. In the liver, the disruption of mitochondrial function causes micro-vesicular and macro-vesicular steatosis with triglyceride accumulation (Lewis et al., 1997; Tennant et al., 1998).

The other cause of the hepatic injury is believed to be inhibition of fatty acid β oxidation and mitochondrial dysfunction (Fromenty and Pessayre, 1995; Silva et al., 1998). In addition, drugs and toxins can inhibit mitochondrial respiratory chain function thereby reducing oxidative phosphorylation and depleting intracellular ATP levels. Inhibition of normal respiratory chain enzymic activity can also generate excessive reactive oxidative species (ROS), causing further cellular injury.

Mitochondria also play a major role in apoptotic pathways, through induction of the mitochondrial permeability transition pore, which results in a rapid increase in mitochondrial membrane permeability and release of cytochrome c and other proapoptotic factors (Lemasters et al., 1998). Thus, mitochondria participate in many forms of hepatic injury. Drugs and toxins that induce mitochondrial permeability transition include salicylates, valproic acid, ethanol, hydrophobic bile acids, and ROS in general. Importantly, substances that block induction of the mitochondrial permeability transition, including ursodiol and cyclosporine, may be protective against these forms of injury.

1.1.5. Basis of laboratory diagnostic approaches

The liver is the site for metabolism of carbohydrate, protein and lipids, as well as for the synthesis of many proteins, the conjugation of bilirubin, and detoxification of drugs and other substances. For this reason, the liver function may be assessed by measurement of total and direct bilirubin, total protein and albumin, cholesterol and triglycerides, and urea and ammonia. The plasma levels of liver enzymes are also helpful in assessing the liver function (Dufour et al 2000a and b; Limdi and Hyde, 2003; Pratt and Kaplan, 2005; Arneson and Brickell, 2007).

1.1.5.1. Bilirubin metabolism and abnormalities

Bilirubin is produced from heme obtained from the destruction of red blood cells (RBCs). It is an insoluble molecule which is found bound to albumin in blood and transported to hepatocytes to be conjugated to glucuronic acid where it can be excreted through specific transporter into bile ducts to the intestine by feces or urine in the form of urobilinogen (Roche and Kobos 2004; Wolkoff, 2005). The increase in bilirubine level in the blood is called hyperbilirubinemia and results from three conditions depending on the three phases of bilirubin metabolism;

Prehepatic hyperbilirubinemia is caused by increased hemolysis and increased degradation of heme, e.g., patients with sickle cell anemia and other hemolytic diseases. Prehepatic jaundice is indicated by relatively normal serum conjugated bilirubin, increased unconjugated bilirubin, and increased urinary urobilinogen that correlate normal serum levels of hepatocellular and hepatobiliary enzymes, with the exception of lactate dehydrogenase (LDH) and possibly aspartate transaminase (AST). These two enzymes are found in erythrocytes so, in situations of increased red cell breakdown, these enzyme concentrations are elevated in the serum.

Hepatic hyperbilirubinemia is generally due to defective uptake by hepatocytes resulting in increased serum unconjugated bilirubin and/or defective conjugation of bilirubin in the hepatocyte. Causes include the genetic *Gilbert's* and *Crigler-Najjar syndromes*, *Neonatal jaundice*, and, *viral and chemical damage to hepatocytes*. Hepatic jaundice is indicated by increased serum conjugated and unconjugated bilirubin and increased urinary urobilinogen that correlate with increased serum levels of hepatocellular enzymes.

Posthepatic hyperbilirubinemia (*obstructive jaundice*) is generally due to a defect in transporting conjugated bilirubin and bile out of the liver. It can involve obstruction of the small canaliculi

within the liver, the hepatic bile duct, and the common bile duct. Obstruction of the bile flow can be due to cirrhosis, gallstones or tumors. Posthepatic jaundice is indicated by relatively normal serum unconjugated bilirubin, increased conjugated bilirubin, and decreased urinary urobilinogen that correlate pronounced elevations of hepatobiliary enzymes but normal to slightly elevated serum levels of hepatocellular enzymes.

1.1.5.2. Ammonia Metabolism

Ammonia is produced from the normal metabolism of protein and by some bacteria in intestinal tract. It is converted into urea by parenchymal liver cells and is excreted by the kidneys as the major non-protein nitrogen waste product. Normally, the plasma ammonia level is ~20 μg/dL and plasma urea level is ~20 mg/dL.

Plasma levels of ammonia will rise when the liver is not functioning properly, such as in Reye's syndrome, inherited deficiencies of urea cycle enzymes, cirrhosis, serious drug toxicity, liver tumors or excessive nitrogen turnover from gastrointestinal bleeds. In Reye's syndrome, elevation of hepatic enzymes such as AST and ALT is associated.

1.1.5.3. Liver enzymes and Serum proteins

The liver contains thousands of enzymes, some of which are present in the serum in very low concentrations due to normal rate of tissue wear and tear. Among the enzymes are LDH, ALP, ALT, AST and GGT which are found in different compartments with in the liver and also in different organs. The elevation of a given enzyme activity in the serum is thought to primarily reflect its increased rate of entrance into serum from damaged or hyperactive liver cells. The extent of the damage is indicated by the release of these enzymes from different sources with in a cell.

Serum contains a large variety of proteins and a large amount of total protein, averaging 7.0 g/dL in the adult. There are two main types of proteins: albumin and globulins. Serum albumin level is 4 g/dl and makes up roughly half of the total serum proteins. On the other hand, there are four globulins, α_1 and α_2, β and γ globulins. Concentration of globulins is calculated by subtracting the albumin concentration from the total serum proteins.

1.1.5.4. Coagulation Factors

With the exception of factor VIII, the blood clotting factors are made exclusively in hepatocytes. They have short half-lives that they are the single best acute measure of hepatic synthetic function and helpful in both the diagnosis and assessing the prognosis of acute parenchymal liver disease. Useful for this purpose is the serum prothrombin time, which collectively measures factors II, V, VII, and X. The prothrombin time may be elevated in hepatitis and cirrhosis as well as in disorders that lead to vitamin K deficiency such as obstructive jaundice or fat malabsorption of any kind.

1.1.5.5. Other diagnostic approaches

Other tests include anti-mitochondrial antibodies in primary biliary cirrhosis and anti-smooth muscle and antinuclear factor antibodies in chronic active hepatitis, procollagen type III terminal peptide and hyaluronic acid serum level in hepatic fibrosis. Disordered metabolism inferred from hippuric acid test (a detoxification assay), bromosulphthalein retention test (a dye excretion assay), plasma lipogram (high), and hypoglycemia and galactose, fructose and epinephrine tolerance test (abnormal carbohydrate metabolism assay). They also include plasma and urinary amino acids and their metabolites as abnormal indicators of their hepatic metabolism. Alpha-fetoprotein is produced by the fetal liver and testes indicating hepatitis or hepatocellularcarcinoma.

1.2. Antiretroviral drug induced hepatotoxicity

All three classes of Antiretroviral Therapy (ART), i.e., inhibitors NRTI, NNRTI and PIs have been associated with hepatotoxicity. According to AIDS Clinical Trials Group (ACTG), there are hepatotoxicity range often refers to Grade 1 up to 4 elevations in ALT, AST, ALP and bilirubin.

Table 1.2: ACTG Grading of Liver Toxicity

	ALT/ AST	ALP	Bilirubin
Grade 1	1.25-2.5 × UNL	1.25-2.5 × UNL	>1.0-1.5 × ULN
Grade 2	>2.5-5.0 × UNL	>2.5-5.0 × UNL	>1.5 × ULN
Grade 3	>5.0 × UNL	>5.0 × ULN	>2.5-5 × ULN
Grade 4	>10 × UNL	>10 × ULN	>5 × ULN

The severity of hepatotoxicity may range from the absence of symptoms to liver decompensation, and the outcome can range from spontaneous resolution to liver failure and death (Clark et al., 2002; Kramer et al., 2005). In one study, severe hepatotoxicity with acute hepatic necrosis was recognized in 2% of HIV infected patients dying from liver disease. Furthermore, in a large ACTG cohort of nearly 3000 patients initiating HAART, the most common grade 4 adverse events were liver related; this risk was increased in patients with underlying chronic viral hepatitis (Reisler et al., 2003). Fortunately, the vast majority of episodes of hepatotoxicity are asymptomatic, and most ALT elevations resolve spontaneously, as described for many other medications, probably through a process called 'adaptation' (Kaplowitz, 2002).

The reported incidence of severe liver toxicity of HAART ranges from 2 to 18%. Differences in study outcomes may reflect heterogeneity in patient populations, frequency of liver enzymes determinations, other exogenous exposures (e.g., alcohol), medication prescribing patterns, prevalence of chronic viral hepatitis, and criteria used for defining severe hepatotoxicity (Vincent et al., 2008).

Predisposing factor like alcohol increased risk of hepatotoxicity in a study (Nunez et al., 2001). Chronic use may also predispose to hepatocyte injury by increasing oxidative damage to mitochondrial DNA and depleting stores of glutathione, an important scavenger of free oxygen radicals (Fromenty and Pessayre, 1997). On the other hand, multiple studies have demonstrated that the risk of liver injury is increased in those with aminotranferase elevations prior to initiating HAART (Vincent et al., 2008). Other risk factors associated with hepatotoxicity include older age (Nunez et al., 2001), female gender (Aceti et al., 2002; Martin et al., 2003), first exposure to antiretroviral treatment (Wit et al., 2002) and significant CD4 cell gains following HAART initiation (Sulkowski et al., 2000; Sulkowski et al., 2002). More recently, an association between the presence of advanced stages of liver fibrosis and greater risk of hepatotoxicity has been reported (Aranzabal et al., 2005). The mechanism for this observation is unclear but it could be the consequence of compromised hepatic clearance with subsequent drug overexposure in patients with cirrhosis (Barreiro et al., 2007). Chronic hepatitis B and/or hepatitis C infection has also been found as risk factors in different studies (Vincent et al., 2008).

Hepatitis B and hepatitis C Viruses (HBV and HCV)

Hepatitis B and hepatitis C viruses are two of the five hepatitis viruses (HAV to HEV) causing hepatitis ranging from asymptomatic and inapparent to fulminant and fatal acute infections common to all types, on the one hand, and from subclinical persistent infections to rapidly progressive chronic liver disease with cirrhosis and even hepatocellular carcinoma, common to the bloodborne types (HBV, HCV, and HDV), on the other (Dienstag and Isselbacher, 2005).

Hepatitis B virus is a DNA virus with a remarkably compact genomic structure which is small, circular, 3200-basepair in size. It is now recognized as one of a family of animal viruses, hepadnaviruses (hepatotropic DNA viruses), and is classified as hepadnavirus type 1. It replicate in the liver but exist in extrahepatic sites, contain their own endogenous DNA polymerase, have partially double-stranded and partially single-stranded genomes, are associated with acute and chronic hepatitis and hepatocellular carcinoma, and rely on a replicative strategy unique among DNA viruses but typical of retroviruses. Instead of DNA replication directly from a DNA template, hepadnaviruses rely on reverse transcription (effected by the DNA polymerase) of minus-strand DNA from a "pregenomic"RNA intermediate. Then plus-strand DNA is transcribed from the minus- strand DNA template by the DNA- dependent DNA polymerase and converted in the hepatocyte nucleus to a covalently closed circular DNA, which serves as a template for messenger RNA and pregenomic RNA. Viral proteins are translated by the messenger RNA, and the proteins and genome are packaged into virions and secreted from the hepatocyte.

Hepatitis C virus is a linear, single-strand, positivesense, 9600-nucleotide RNA virus, the genome of which is similar in organization to that of flaviviruses and pestiviruses; HCV is the only member of the genus *Hepacivirus* in the family Flaviviridae. The HCV genome contains a single large open reading frame (gene) that codes for a virus polyprotein of approximately 3000 amino acids. The 5' end of the genome consists of an untranslated region (containing an internal ribosomal entry site) adjacent to the genes for structural proteins, the nucleocapsid core protein and two envelope glycoproteins, E1 and E2/NS1. The 5' untranslated region and core gene are highly conserved among genotypes, but the envelope proteins are coded for by the hypervariable region, which varies from isolate to isolate and may allow the virus to evade host immunologic containment directed at accessible virus-envelope proteins. The 3' end of the genome contains the genes for nonstructural (NS) proteins. The first reported HCV clone, 5-1-1, and the

nucleotide sequence coding for C100-3, the recombinant virus protein used in the first immunoassay for antibodies to HCV, reside within the NS4 gene, and the RNA-dependent RNA polymerase, through which HCV replicates, is encoded by the NS5 region.

Evidence suggests that the clinical manifestations and outcomes after liver injury associated with viral hepatitis are determined by the immunologic responses of the host. The most experimental support HBV associated hepatitis involves cytolytic T cells sensitized specifically to recognize host and hepatitis B viral antigens on the liver cell surface. Cell-mediated immune responses and elaboration by T cells of antiviral cytokines contribute to the containment of infection and pathogenesis of liver injury associated with hepatitis C.

1.2.1. NRTIs hepatotoxicity

Mechanisms of drug injury observed with NRTI mainly include mitochondrial toxicity and hypersensitivity reactions. Early clinical trial data from the late 1980s demonstrated that NRTI may be associated with high rates of moderate to severe hepatotoxicity, ranging from 7% with zidovudine, 9–13% with stavudine and 16% with didanosine (Ogedegbe and Sulkowski, 2003). Newer NRTI such as emtricitabine, abacavir and tenofovir are associated with a low incidence of mild asymptomatic aminotransferase elevations (Birkus, 2002). Mitochondrial toxicity is an infrequent but distinctive type of hepatotoxicity associated with the use of NRTI that may evolve to acute liver failure with severe hepatomegaly and lactic acidosis (Brinkman et al., 1998). This complication generally occurs after several weeks or months of NRTI treatment. However, nucleoside analogues differ widely in their propensity to induce mitochondial toxicity. In-vitro data support additive or synergistic mitochondrial toxicity of some NRTI combinations (Walker et al., 2002) such as stavudine and didanosine (ter Hofstede et al., 2000; Gisolf et al., 2000; Walker et al., 2002).

Incidents of unexplained liver disease in HIV-infected individuals have recently been reported in which clinical manifestations of portal hypertension are often predominant. Didanosine exposure seems to be involved in almost all and nodular regenerative hyperplasia is a frequent histological finding (Maida et al., 2006, Mallet et al., 2007).

1.2.2. NNRTIs hepatotoxicity

Although registration trials of NVP or EFV demonstrated acceptable toxicity profiles, postmarketing reports of severe hepatotoxicity associated with nevirapine have focused attention on this particular agent. Two distinct patterns of drug injury associated with EFV use have emerged: hypersensitivity reactions and direct drug related toxicity (Gonzalez et al., 2002).

Hypersensitivity reactions: In patients taking NVP, the overall incidence of symptomatic events involving the liver enzymes is approximately 5% (Stern et al., 2003; Ritchie et al., 2006). However, severe liver toxicity, occurring with early latency, has been reported in HIV-infected and HIV-seronegative individuals. In an HIV treatment trial assessing the efficacy and safety of Emtriva, a higher incidence of hepatotoxicity was observed in patients assigned to the NVP arm than in those in the EFV arm (Sanne, 2000). Hepatotoxicity predominated in black women, often in association with rash and fever and was consistent with a drug hypersensitivity reaction. Further analysis revealed that these events appeared to be linked to nevirapine use in women with CD4 cell counts >250 cells/ml, emphasizing the importance of host immunity and neoantigen recognition in hypersensitivity reactions (Leith et al., 2005). Boehringer-Ingelheim subsequently released a warning on the risk of severe liver toxicity, in some occasions with fatal outcome, and currently only recommends the use of NVP in women with a CD4 cell count <250 cells/ml and in men with <400 cells/ml. Interestingly, a recent study has refuted the role of immunocompetence as a risk factor for nevirapine toxicity (Manfredi and Calza, 2006).

Other risk factors for nevirapine-associated hepatotoxicity include low body mass index (Sanne et al., 2005) and host genetics; persons with an HLA-DRB1*0101 background have an increased propensity for developing nevirapine-associated hypersensitivity (De Maat et al., 2007; Johnson et al., 2002; Martin et al., 2005).

Idiosyncratic drug-related toxicity: In other studies, a different pattern of drug injury with NVP use has emerged, with onset of liver enzyme elevations occurring beyond 16 weeks of therapy, consistent with direct or idiosyncratic host-mediated liver injury (Martı´nez et al., 2000; Sulkowski et al., 2002; Martı´n et al., 2003). This late onset of hepatotoxicity with NNRTI is more common in patients with underlying chronic viral Hepatitis B Virus, Hepatitis C Virus (HBV) and/or (HBC) infection, as has been described with many other antiretroviral agents.

In patient populations that vary in terms of chronic viral hepatitis prevalence, NNRTI-associated liver injury can vary from 15% (Sulkowski et al., 2002) to as low as 3% (Palmon et al, 2002). Specific genetic polymorphisms of metabolizing enzymes and drug transporters may also increase the risk of this complication (Rotger et al., 2005; Ritchie et al., 2006).

It should be highlighted that hepatotoxicity with either NVP or EFV does not appear to increase the risk of developing liver injury on exposure to the alternative NNRTI (Soriano et al., 2000; Manosuthi et al., 2006). The current study emphasizes more on NNRTIs, particularly on the hepatotoxicity of EFV and NVP.

1.2.3. Protease inhibitors

The phenomenon of hepatotoxicity became more evident after the introduction of PI drugs. Rates of hepatotoxicity from registration trials of various PI have ranged from 1% to 9.5%, but few patients had serious liver-related outcomes (Sulkowski, 2004). In comparison with other drugs in its class, full-dose ritonavir has consistently been shown to be more hepatotoxic (Sulkowski et al., 2000; Bonfanti et al., 2001; Wit et al., 2002). However, the use of low-dose ritonavir for pharmacokinetic boosting of other PI drugs appears to be safe (Cooper et al., 2002).

Although there are a few case reports of liver-related toxicity with indinavir, these were in association with advanced liver disease; dose reduction is recommended in patients with cirrhosis. Several cases of clinical hepatitis and hepatic decompensation, including some fatalities, have been associated with the use of tipranavir, particularly in patients with chronic HCV infection (Kandula et al., 2005; Hicks et al., 2006). Nelfinavir, saquinavir, atazanavir, fosamprenavir, lopinavir and darunavir are associated with a relatively safer liver toxicity profile (Vincent et al., 2008). APV has occasionally been associated with drug-related hypersensitivity reactions but only sporadically with severe hepatotoxicity (Goodgame et al., 2000).

1.3. Significance of the study

The use of combination drug regimens is likely to cause hepatotoxicity. Thus, it is essential to evaluate the frequency, type and magnitude of hepatotoxicity in HIV patients on ART in order to determine risk status in our population. The findings of the present study are anticipated to cast light on the importance of measuring liver function tests (LFTs) for patients on ART and could also be a stepping stone for future large scale prospective studies in the country.

1.4. Hypothesis

This study hypothesized that there is high prevalence of hepatotoxicity in Ethiopian HIV infected patients receiving HAART when the duration of therapy increases.

1.5. Objective of the study

1.5.1. General objective

The general objective of the study is to assess the liver functions of HIV-infected patients receiving HAART at TASH.

1.5.2. Specific objectives

1. To assess the changes of liver function parameters of HIV-infected patients receiving HAART as compared to control values from the local laboratory of TASH.
2. To assess regimen-specific impact of HAART on liver toxicity.
3. To assess the specific time-dependent regimen impact of the different HAART regimens on liver toxicity.
4. To assess the nature of correlation among the LFTs tests used.
5. To assess the possible worsening due to different types of co-infections and/or comorbid cancers.

2. Materials and Methods

2.1. Study Design

A cross sectional retrospective study was conducted on medical records of HIV infected patients receiving HAART.

2.2. Study Subject

All HIV-infected patients receiving HAART at VCT center of TASH were included for the study. The main group of the study was HIV-infected patients on HAART which were subdivided according to regimen of treatment: patients taking NVP based regimen and those taking EFV based. The duration of the treatment was from 1-18 month.

2.3. Study Area

The study was conducted in ART clinic in collaboration with voluntary counseling and testing (VCT) center of TASH in Addis Ababa from June 2009 to August 2010.

2.4. Sample Size Determination

The sample size for the present study was determined based on the standard formula for determination of sample size for estimating proportion. In this study the proportion of HIV patients is taken as 4.6% [*Hoffmann et al, 2007*], and assuming a 95% confidence interval and 1.5% marginal error, the calculated sample size required for the study is estimated to be 750.

$$n= p\ (1-p)\ (Z_{\alpha/2}/E)^{2}$$

Where: n is sample size, p is proportion, Z is confidence interval, and E is marginal error (Daniel, 1995).

2.5. Data Collection

All necessary information was collected from patient's medical record available at TASH using non-probability sampling method on structured formats (Annex I) consisting parts for collection of data on demographic characteristics (age and sex) and laboratory investigations ($CD4^{+}$ cells count, ALT, AST, ALP, Total Bilirubin) about sampled individuals.

2.6. Selection and exclusion criteria

2.6.1. Inclusion criteria

- All HIV patients, above the ages of 18 who are receiving HAART in VCT center of TASH since 1997 EC were selected.

2.6.2. Exclusion criteria

- Those with age below 18
- women who were pregnant
- Those who drink alcohol frequently
- Those who are on other medications
- Those who were infected with hepatitis B and C, and tuberclosis were excluded.

2.7. Specimen Collection and storage

The blood collection was done in the morning 8 to 11 AM, after 8 to 12 hours overnight fasting. About 10 ml venous blood was collected aseptically from each study participant into two tubes: a 5 ml vacutainer plain tube and a 5 ml vacutainer di-Sodium-EDTA anti-coagulated tube. Each blood specimen vial was labeled with the Study participant lab serial number.

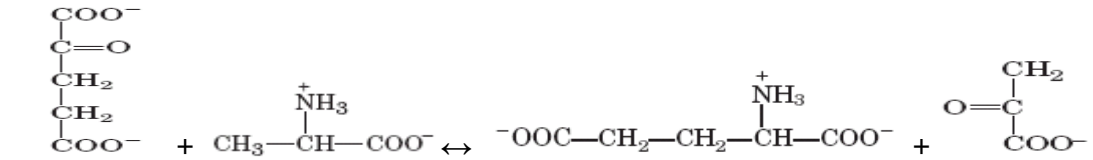
Serum was separated from the plain tube with centrifugation at 3500 rpm (1000g) for 10 min following coagulation at room temperature for a minimum of 2 hours minute standing upright. The serum was separated into a sterile test tube aseptically using sterile Pasteur pipettes and Clinical Chemistry assay was done immediately.

2.8. Methods for biochemical investigations

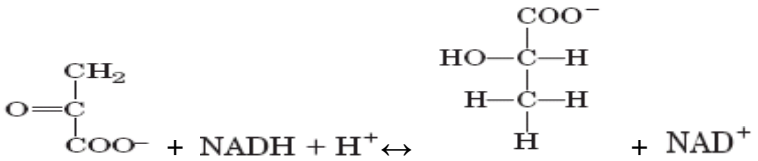
2.8.1. Assessment of the Alanine Transaminase (ALT)

Principle: The method utilizes a coupled reaction involving pyridoxal phosphate (PLP) and lactate dehydrogenase (LDH) at 37 ^{o}C with continuous monitoring of the decrease in absorbance at 340 nm due to consumption of $NADH.H^{+}$. Serum free from hemolysis (erythrocyte is a source of ALT) is used. Maintaining substrate concentration in excess, as well as stable temperature, are also critical for accurate enzyme analysis. Stability of the enzyme activity can be maintained by refrigeration of the sample for up to 3 days and freezing the sample for up to 30 days. In the first

reaction, ALT reversibly converts alanine into pyruvate couple with conversion of α-ketoglutarate into glutamate in the presence of PLP. In the second reaction, pyruvate reacts with $NADH.H^+$ to produce lactate and NAD^+ catalyzed by lactate dehydrogenase. The normal range is 8 - 45 U/L for adults (Dufour et al 2000a and b; Arneson and Brickell, 2007).



α-Ketoglutarate L-Alanine L-Glutamate Pyruvate



Pyruvate L-Lactate

Figure 2.1: Reaction principle for the determination of alanine aminotransferase (ALT)

Reagents:

Reagent 1: TRIS buffer pH 7.5, 110 mM/L containing L-alanine, 600 mM/L and LDH, 1500 U/L.

Reagent 2: Alpha-ketoglutarate, 16 mM/L and NADH, 0.24 mM/L.

Working reagent: 10 volumes of reagent 1 are mixed with one volume of reagent 2.

Procedure:

1. All reagents and samples were brought to room temperature.
2. 100 µL of serum and 1 mL of working reagent were mixed.
3. Incubation of the mixture at 37 ^oC had taken place for 1 minute.
4. Using distilled water as blank the spectrophotometer was adjusted to zero at 340 nm.
5. The change of absorbance per minute (ΔA/min) was measured for a period of 3 minutes was recorded at 340 nm. When ΔA/min exceeds 0.150, the test was repeated using serum diluted 1/10 with saline solution (9 g/L) and the result was multiplied by 10.

Calculation: ALT concentration (U/L) was calculated using the following formula:

ALT activity (U/L) = ΔA/min X (TV/SV) X (10^6/ϵ) = ΔA/min X 1746

Where: ΔA/min = the change of absorbance per minute,

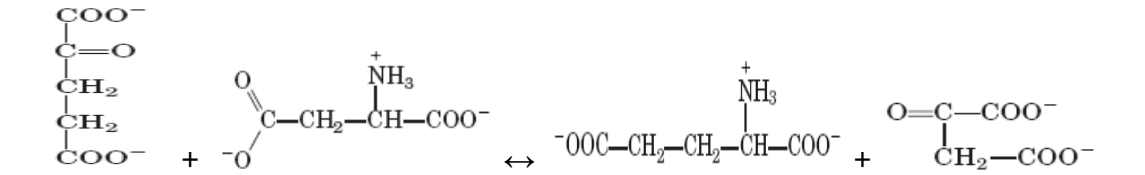
TV = the total volume,

SV = the specimen volume, and

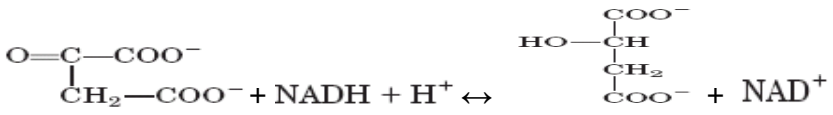
ϵ = the molar absorpitivity for NADH (6.3 X 10^3 mol^{-1}. cm^{-1})

2.8.2. Assessment of the Aspartate Transaminase (AST)

Principle: The method utilizes a coupled reaction involving pyridoxal phosphate (PLP) and malate dehydrogenase at 37 ^oC (rate of the product formation differs with temperature) with continuous monitoring of the decrease in absorbance at 340 nm due to consumption of $NADH.H^+$. Serum free from hemolysis (erythrocyte is a source of AST) is used. Alcohol and drugs such as opiates, salicylates, or ampicillin may increase AST activity. In the first reaction, AST reversibly converts aspartate into oxaloacetate; coupled with conversion of α-ketoglutarate into glutamate, in the presence of PLP. In the second reaction, the produced oxaloacetate reacts with $NADH.H^+$ to produce malate and NAD^+ catalyzed by malate dehydrogenase. The rate of decrease in UV absorbance due to $NADH.H^+$ is monitored at 340 nm. The normal range for adults is 8 - 40 U/L (Dufour et al 2000a and b; Arneson and Brickell, 2007).



α-Ketoglutarate L-Aspartate L-Glutamate Oxaloacetate



Oxaloacetate L-Malate

Figure 2.2: Reaction principle for the determination of aspartate aminotransferase (AST)

Reagents:

Reagent-1: TRIS buffer pH 7.8, 88 mM/L; L-aspartate, 260 mM/L; and malate dehydrogenase, 900 U/L.

Reagent-2: Alpha-ketoglutarate, 12 mM/L and NADH, 0.24 mM/L.

Working reagent: 10 volumes of reagent 1 are mixed with one volume of reagent2

Procedure:

1. All reagents and samples were brought to room temperature.
2. 100 µL of serum and 1 mL of working reagent were mixed.
3. Incubation of the mixture had taken place at 37 ^{o}C for 1 minute.
4. Using distilled water as blank the spectrophotometer was adjusted to zero at 340 nm.
5. The change of absorbance per minute (ΔA/min) for a period of 3 minutes were recorded. When ΔA/min exceeds 0.150, the test was repeated using serum diluted 1/10 with saline solution (NaCl 9 g/L) and the result was multiplied by 10.

Calculation: The AST level (U/L) was calculated using the following formula:

$$\textbf{AST activity (U/L)} = \Delta A/min \times (TV/SV) \times (10^6/\epsilon) = \Delta A/min \times 1746$$

Where: ΔA/min = the change of absorbance per minute,

TV = the total volume,

SV = the specimen volume, and

ϵ = the molar absorpitivity for NADH (6.3 X 10^3 mol^{-1}. cm^{-1})

2.8.3. Assessment of the Alkaline Phosphatase (ALP)

Principle: The modified method of Bowers and McComb is utilized based on the ability of ALP to hydrolyze 4-nitrophenyl phosphate into 4-nitrophenoxide in presence of water and Mg^{2+} at pH 10.3 and 37 ^{o}C with continuous or endpoint monitoring of the increase in absorbance at 405 nm due to production of 4-nitrophenoxide. Maintaining substrate concentration in excess, as well as stable temperature, are critical for accurate enzyme analysis. There may be slight variation due to gender. Serum within less than 3 hours of collection may be used. Analysis of fresh serum is preferred since serum show alkalinity increases over time and leads to consequent time-dependent increases in ALP activity. Since lipids, hemoglobin, or bilirubin absorb light at 405

nm, they may interfere. Since ALP is found in high concentration in hepatobiliary cells, inflammation or obstruction of the biliary ducts (cholestasis, i.e., obstruction of the flow of bile) and disruption of their cells causes the release of ALP into the circulation to a level 3 - 10 times the normal levels. The normal range for adult male ranges from 80 – 306 U/L and 80 – 306 U/L in adult females (Dufour et al ,2000a and b; Arneson and Brickell, 2007).

4 – nitrophenyl phosphate + $H_2O \rightarrow$ 4- nitrophenoxide +P_i

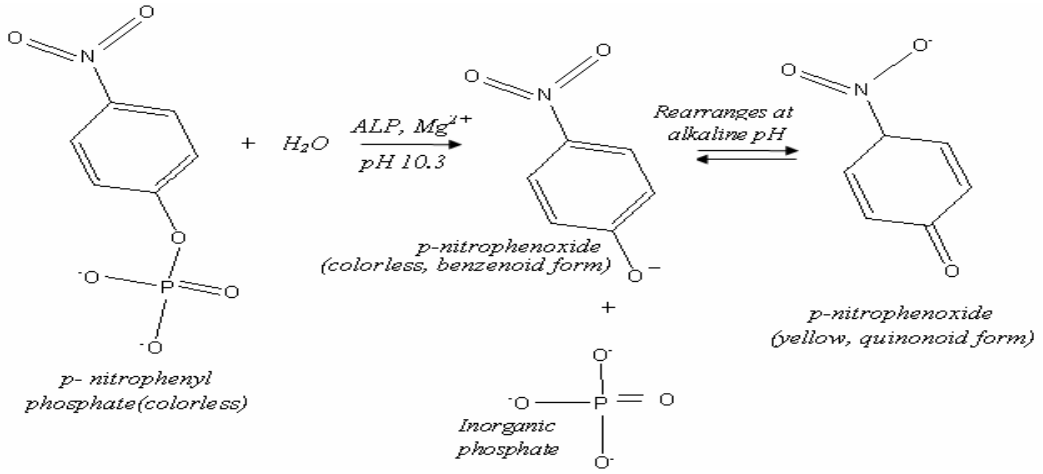


Figure 2.3: Reaction principle for the determination of Alkaline phosphatase (ALP)

Reagents:

Reagent-1: DEA buffer pH 10.2, 1.25 mM/L and magnesium chloride 0.625 mM/L.

Reagent-2: 4-nitrophenylphosphate 50 mM/L.

Working reagent: 4 volumes of reagent 1 are mixed with one volume of reagent 2.

Procedure:

1. All reagents and sapmples ware brought to room temperature.
2. 20 µL of serum was mixed with 1 mL of working reagent
3. The mixture was incubated at 37 ^{o}C for 1 minute.
4. Using distilled water as blank the spectrophotometer was adjusted to zero at 405 nm.

5. The change of absorbance per minute (ΔA/min) for a period of 3 minutes was recorded. When ΔA/min exceeds 0.250, the test was repeated using serum diluted 1/10 with saline solution (9 g/L) and the result was multiplied by 10.

Calculation: The ALP activity in U/L is calculated using the following formula:

ALP activity (U/L) = ΔA/min x (TV/SV) x (10^6/ϵ) = ΔA/min x 8095

Where: ΔA/min = the change of absorbance per minute,

TV = the total volume,

SV = the specimen volume,

ϵ = the molar absorpitivity for NADH (6.3 X 10^3 mol^{-1}. cm^{-1})

2.8.4. Assessment of the bilirubin level

Principle: The detection of the three forms of bilirubin (glucuronic acid-unconjugated, glucuronic acid-conjugated and albumin-bound) depends largely on the difference in their water solubility. The fast and direct reacting glucuronic acid-conjugated and albumin-bound delta bilirubins are soluble in water. The less water soluble glucuronic acid-unconjugated requires a reaction *accelerator* - usually an alcohol that is mostly methanol or sodium benzoate-caffeine mixture - that facilitate solubilizing bilirubin. Therefore, the so called "*Direct Bilirubin*" reflects serum, plasma, spinal fluid, or urine level of glucuronic acid-conjugated and albumin-bound bilirubin. The "*Total Bilirubin*" reflects the serum, plasma, spinal fluid, or urine level of all forms of bilirubin including the "*Indirect Glucuronic acid-Unconjugated Bilirubin"* measured after the addition of the accelerator. A diazonium salt, e.g., diazotized sulfanilic acid reacts with bilirubin to produce the colored azobilirubin. Lipemia may falsely elevate bilirubin measurement. Bilirubin may be broken down by light or heat and sample should be protected from these environmental conditions. The normal range for adult total bilirubin is 0.1 - 0.8 mg/dL (Dufour et al 2000a and b; Arneson and Brickell, 2007).

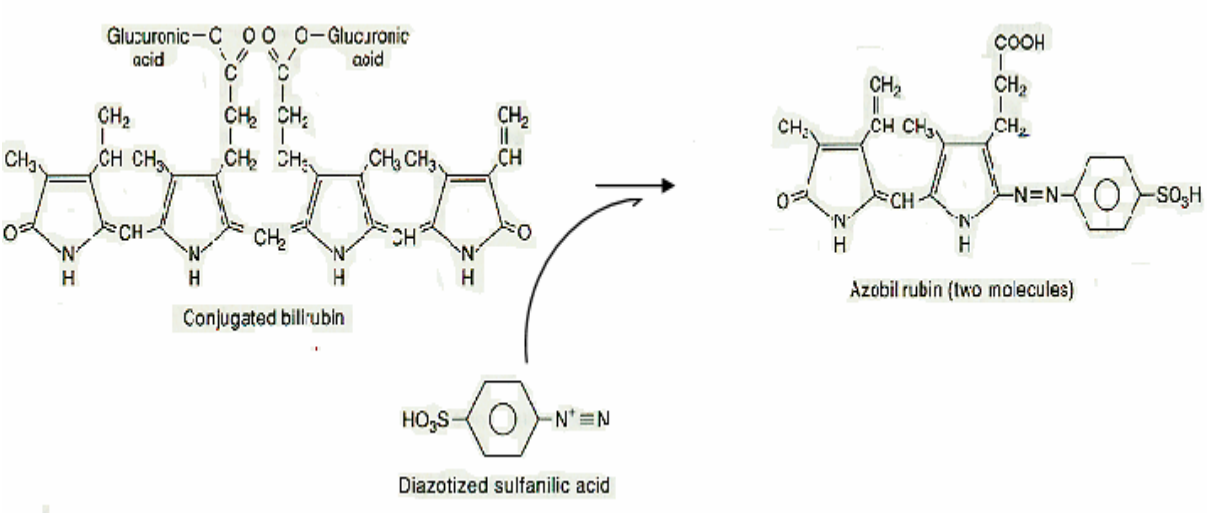


Figure 2.4: Reaction principle for the determination of bilirubin

Reagents:

Reagent-1: 3% methanol, 0.24 mM HCL and 29 mM Sulphanilic acid.

Reagent-2: 11.6 mM sodium nitrite.

Working reagent for total bilirubin is prepared by adding of 1 mL reagent 2 with 4 mL of reagent1.

Procedure:

Pipette in to reaction cell (cuvette)	Standard	Sample	Sample blank
All samples and reagents were brought to room temprature			
Standard	100 µl	-	-
Sample	-	100 µl	100 µl
Reagent 1	1 ml	1 ml	1 ml
Working reagent	1 ml	1 ml	-
Mixed thoroughly, allowed to stand for 2 minits at room temprature, The absorbance was measure against sample and reagent blanked at 540 nm.			

Calculation: The bilirubin concentration was calculated form the following equation,

$$\mathbf{Cs = As - Asb\ X\ Cst\ /Ast}$$

Where: Cs is concentration of sample

Cst = concentration of the standard

As = absorbance of sample

Asb = absorbance of sample blank

Ast = the absorbance of standard

2.9. Statistical analysis

All the data collected were coded and entered into Excel data sheet on computer. Double entry method was applied to preserve data quality. Analysis was done using the statistical package for the Social Science (SPSS Cary, NC, USA) version 13. Statistical analysis was performed as follows: base line characteristics were compared between NVP and EFV groups using x^2 test for categorical variables and Mann-Whitney test for continuous variables. Counts and percentages of patients were also used. The Spearman rank correlation coefficient was used to establish the degree of correlation between continuous variables and multivariant logistic regression was used to test if age and sex are risk factors for hepatotoxicity. Since the data collection is non parametric, the results are expressed in median. The minimum level of statistical significance was set at p-value <0.05.

2.10. Ethical Clearance

The ethical clearance was obtained from the Institutional Research Board (IRB) of the Addis Ababa University Faculty of Medicine. In order to maintain the confidentiality, a nurse (unlinked to the research) collected the necessary clinical information from the patient's clinical data record and each patient was assigned a number code. Laboratory data was collected from the log book of the Black Lion hospital laboratory by the investigator.

3. Results

3.1. Characteristics of patients in NVP and EFV groups before the start of HAART

Seven hundred fifty HIV positive patients who were on HAART were included in the study. Of these 521 were receiving NVP based regimen while 229 were receiving EFV based regimen. In both drug regimen groups, the proportion of female was higher than that of male. The median values of all variables: CD4, ALT, AST, ALP, TB in the two groups did not differ significantly (Table 3.1).

Table 3.1: Characteristics of variables for NVP and EFV groups before the start of HAART

Variables	NVP	EFV	P – value
Median Age (years)	35 (30 – 40)	35 (30 – 40)	0.45
Male	194 (37.2%)	95 (41.5%)	0.0001
Female	327 (62.8%)	134 (58.5%)	0.0001
n (%)	521 (69.5%)	229 (30.5%)	0.0001
P - value	0.001	0.001	
CD4 (cells/ml^3)	122 (71 – 173)	101 (53 – 170)	0.08
ALT (U/L)	24 (15 – 34)	27 (17 – 48)	0.06
AST (U/L)	30 (22 – 42)	34 (23 – 54)	0.07
ALP (U/L)	188 (158 – 255)	199 (142 – 283)	0.77
TB (U/L)	0.60 (0.50 – 0.60)	0.60 (0.40 – 0.70)	0.72

Note: *All figures for LFTs and age are present in median, while for male and female are in counts*

3.2. Population distribution and LFT median values before the start of HAART

Table 3.2 presents population distribution and median values of LFTs before the start of HAART of those patients who were receiving different combinations of ART drugs, 1a (n = 340), 1b (n = 92), 1c (n =181) and 1d (n = 137). 1a and 1c are NVP based while 1b and 1d are EFV based combination regimens.

Table 3.2: Population distribution and LFT characteristics before the start of HAART

Drug type	n	Median value of LFTs before the start of HAART			
		ALT (U/L)	AST (U/L)	ALP (U/L)	TB (U/L)
1a	340	23	31	189	0.6
1b	92	37	34	229	0.6
1c	181	24	29	189	0.5
1d	137	23	36	188	0.55

3.3. Population distribution and duration of therapy

Of the total patients included in the study 45.3%, 20.8%, 22.9%, and 10.9% were on their 3rd, 6th, 12th and 18th months of therapy respectively (Table 3.3). At each duration of therapy,a greater number of patients was on NVP treatment than EFV.

Table 3.3: Population distribution and duration of therapy

Duration of therapy (month)	**Drug type**		**n (%)**
	NVP	**EFV**	
3rd	249 (73.2)	91 (26.8)	340 (45.3)
6th	98 (62.8)	58 (37.2)	156 (20.8)
12th	109 (63.4)	63 (36.6)	172 (22.9)
18th	65 (79.3)	17 (20.3)	82 (10.9)

3.4. Proportion of patients receiving NVP and EFV with in each hepatotoxicity grade

Of the 750 patients recruited for the study, data for the liver function tests were available as, for ALT, 703 (93.7%); for AST, 702 (93.6%); for ALP, 652 (86.9%) and for TB, 655 (87.3%). Among the population for which data was available for liver function tests, ALT, AST, ALP and TB, 7.3%, 10.5%, 8.9% and 4.1% respectively had developed hepatotoxicity (1 – 4 grades).

The proportion of patients with in each hepatotoxicity grade is higher for NVP users as compared to EFV group (Figure 3.1). In addition, the table shows that more severe hepatotoxicity (grade 3 and 4) cases were seen in NVP users: ALT, 4 patients; AST, 5 patients; ALP, 1 patient and TB, 3 patients than EFV in which only one case of Grade 3 hepatotoxicity for ALT was observed.

3.5. Proportion of patients at different duration of therapy receiving NVP and EFV

Figure 3.2 shows proportion of patients on different duration of intake of NVP based regimen with the trend of hepatotoxicity. Results before the start of therapy showed higher proportion of patients in each hepatotoxicty grades and it generally decreased with time. After initiation of therapy, severe cases of hepatotoxicity were reported at 3rd and 12th month of duration of therapy for all LFTs. Values of ALT, AST, ALP and TB showed 3, 4, 1 and 2 severe hepatotoxicity cases at the 3rd month of therapy, respectively, while at 12th month, there was one case each as ALT, AST and TB measured. Like wise in those taking EFV based HAART, proportion of all grades of hepatotoxicity is higher before start of therapy decreasing with time. After initiation of therapy, only one case of severe hepatotoxicity at 12th month for ALT enzyme was found, as illustrated in Figure 3.3.

3.6. The change of LFTs with duration of therapy and HAART types

The median values of all LFTs were below the UNL level. No hepatotoxicity was indicated in all cases of the drugs with time as can be seen from Figure 3.4.

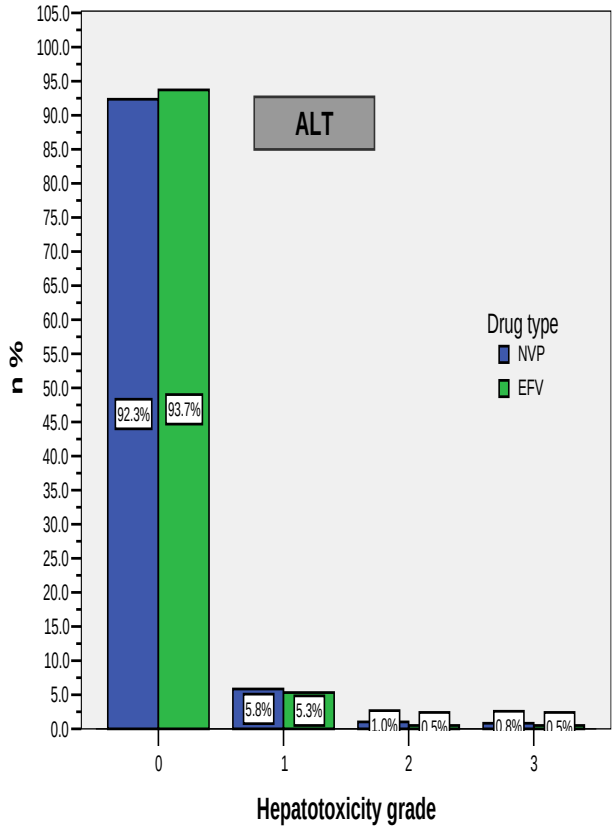


Figure 3.1 A

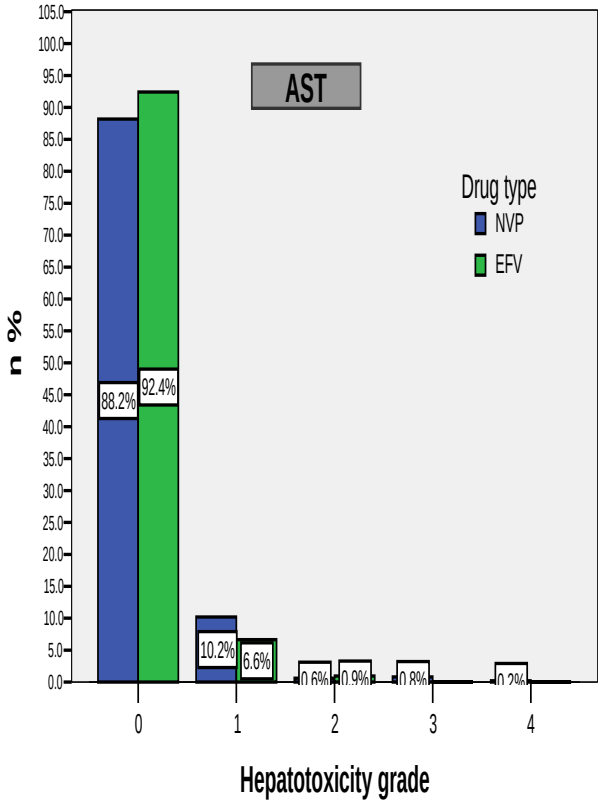


Figure 3.1 B

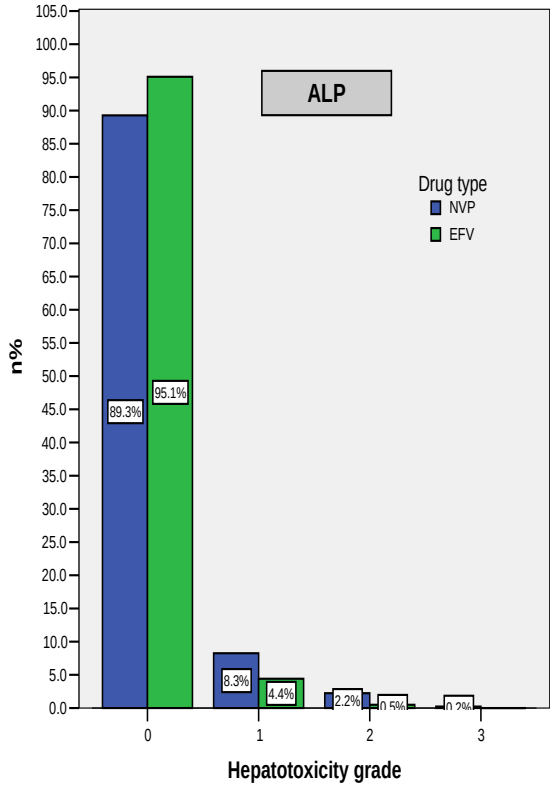


Figure 3.1 C

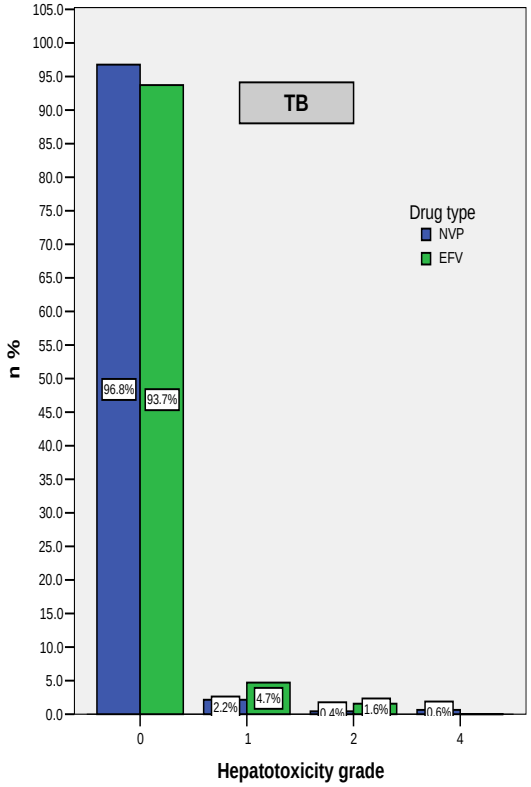


Figure 3.1 D

Figure 3.1: Proportion of patients taking NVP and EFV based regimen in the four hepatotoxicity grades: **A** for ALT, **B** for AST, **C** for ALP and **D** for T

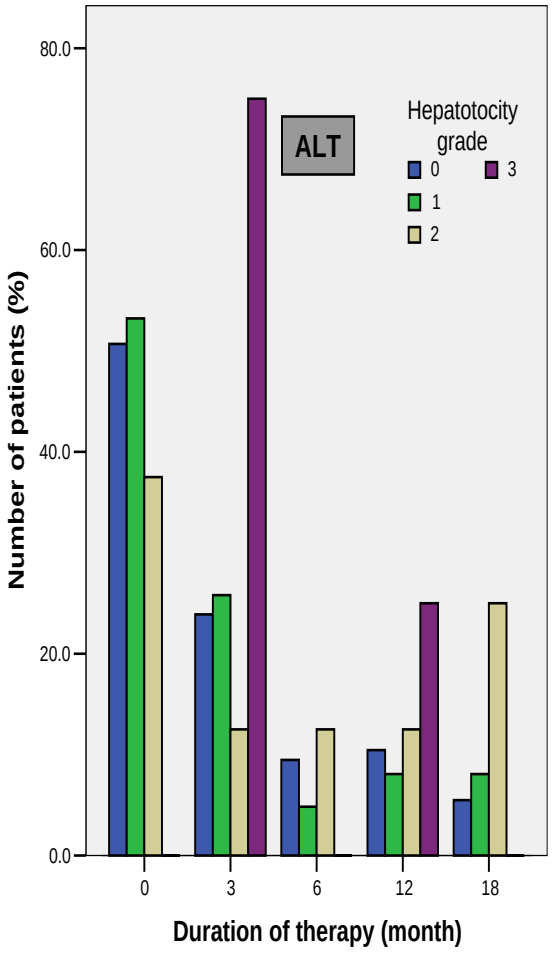


Figure 3.2 A

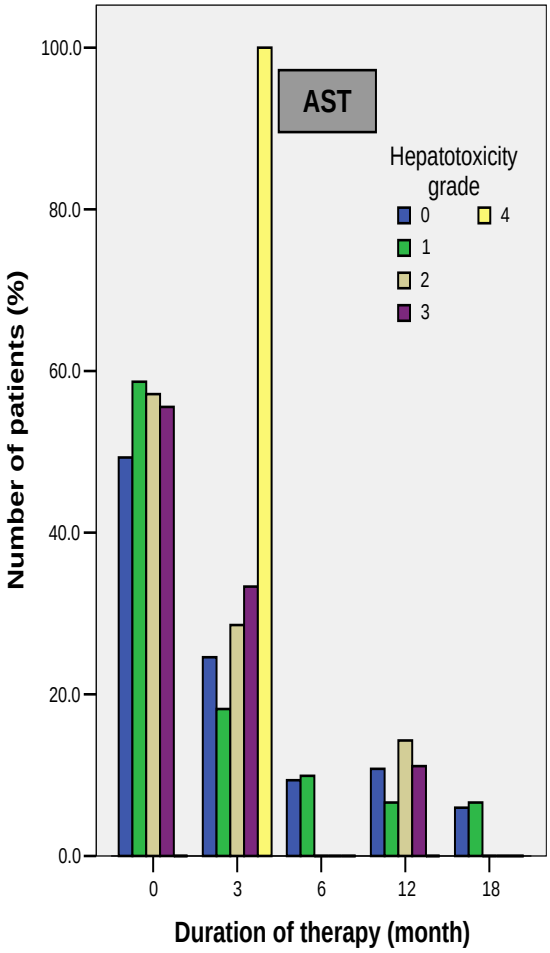


Figure 3.2 B

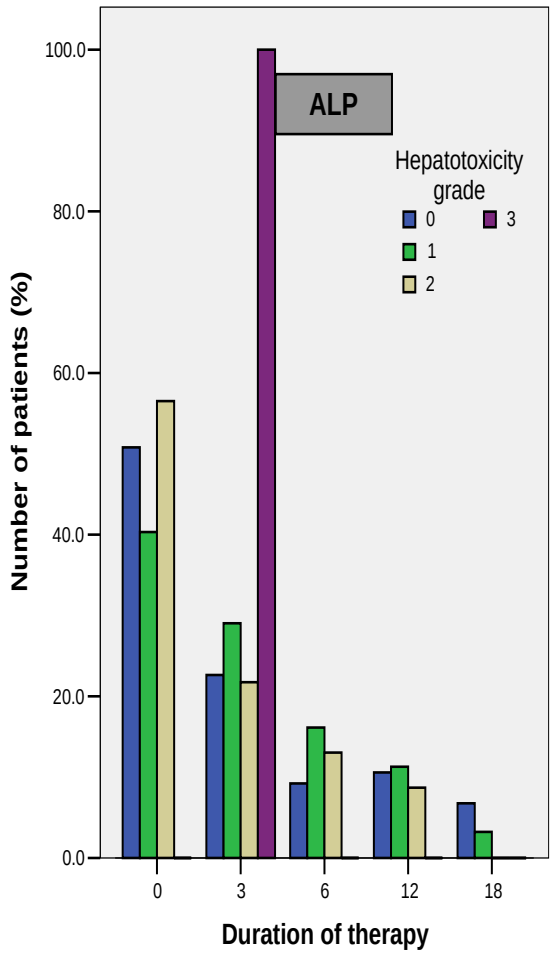


Figure 3.3 C

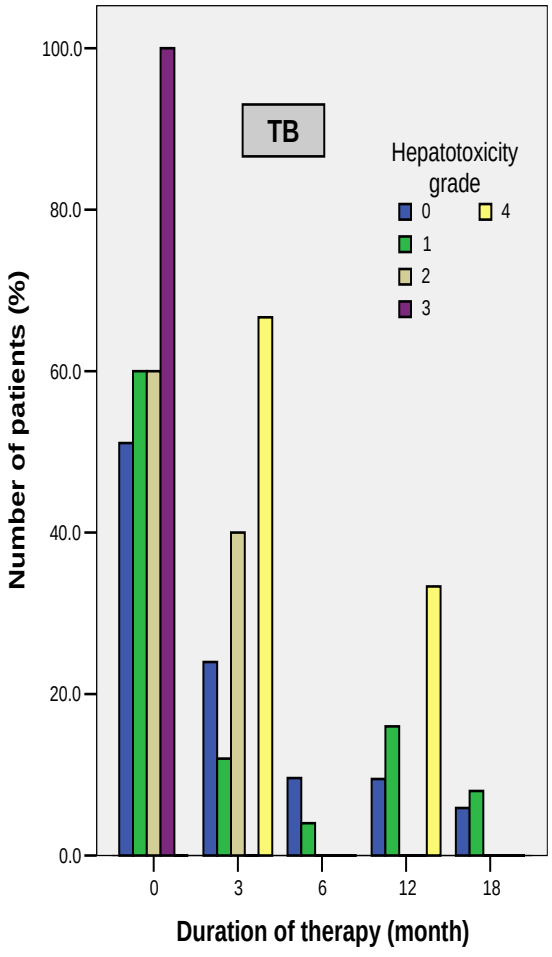


Figure 3.3 D

Figure 3.2: Hepatotoxicity of NVP and duration of therapy for the four LFTs: **A-** ALT**, B-** AST**, C-** ALP and **D-** TB

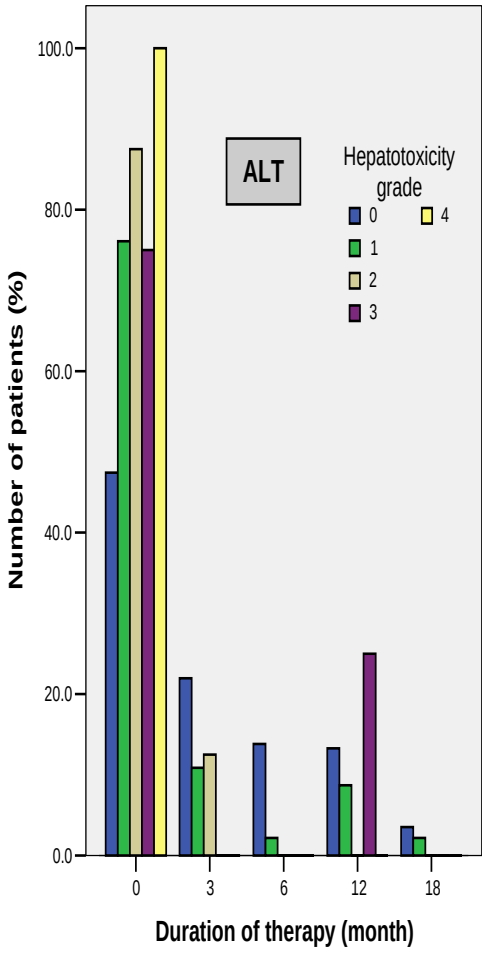


Figure 3.3 A

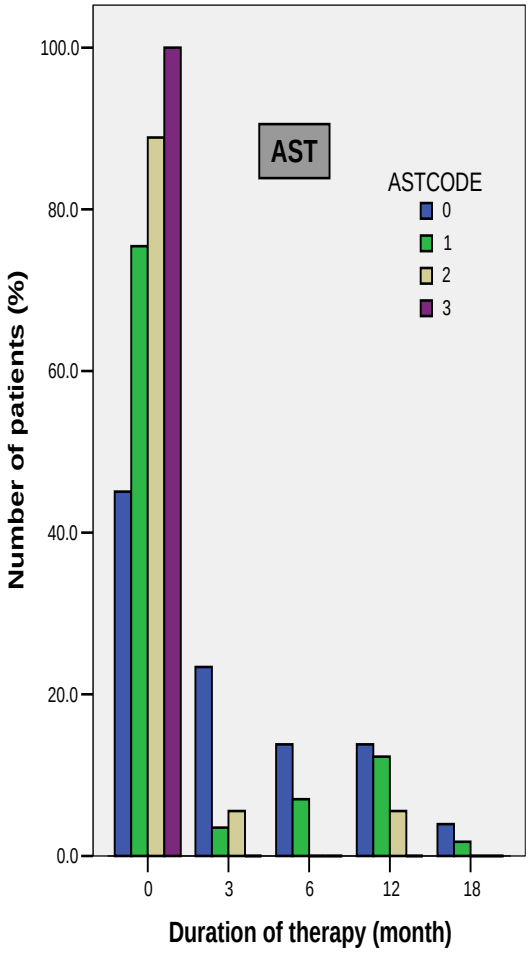


Figure 3.3 B

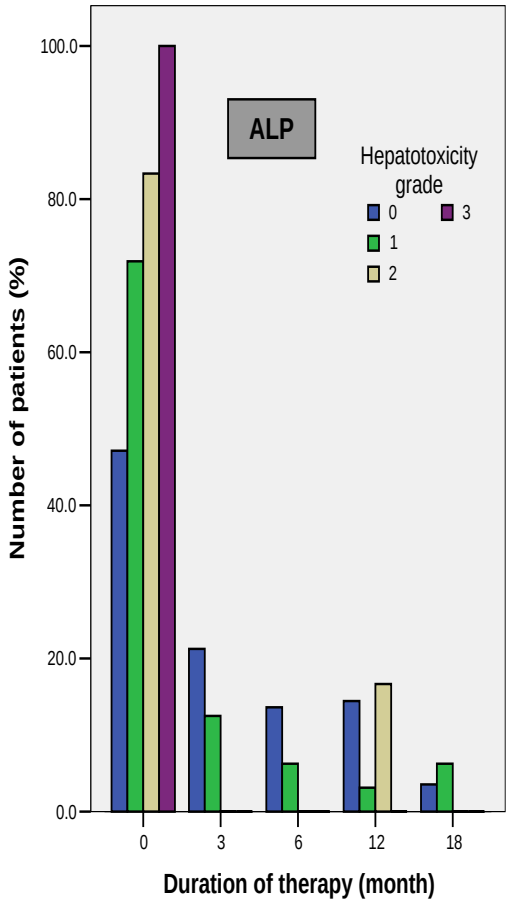


Figure 3.3 C

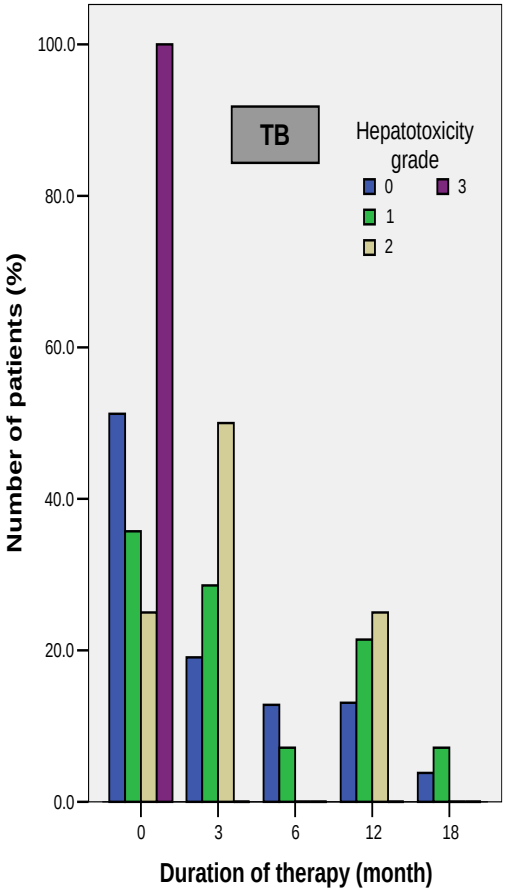


Figure 3.3 D

Figure 3.3: Hepatotoxicity EFV and duration of therapy for the four LFTs**, A-** ALT**, B-** AST**, C-** ALP and **D-** TB

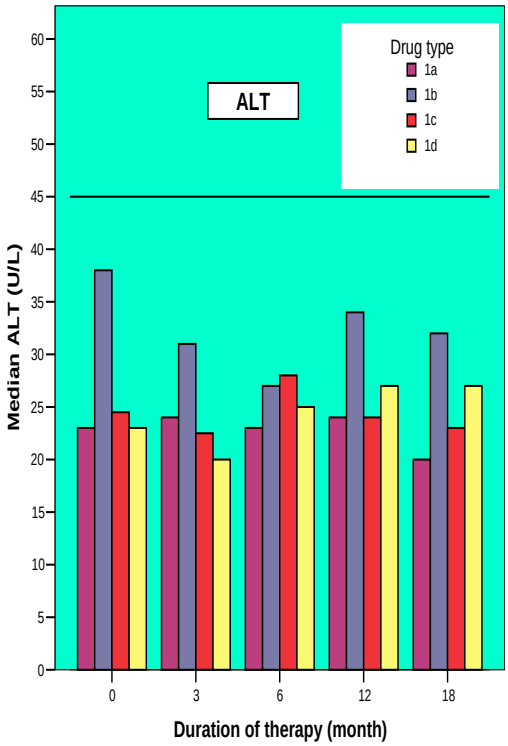


Figure 3.4 A

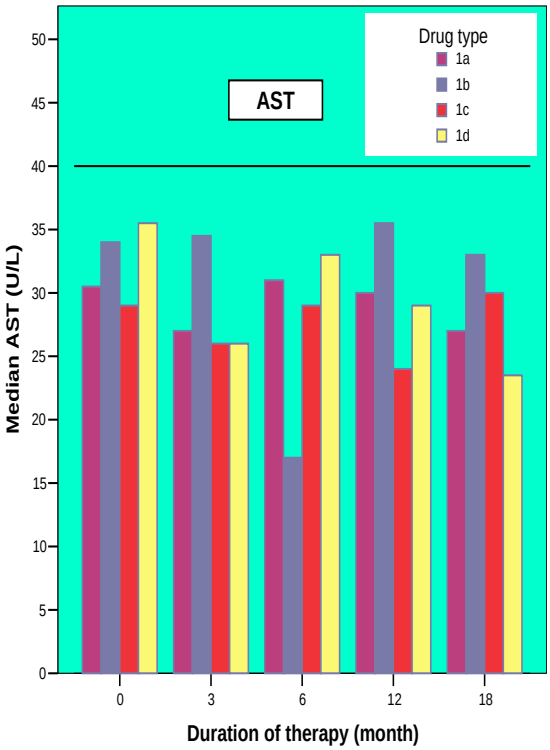


Figure 3.4 B

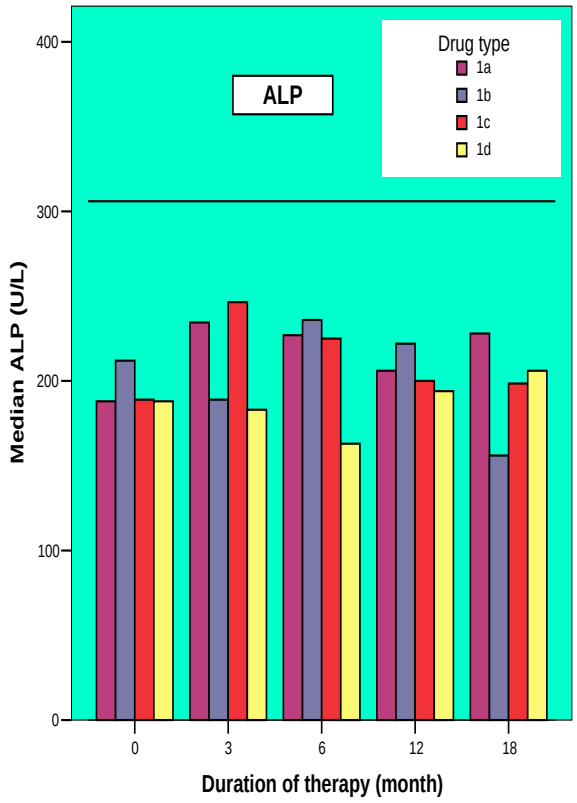


Figure 3.4 C

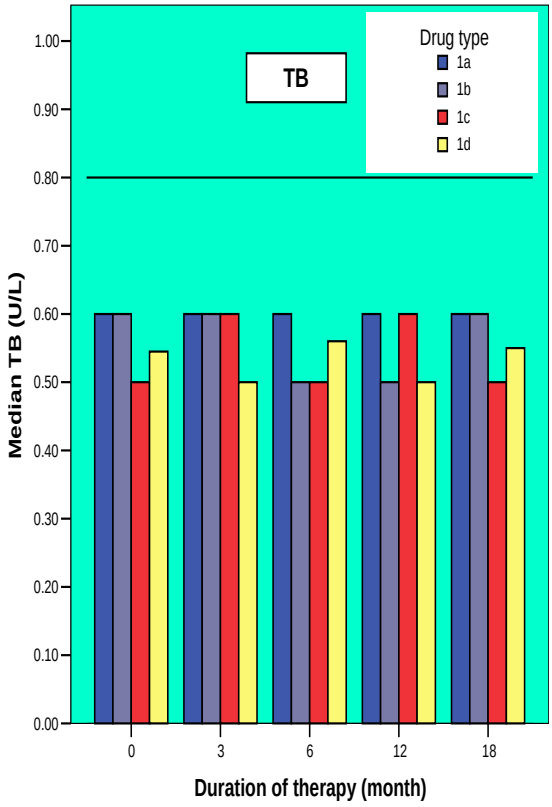


Figure 3.4 D

Figure 3.4: Pattern LFTs change with time for the four drugs: **A** – ALT, **B** – AST**, C** – ALP and **D** – TB

3.7. Correlation of pattern of change of the four LFTs with each other

As can be seen in Table 3.7, there was a positive correlation between the LFTs with significant difference ($p < 0.05$) but the correlation seen between TB and other LFTs was not significant ($p > 0.05$).

Table 3.4: Correlation of the four LFTs with each other in patients who have already started HAART

Enzyme		ALT	AST	ALP	TB
ALT	r	1	0.592	0.184	0.08
	p	-	0.0001	0.0001	0.07
AST	r	0.592	1	0.245	0.05
	p	0.0001	-	0.0001	0.27
ALP	r	0.184	0.245	1	0.08
	p	0.0001	0.0001	-	0.08
TB	r	0.079	0.048	0.077	1
	p	0.069	0.267	0.075	-

3.8. Risk factor association of hepatotoxicity of NVP based regimen with sex and age

Table 3.8 shows the association of sex and age with hepatotoxicity as calculated by multivariant logistic regression test. Neither of the variables was found to be risk factor for hepatotoxicity as can be seen from odd's ratio (OR).

Table 3.5: Risk factors for hepatotoxicy

Parameters	Variables	OR	CI (95%)	p - value
ALT	Sex	0.88	0.44 – 1.74	0.71
	Age	0.84	0.37 – 1.91	0.67
AST	Sex	0.77	0.43 – 1.35	0.36
	Age	0.26	0.77 – 2.60	0.26
ALP	Sex	0.89	0.48 – 1.68	0.73
	Age	0.81	0.38 – 1.72	0.59
TB	Sex	0.83	0.28 – 2.42	0.73
	Age	0.79	0.21 – 2.94	0.73

4. Discussion

4.1. Hepatotoxicity of NVP and EFV based regimens

In this study, it was found that there were patients with hepatotoxicity, even though the larger proportion of the population was in normal range. Most of the hepatotoxity cases were found to be in NVP user population when compared with EFV users. This is in agreement with Van et al., (2004), who compared the frequency of efavirenz- and nevirapine-induced toxicity in which 1217 naive patients were assigned to receive nevirapine 200 mg/12 h, nevirapine 400 mg/24 h, efavirenz 600 mg/24 h or nevirapine 400 mg/day in combination with efavirenz 800 mg/day, for 48 weeks. The subgroup of patients treated with efavirenz presented hypertransaminasaemia of grade 3 or above, according to ACTG criteria, with the lowest frequency of all the arms (4.5%), whereas the patients treated with nevirapine-based ART, once and twice per day, presented frequencies of 13.2% and 7.8%, respectively. One patient assigned nevirapine twice daily died due to an episode of fulminant hepatitis attributed to the use of this antiretroviral drug. There were no liver-related deaths in those subjects treated with efavirenz.

In our study, patients with severe hepatotoxicity grades were NVP users 4 (0.8%) out of 496 patient more than EFV users (0.5%) out of 207 patients. The NEFA trial evaluating the strategy of replacing a protease inhibitor (PI) by nevirapine, efavirenz or abacavir in patients with suitable virological control, only 0.6% of the patients who received efavirenz showed grade 3 or 4 hepatotoxicity compared with 4% of those treated with nevirapine. The trial evaluated the grade of hepatotoxicity using the ACTG definition. None of the patients treated with efavirenz and 3% of those treated with nevirapine suspended therapy due to increased ALT and/or AST (Martinez et al., 2003). The Johns Hopkins University in Baltimore, USA, also prospectively analyzed the incidence of severe liver toxicity in 568 patients treated with efavirenz or nevirapine. Of those who received efavirenz, 8% had severe hepatotoxicity, compared with 15.8% in the nevirapine group (Martin-Carbonero et al., 2003). Of the 298 patients included in the Spanish cohort from the Hospital Carlos III, who had received NNRTI-based ART six of the 136 (4%) patients receiving efavirenz and 20 (12%) of the 162 receiving nevirapine developed severe hypertransaminasaemia (Sulkowski et al., 2002). In a prospective cohort study carried out in Italy, 14 in which patients treated with either of the two NNRTI were followed up for 18 months, 17% and 52% of patients who received efavirenz and nevirapine, respectively, presented increased levels of ALT and/or AST of at least twice baseline (Manfredi et al., 2006). Another recent survey of Sulkowski, (2003), regarding 312 patients prescribed EFV and 256 prescribed NVP demonstrated severe hepatotoxicity in 15.6% of the NVP-treated group versus 8% of patients receiving EFV.

4.2. Impact of the duration of therapy on hepatotoxicity of NVP and EFV

The impact of the duration of HAART was also assessed for both patients taking NVP and EFV based regimen in this study. After the start of therapy, patients at 3rd month after treatment happen to show higher proportion of hepatotoxicity grade than the rest of duration of therapy. Non-nucleoside reverse transcriptase inhibitors in general can cause hepatitis in the first 2-3 months of therapy, sometimes as part of a hypersensitivity reaction (Martinez et al., 2001). This actually extends up to 12 month (Nathwani and Kaplowitz, 2006). In Evy Yunihastuti et al., (2009) study, the highest incidence of heepatotoxicity was observed during the first three months after starting HAART. The median duration of treatment before the detection of grade 3-4 liver enzyme elevation was 20 weeks (minimum-maximum 2-80 weeks). A warning has been recently added to the product information in Europe and the USA advising clinicians to monitor liver chemistry tests during the first 8-12 weeks of therapy because of it causes fulminant hepatitis leading to hepatic failure and death, and with later onset of direct drug-related hepatotoxicity leading to liver enzymes elevations (Boehringer-Ingelheim International, 2004).

Two types of antiretroviral-associated hepatotoxicity are actually discussed: an early and a late onset. The early occurring form (less than 12 weeks after initiation of therapy) frequently goes along with rash, eosinophilia, fever and arthralgia and seems to be based on an immunemediated mechanism. The second form with a late onset (after more than 12 weeks of therapy) is supposed to rely on an intrinsic toxic effect of the drug (Martinez et al. 2002).

According to Law et al., (2003), cohort study, antiretroviral therapy was not permanently modified or stopped in any of the 40 patients who experienced severe hepatotoxicity. At week 48 following commencement of trial antiretroviral therapy, all 40 patients were receiving their original antiretroviral regimen assigned at baseline. Despite development of severe hepatotoxicity, and continuation of original antiretroviral therapy regimens, median ALT level in these patients had returned towards baseline level by week 48. No episodes of clinical acute hepatitis or deaths related to severe hepatotoxicity were seen in these patients during the study period. This may explain the decrease in LFTs after 3rd month through out the 18th month in our study. The reason could be because hepatocytes promote mechanisms of cytoprotection, such as the formation of heat shock proteins, which protect the liver against toxic metabolites (Bissell et al., 2001). Heat-shock proteins, or molecular chaperones, are proteins induced by various forms of stress including drugs and are important for normal folding of nascent proteins, for protein degradation, and intracellular trafficking. As such, they may exert cytoprotective functions and underlie a tolerance towards potentially damaging toxicants. Although no human polymorphisms in these proteins related to idiosyncratic drug reactions have been described to date, an increase in heat shock

proteins may help the liver adapt to and minimize drug cytotoxicity. This cytoprotective response may explain the spontaneous normalization of liver enzymes that may occur despite maintenance of HAART (Bissell et al., 2001). Alternatively, the rise and fall of serum aminotransferase concentrations after initiation of medications may be related to a phenomenon of 'adaptation', whereby liver function tests normalize despite ongoing drug exposure (Kaplowitz, 2004). This pattern of liver enzyme changes following commencement of antiretroviral therapy is also consistent with other studies (den Brinker et al., 2000; Gisolf et al., 2000; Bruck et al., 2008; Haas et al., 2006; Ritchie et al., 2006).

4.3. Risk factors for hepatotoxicity of NVP associated with sex and age

With regard to nevirapine in particular, some factors are associated with severe liver toxicity during therapy in many studies. Among the risk factors are sex and age. In current study, risk factors for all patients with at least grade 1 hepatotoxicity was determined and by multivariant logistic regression it was found that both were not risk factors for hepatotoxicity of NVP. Although this is supported by different studies (Reisler et al., 2001; Bartlett, 2001; Miguez-Burbano et al., 2001; Hernandez et al., 2000; Mark et al., 2002; Brück et al., 2008), older age and female sex is associated with hepatotoxicity in other studies. Why women have a higher incidence of liver enzyme elevation than men is unclear but, certainly, the effect of gender does not seem to be related to lower body weight in female patients (Wit et al., 2002). Multivariant analysis by Biglino et al., (2000) and Hoffman-Terry et al., (2000) several factors in addition to age and sex were related to the development of acute hepatic disease. Puott and colleagues, (2000), from Brescia also examined the incidence, etiology, and outcome of "life-threatening" hepatotoxicity in 755 previously treatment-naive patients receiving combination antiretroviral therapy. The investigators defined life-threatening as the development of liver failure or 10-fold elevations of LFTs (or 5-fold elevations if initially abnormal). They documented 26 cases of life-threatening hepatotoxicity, for an incidence of 4.2 per 100 person-years of treatment. Significant risk factors included age older than 35 years. The result in current study may be because of the fact that study populations included in the study are mostly in similar age group.

4.4. Correlation of pattern of changes of the four LFTs with each other

As can be expected the correlation of the three liver enzymes and bilirubin with each other is significantly positive except for TB correlation with the rest of parameters which was insignificant. Any injury to Hepatocytes increases the level of ALT, AST and ALP in blood simeltaneously. Bilirubin raises when only there is cholestatic liver disease. Martinz et al., (2001) also found very high correlation between ALT and AST (correlation coefficient 0.83) and no correlation between TB and the rest of LFTs.

5. Limitations of the study

1. Data on LFTs of patients, who has not started HAART yet, was not available as a control group for the study.
2. The effect of comorbid diseases on hepatotoxicity was not assessed because there was no enough number of samples available.

6. Conclusion

In conclusion, it is suggested that hepatotoxicity is more common in patients receiving NVP based regimens than EFV based regimens. In addition, early hepatotoxicity is more, when both NVP and EFV drugs are used, rather than late hepatotoxicity i.e., the effect of hepatotoxicity decreases with time. On the other hand, sex and age are not risk factors for hepatotoxicity of NVP.

7. Recommendations

- As NVP happen to cause hepatotoxicity more, close monitoring of patients is needed.
- Frequent monitoring of liver enzymes after initiation antiretroviral therapy is recommended because of sever early hepatotoxicity was resulted from the study.

8. References

Andrew Rambaut, David Posada, Keith A. Crandall and Edward C. Holmes. The causes and consequences of HIV evolution. Nature Reviews Genetics . 2004; 5: 52-61.

Anthony S. Fauci, H. Clifford Lane. Human immunodeficiency virus disease: AIDS and related disorders. In, Harrison's Principles of Internal Medicine (Kasper DL, editors), pp 1076 – 39, 16th edition, 2005. McGraw-Hill Medical Publishing Division, NY.

Aranza´bal L, Casado J, Moya J, Quereda C, Diz S, Moreno A. Influence of liver fibrosis on highly active antiretroviral therapy-associated hepatotoxicity in patients with HIV and hepatitis C virus coinfection. Clin Infect Dis 2005; 40:588 – 593.

Arneson W and Brickell J. Assessment of Liver Function. In, Clinical Chemistry; A laboratory perspective (Arneson W and Brickell J, editors), pp233-66, chapter 7, 2007. FA Davis company, Philadelphia.

Arribas JR, Ibanez C, Ruiz-Antoran B, Pena JM, Esteban-Calvo C, Frias J, Vazquez JJ. . Acute hepatitis in HIV-infected patients during ritonavir treatment. AIDS 1998, 12 (13): 1722- 24.

Bailey A. and M. Fisher. Current use of antiretroviral treatment. British Medical Bulletin 2008; 87: 175–192.

Barbaro G, Di Lorenzo G, Asti A, Ribersani M, Belloni G, Grisorio B, et al. Hepatocellular mitochondrial alterations in patients with chronic hepatitis C: ultrastructural and biochemical findings.J Am. Gastroenterol 1999, 94(8): 2198-205.

Barreiro P, Rodriguez-Novoa S, Labarga P, Ruiz A, Jime´nez- Na´cher I, Martı´n-Carbonero L, et al. Influence of the stage of liver fibrosis on plasma levels of antiretrovirals in HIV patients with chronic hepatitis C. J Infect Dis 2007; 195:973–979.

Bartlett J. Severe liver toxicity in patients receiving two nucleoside analogues and a non-nucleoside reverse transcriptase inhibitor [Abstract 19]. In: Program and abstracts of the 8th Conference on Retroviruses and Opportunistic Infections 2001; February 4-8. Chicago, IL.

Biglino A, Degioanni M, Valle M, Crivelli P. Prospective evaluation of liver damage in a cohort of HIV/HCV coinfected patients treated with protease inhibitors (PIs). Program and abstracts of the XIII International AIDS Conference; July 9-14, 2000; Durban, South Africa. Abstract TuPpB1162.

Birkus G, Hitchcock M, Cihlar T. Assessment of mitochondrial toxicity in human cells treated with tenofovir: comparison with other nucleoside reverse transcriptase inhibitors. Antimicrob Agents Chemother 2002; 46:716–723.

Bissell D, Gores G, Laskin D, Hoofnagle J. Drug-induced liver injury: mechanisms and test systems. Hepatology, 2001, 33:1009–1013.

Boehringer-Ingelheim International Viramune product monograph, version 3.0. Ingelheim am Rhein, Germany: Boehringer-Ingelheim International GmbH, 2004.

Bonfanti P, Landonio S, Ricci E, Martinelli C, Fortuna P, Faggion I, et al. Risk factors for hepatotoxicity in patients treated with highly active antiretroviral therapy. J Acquir Immune Def Syndr 2001; 27:316–318.

Boyle BA, Mitruka K. In: Ronald Baker Ed, Toxicities & Side effects. Guide to management of Nucleoside Analogue (NA). 2003; 4-25.

Braun N, Leaf HL, Wieczorek RL, Margolis DM. Severe hepatitis in three AIDS patients treated with indinavir. Lancet 1997, 349(9056):924-5.

Brinkman K, ter Hofstede H, Burger D, Smeitink J, Koopmans P. Adverse effects of reverse transcriptase inhibitors: mitochondrial toxicity as common pathway. AIDS 1998; 12:1735–1744.

den Brinker M, Wit WNM, Wertheim-van Dillen PME, Jurriaans S, Weel J, van Leeuwen R. Hepatitis B and C virus co-infection and the risk for hepatotoxicity of highly active antiretroviral therapy in HIV-1 infection. AIDS, 2000; 14:2895-2902.

Brück S., S. Witte2, J. Brust3, D. Schuster3, F. Mosthaf4, M. Procaccianti4, J.A. Rump5, H. Klinker6, D. Petzold1, M. Hartmann1. Hepatotoxicity in patients prescribed efavirenz or neverapine. Eur J Med Res, 2008; 13: 343-348.

Clark S, Creighton S, Portmann B, Taylor C, Wendon J, Cramp M. Acute liver failure associated with antiretroviral treatment for HIV: a report of six cases. J Hepatol 2002; 36:295–301.

Cooper C, Parbhakar M, Angel J. Hepatitis associated with antiretroviral therapy containing dual versus single protease inhibitors in individuals coinfected with hepatitis C virus and HIV. Clin Infect Dis 2002; 334:1259–1263.

Demographic and Health Survey, 2005, Ethiopia.

Dienstag JL and Isselbacher KJ. Toxic and drug-induced hepatitis. In, Harrison's Principles of Internal Medicine (Kasper DL et al, editors), pp1838-44, Part XII, 16th edition, 2005. McGraw-Hill Medical Publishing Division, NY.

Dufour DR, et al: Diagnosis and monitoring of hepatic injury I: Performance characteristics of laboratory tests. Clin Chem 2000a; 46:2027–2049.

Dufour DR, et al: Diagnosis and monitoring of hepatic injury II: Recommendations for use of laboratory tests in screening, diagnosis, and monitoring. Clin Chem 2000b; 46:2050–2068.

Evy Yunihastuti, Rino A. Gani, LA Lesmana, Heru Sundaru, Samsuridjal Djauzi. Grade 3-4 Liver Enzyme Elevation during HAART in HIV and Hepatitis C Co-infected Adults. Acta Med Indones-Indones J Intern Med, 2009; 44(3):130-135.

Freiman JP, Helfert KE, Hamrell MR, Stein DS. Hepatomegaly with severe steatosis in HIV seropositive patients. AIDS 1993, 7(3):379-85.

Furst SM, Luedke D, Gaw HH, Reich R, Gandolfi AJ. Demonstration of a cellular immune response in halothane-exposed guinea pigs. Toxicol Appl Pharmacol 1997;143:245-255.

Gisolf E, Dreezen C, Danner S, Weel JL, Weverling G, and the Prometheus Study Group. Risk factors for hepatotoxicity in HIV-1-infected patients receiving ritonavir and saquinavir with or without stavudine. Clin Infect Dis 2000; 3:1234– 1239.

Gonzalez de Requena D, Nun˜ez M, Jimenez-Nacher I, Soriano V. Liver toxicity caused by nevirapine. AIDS2002; 16:290–291.

Goodgame J, Pottage J, Jablonowski H, Hardy W, Stein A, Fischl M, et al. Amprenavir in combination with lamivudine and zidovudine versus lamivudine and zidovudine alone in HIV-1-infected antiretroviral-naive adults. Antivir Ther 2000; 5:215–225.

Haas D, Bartlett J, Andersen J, Sanne I, Wilkinson G, Hinkle J, et al. Pharmacogenetics of nevirapine-associated hepatotoxicity: an adult ACTG collaboration. Clin Infect Dis, 2006; 43:783–786.

Hernandez LV, Gilson I, Jacobson J, Affi A, Puetz TR, Dindzans VJ. Antiretroviral hepatotoxicity in human immunodeficiency virus-infected patients. Aliment Pharmacol Ther, Oct 2000; 15(10):1627-32.

Hicks C, Cahn P, Cooper D, Walmsley SL, Katlama C, Clotet B, et al. Durable efficacy of tipranavir-ritonavir in combination with an optimised background regimen of antiretroviral drugs for treatment-experienced HIV-1-infected patients at 48 weeks in the Randomized Evaluation of Strategic Intervention in multidrug reSistant patients with Tipranavir (RESIST) studies: an analysis of combined data from two randomised open-label trials. Lancet 2006; 368:466– 475.

Hoffmann, Christopher, Charalambus, Salome, Thio, Chloe L., Chorchyard, Gavin J., Chaission, Richard E, Grant, Alison D. Hepatotoxicty in an African antiretroviral therapy cohort: the effect of tuberculosis and hepatitis B. AIDS 2007; 21(10): 1301-1308.

Hoffmann-La Roche Ltd. HIV Lifecycle. HIV 2006; 40:284-7.

Hoffman-Terry M, Smith A, Badillo K, Wasser T. Hepatotoxicity of non-nucleoside reverse transcriptase inhibitor (NNRTI) containing. Program and abstracts of the XIII International AIDS Conference; July 2000; 9-14, Durban, South Africa. Abstract MoPeB2148.

ter Hofstede H, de Marie S, Foudraine N, Danner S, Brinkman K. Clinical features and risk factors for lactic acidosis following long term antiretroviral therapy: 4 fatal cases. Int J STD AIDS 2000; 11:611–616.

Johnson S, Chan J, Bennett C. Hepatotoxicity after prophylaxis with a nevirapine-containing antiretroviral regimen. Ann Intern Med 2002; 137:146–147.

Kakuda TN. Pharmacology of nucleoside and nucleotide reverse transcriptase inhibitor-induced mitochondrial toxicity. Clin Ther 2000; 22 (6): 685–708.

Kandula V, Khanlou H, Farthing C. Tipranavir: a novel secondgeneration nonpeptidic protease inhibitor. Expert Rev Anti Infect Ther 2005; 3:9–21.

Kaplowitz N. Drug-induced liver disorders: introduction and overview. In: Kaplowitz N, De Leve L, editors. Drug-induced liver disease. New York: Marcel Dekker; 2002; pp. 1–13.

Kaplowitz N. Drug-induced liver injury. Clin Infect Dis, 2004; 38 (suppl 2):44–48.

Kenna JG. Immunoallergic druginduced hepatitis: lessons from halothane.J. Hepatol. 1997; 26(Suppl. 1):5–12

Knowles SR, Uetrecht J, Shear NH. Idiosyncratic drug reactions: the reactive metabolite syndromes. Lancet 2000; 356:1587-1591.

Kontorinis N, Dieterich D. Hepatotoxicity of antiretroviral therapy. AIDS Rev, 2003; 5 (1): 36–43.

Kramer J, Giordano T, Souchek J, El-Serag H. Hepatitis C coinfection increases the risk of fulminant hepatic failure in patients with HIV in the HAART era. J Hepatol 2005; 42:309– 314.

Kuby, J., Kindt, T., Goldsby, R. and Osborna B. (2007) Hypersensitivity Reactions. In: Immunology. 6th ed. 371-400. W. H. Freeman and Company, New York.

Law WP, Dore GJ, Duncombe CJ et al. Risk of severe hepatotoxicity associated with antiretroviral therapy in the HIV-NAT Cohort, Thailand, 1996–2001. AIDS 2003; 17: 2191–9.

Lai KK, Gang D, Zawacki J, Cooley T. Fulminant hepatic failure associated with 2', 3'-dideoxyinosine. Ann Inter Med, 1991; 115 (4): 283-84.

Leith J, Piliero P, Storfer S, Mayers D, Hinzmann R. Appropriate use of nevirapine for long-term therapy. J Infect Dis 2005; 192:545–546.

Lemasters JJ, Nieminen AL, Qian T, Trost LC, Elmore SP, Nishimura Y, Crowe RA, et al. The mitochondrial permeability transition in cell death: a common mechanism in necrosis, apoptosis and autophagy. Biochim Biophys Acta 1998; 1366:177-196.

Lewis W, Dalakas MC. Mitochondrial toxicity of antiretroviral drugs. Nat Med, 1995, 1: 1417-422.

Lewis W, Griniuviene B, Tankersley KO, Levine ES, Montione R, Engelman L, de Courten-Myers G, et al. Depeletion of mitochondrial DNA, destruction of mitochondria, and accumulation of lipid droplets result from fialuridine treatment in woodchucks (Marmota monax). Lab Invest 1997; 76:77-87.

Limdi JK, Hyde GM: Evaluation of abnormal liver function tests. Postgrad Med J 2003; 79 (932):307–312.

De Maat M, Mathot R, Veldkamp A, Huitma A, Mulder J, Meenhorst P, et al. Hepatotoxicity following nevirapine containing regimens in HIV-1-infected individuals. Pharmacol Res 2002; 46:295–300.

Maida I, Nu´n˜ez M, Rios MJ, Martı´n-Carbonero L, Sotgiu G, Toro C, et al. Severe liver disease associated with prolonged exposure to antiretroviral drugs. J Acquir Immune Defic Syndr 2006; 42:177–182.

Mallet V, Blanchard P, Verkarre V, Vallet-Pichard A, Fontaine H, Lascoux-Combe C, et al. Nodular regenerative hyperplasia is a new cause of chronic liver disease in HIV-infected patients. AIDS 2007; 21:187–192.

Manfredi R, Calza L. Nevirapine versus efavirenz in 742 patients: no link of liver toxicity with female sex, and a baseline CD4 cell count greater than 250 cells/microliter. AIDS 2006; 20:2233–2236.

Manosuthi W, Thongyen S, Chumpathat N, Muangchana K, Sungkanuparph S. Incidence and risk factors of rash associated with efavirenz in HIV-infected patients with preceding nevirapine-associated rash. HIV Med 2006; 7:378–382

Mark S. Sulkowski, David L. Thomas, Shruti H. Mehta, Richard E. Chaisson, and Richard D. Moore. Hepatotoxicity Associated With Nevirapine or Efavirenz-Containing Antiretroviral Therapy: Role of Hepatitis C and B Infections. Hepatology, 35(1): 182-89, 2002.

Martin A, Nolan D, James I, Cameron P, Keller J, Moore C. Predisposition to nevirapine hypersensitivity associated with HLA-DRB1M0101 and abrogated by low CD4 T-cell counts.AIDS 2005; 19:97–99.

Martin-Carbonero L, Nun˜ez M, Gonza´lez-Lahoz J, Soriano V. Incidence of liver injury after beginning antiretroviral therapy with efavirenz or nevirapine. HIV Clin Trials 2003, 4 (2): 115–20.

Martı´nez E, Arnaiz JA, Podzamczer D, David D, Esteban R, Pere D, Hernando K, Melcior, Enric P, Lluis F, Josep M, Ferran S, Cristóbal R, Cristina C, Manuel J, Miquel A, Ana C, Elisa de L, José M. Substitution of nevirapine, efavirenz, or abacavir for protease inhibitors in patients with human immunodeficiency virus infection. N Engl J Med; 2003; 349 (11): 1036–46.

Martinez E, Blanco J, Arna´iz J, Pe´rez-Cuevas J, Mocroft A, Cruceta A, et al. Hepatotoxicity in HIV-infected patients receiving nevirapine-containing antiretroviral therapy. AIDS 2001; 15:1261–1268.

McKenzie R, Fried MW, Sallie R, Conjeevaram H, Di Bisceglie AM, Park Y, Savarese B, et al. Hepatic failure and lactic acidosis due to fialuridine (FIAU), an investigational nucleoside analogue for chronic hepatitis B.N Engl J Med 1995;333:1099-1105.

Melamed AJ, Muller RJ, Gold JW, Campbell SW, Kleinberg ML, Armostrong D. Possible zidovudine-induced hepatotoxicity. JAMA 1987; 258 (15): 2063.

Nathwani R, Kaplowitz N. Drug hepatotoxicity. Clin Liver Dis 2006; 10:207–217.

Nunez M, Lana R, Mendoza J, Martı´n-Carbonero L, Soriano V. Risk factors for severe hepatic injury following the introduction of HAART. J Acquir Immune Def Syndr 2001; 27: 426–431.

Olano JP, Borucki MJ, Wen JW, Haque AK. Massive hepatic steatosis and lactic acidosis in a patient with AIDS who was receiving zidovudine. Clin Infect Dis 1995; 21 (4): 973-76.

Ogedegbe A, Sulkowski M. Antiretroviral-associated liver injury. Clin Liver Dis 2003; 7:475–499.

Palella FJ Jr, Delaney KM, Moorman AC, Loveless MO, Fuhrer J, Satten GA, Aschman DJ,Holmberg SD. "Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. HIV Outpatient Study Investigators." N Eng J Med 1998; 338: 853-60.

Palmon R, Koo B, Shoultz D, Dieterich D. Lack of hepatotoxicity associated with nonnucleoside reverse transcriptase inhibitors. J Acquir Immune Defic Syndr 2002; 29:340–345.

Podzamczer D, Ferrer E, Consiglio E et al. A randomized clinical trial comparing nelfinavir or nevirapine associated to zidovudine/lamivudine in HIV-infected naı¨ve patients (The Combine Study). Antivir Ther 2002, 7 (2): 81–90.

Pratt DS and Kaplan MM. Evaluation of liver function. In, Harrison's Principles of Internal Medicine (Kasper DL et al, editors), pp1813-16, Part XII, 16th edition, 2005. McGraw-Hill Medical Publishing Division, NY.

Puoti M, Torti C, Ripamonti D, et al. Life threatening hepatoxicity during combination antiretroviral treatment: incidence, risk factors and outcome. Program and abstracts of the XIII International AIDS Conference 2000; July 9-14, Durban, South Africa. Abstract TuPpB1160.

Reisler R, Liou S, Servoss J, Robbins G, Theodore D, Murphy R, Chung R. Incidence of hepatotoxicity and mortality in 21 adult antiretroviral treatment trials [abstract 43]. 1st International AIDS Society on HIV Pathogenesis and Treatment 2001; July 8 -11, Buenos Aires, Argentina.

Reisler R, Han C, Burman W, Tedaldi E, Neaton J. Grade 4 events are as important as AIDS events in the era of HAART. J Acquir Immune Defic Syndr 2003; 34:379–386.

Ritchie M, Haas D, Motsinger A, Donahue JP, Erdem H, Raffanti S, et al. Drug transporter and metabolizing enzyme gene variants and NNRTI hepatotoxicity. Clin Infect Dis 2006; 43:779–782.

Roche SP, Kobos R: Jaundice in the adult patient. Am Fam Physician 2004; 69:299–304.

Robbins GK, Victor De Gruttola, Sc.D., Robert W. Shafer, Laura M. Smeaton, Sally W. Snyder, Carla Pettinelli, Michael P. Dubé, Margaret A. Fischl, Richard B. Pollard, Robert Delapenha, Linda Gedeon, Charles van der Horst, Robert L. Murphy, Mark I. Becker, Richard T. D'Aquila, Stefano Vella, Thomas C. Merigan, Martin S. Hirsch. "Comparison of sequential three-drug regimens as initial therapy for HIV-1 infection." N Engl J Med 2003; 349: 2293-03.

Rotger M, Colombo S, Furrer H, De´costerd L, Buclin T, Telenti A. Influence of CYP2B6 polymorphism on plasma and intracellular concentrations and toxicity of efavirenz and nevirapine in HIV-infected patients. Pharmacogenet Genomics 2005; 15:1–5.

Sanne I, Mommeja-Marin H, Hinle J, Bartlett J, Lederman M, Maartens G, et al. Severe hepatotoxicity associated with nevirapine use in HIV-infected subjects. J Infect Dis 2005; 191:825–829.

Sanne I, on behalf of the FTC-302 Study Investigators and the FTC-302 Independent Clinical Steering Committee. Severe liver toxicity in patients receiving two nucleoside analogues and a nonnucleoside reverse transcriptase inhibitor. AIDS 2000; 14 (suppl 4):12.

Silva MF, Ruiter JP, Illst L, Jakobs C, Duran M, de Almeida IT, Wanders RJ. Valproate inhibits the mitochondrial pyruvate-driven oxidative phosphorylation in vitro. J Inherit Metab Dis 1998; 20:397-400.

Sokol RJ, Treem WR. Mitochondria and childhood liver diseases. J Pediatr Gastroenterol Nutr 1999; 28:4-16.

Soriano V, Dona C, Barreiro P, Gonzalez-Lahoz J. Is there cross-toxicity between nevirapine and efavirenz in subjects developing rash? AIDS 2000; 14:1672–1673.

Stern J, Robinson P, Love J, Lanes S, Imperiale M, Mayers D. A comprehensive hepatic safety analysis of nevirapine in different populations of HIV-infected patients. J Acquir Immune Defic Syndr 2003; 34 (suppl 1):21–33.

Sulkowski M. Drug-induced liver injury associated with antiretroviral therapy that includes HIV-1 protease inhibitors. Clin Infect Dis 2004; 38 (suppl 2):90–97.

Sulkowski MS. Hepatotoxicity associated with antiretroviral therapy containing HIV-1 protease inhibitors. Semin Liver Dis 2003; 23 (2): 183-93.

Sulkowski MS, Thomas DL, Chaisson RE, Moore RD. Hepatotoxicity associated with antiretroviral therapy in adults infected with human immunodeficiency virus and the role of hepatitis C or B virus infection. JAMA 2000; 283: 74-80.

Sulkowski MS, Thomas DL, Metha SH, Chaisson RE, Moore RD. Hepatotoxicity associated with nevirapine or efavirenz-containing antiretroviral therapy: role of hepatitis C or B virus infection. Hepatology 2002; 35 (1): 182-89.

Tennant BC, Baldwin BH, Graham LA, Ascenzi MA, Hornbuckle WE, Rowland PH, Tochkov IA, et al. Antiviral activity and toxicity of fialuridine in the woodchuck model of hepatitis B virus infection. Hepatology 1998; 38:179-191.

UNAIDS (2008) UNAIDS Sub-Saharan Africa AIDS epidemic update.

van Leth F, Phanuphak P, Ruxrungthan K, Baraldi E, Miller S, Gazzard B, Cahn P, Lalloo UG, van der Westhuizen IP, Malan DR, Johnson MA, Santos BR, Mulcahy F, Wood R, Levi GC, Reboredo G, Squires K, Cassetti I, Petit D, Raffi F, Katlama C, Murphy RL, Horban A, Dam JP, Hassink E, van Leeuwen R, Robinson P, Wit FW, Lange JM. Comparison of first-line antiretroviral therapy with regimens including nevirapine, efavirenz, or both drugs, plus stavudine and lamivudine: a randomized open-label trial, the 2NN study. Lancet 2004, 363 (9417): 1253–63.

Vincent Soriano, Massimo Puoti, Pilar Garcia-Gasco, Juergen K. Rockstroh, Yves Benhamou, Pablo Barreiro and Barbara McGovern. Antiretroviral drugs and liver injury. AIDS 2008, 22:1–13

Walker U, Setzer B, Venhoff N. Increased long-term mitochondrial toxicity in combinations of nucleoside analogue reverse transcriptase inhibitors. AIDS 2002; 16:2165–2173.

Wit FW, Weverling GJ, Weel J, Jurriaans S, Lange JM. Incidence of and risk factors for severe hepatotoxicity associated with antiretroviral combination therapy. J Infect Dis 2002; 1: 186.

Zimmerman H. Drug-induced liver disease. In: Schiff E, editor. Schiff's Diseases of the Liver, 1999; Vol. 8, pp. 973–1064. Lippincott- Raven Publishers, Philadelphia.

ANNEX

DATA COLLECTION FORMAT

Addis Ababa University, Faculty of Medicine

Department of Biochemistry

Data collection format prepared for the study of liver function in HIV-infected patients receiving combination antiretroviral therapy in Tikur Anbassa Specialized Hospital.

1. Patient Identification: Format No. _____

1.1 Patient unique ART No. _____ Sex: Male____ Female____ Age_____

1.2 Address: Region _____ Woreda/Subcity _____ Kebele ___

2. Clinical Data:

2.1 Date of HIV positive confirmed ___/___/_____ (dd/mm/yy)

2.1 When the patient is medically eligible and ready for HAART __/__/_ (dd/mm/yy)

2.1 Recommend type of HAART (dose/code): _____

3. Patient HAART follow up:

Follow up date (dd/mm/yy)	Months on HAART	WHO Stage1-4	HAART			Other Illness	Other medication dispensed
			Dispense (dose/code)	Side effect	Reason for change		

4. Laboratory Investigation Results:

Date of test done (dd/mm/yy)	Months on disease at test	Months on HAART at test	CD4/mm^3	ALT, mg/dL	AST, mg/dL	ALP, mg/dL	Total Bilirubin mg/dL	Direct Bilirubin mg/dL	Total protein g/dL	Albumin g/dL

ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; ALP, Alkaline phosphatase; CD4, Cluster of differentiation four.