Addis Ababa University

College of Health Sciences

School of Pharmacy

Department of Pharmacology and Clinical Pharmacy

Evaluation of protease inhibitors based anti-retroviral regimen induced liver injury at Zewditu memorial hospital.

By: Lemma Bose

A Thesis Submitted to the Department of Pharmacology and Clinical Pharmacy, School of Pharmacy, College of Health Sciences, Addis Ababa University in partial fulfillment of the requirements for the Master of Science degree in pharmacology (clinical).

September, 2019

Addis Ababa, Ethiopia

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School of Graduate studies

This is to certify that the thesis prepared by Lemma Bose, entitled: Evaluation of protease inhibitors based anti-retroviral regimen induced liver injury at Zewditu memorial hospital and submitted in partial fulfillment of the requirements for degree of Master of Science in pharmacology (clinical) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

Signed by Examining Committee:

Advisor: Dr. Solomon Mequanente (PhD) Signature _____ Date _____

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Chair of Department

ABSTRACTS

***Background**: The inclusion of protease inhibitors (PIs) in therapeutic regimens has a significant impact on the success of the treatment; these classes have been associated with liver-related injury in some individuals. Rates of hepatotoxicity from registration trials of various PIs have ranged from 1% to 9.5%, of which few patients had serious liver-related outcomes.*

*O**bjective**: To evaluate the incidence, severity, patterns, time dependent and risk factors of PIs based anti-retroviral regimens induced liver injury.*

***Methods**: An observational cohort prospective study was conducted at anti-retroviral treatment clinic of Zewditu memorial hospital. All patients that were HIV positive and candidate for PI based regimens, and fulfilling other eligibility criteria were included in the study. The study was conducted from March 1, 2018 to December 30, 2018 (including follow up period for total of 6 months).*

***Results**: Out of 142 study participants, overall, liver injury of any grade was observed in 25 (17.6%) participants. From 35 PI based naïve participants, 3 patients developed liver injury during the study period. Majority of the participants 21 (84%) developed cholestatic pattern of liver injury. Overall incidence of grade 1, 2 and 3 were 16 (64%), 6 (24%) and 3 (12%) respectively. The incidence of liver injury was high (57.1%; 4/7) within 25-48 weeks from the starting PI based regimens. Cotrimoxazole prophylactic therapy and previous first line on AZT/3TC/NVP were significantly associated with liver injury.*

***Conclusion:** Overall, liver injury of any grade was observed in 25 (17.6%) participants while only 3 (12%) participants developed grade-3 liver injury. None of the participants developed grade-4 liver injury and no liver related death was reported during the study period.*

***Recommendation:** Regular follow up of liver function tests is important to monitor liver toxicities in HIV positive patients, particularly with PIs-based regimen. Further study need to be conducted with large sample size and long term follow up to identify the mechanism and risk factors associated with PIs.*

***Keywords:** Incidence, Severity, Protease enzyme inhibitor, Drug induced liver injury, Liver injury.*

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Contents

ABBREVIATIONS AND ACRONYMS

3TC: Lamivudine

ABC: Abacavir

ADR: Adverse Drug Reaction

AETC: AIDS Education and Training Centers

AIDS: Acquired Immunodeficiency Syndrome

ALP: Alkaline Phosphatase

ALT: Alanine Aminotransferase

ART: Anti-retroviral Treatment

ARV: Anti-retroviral

AST: Aspartate Aminotransferase

AT: Aminotransferase

ATV: Atazanavir

BMI: Body Mass Index

CDC: Centers for Disease Control and prevention

D4t: Stavudine

DILI: Drug Induced Liver Injury

EFV: Efavirenz

FDA: Food and Drug Administration

FMHACA: Food, Medicine and Healthcare Administration and Control Authority

FTC: Emtricitabine

HAART: Highly Active Ant-retroviral Treatment

HBV: Hepatitis B Virus

HCV: Hepatitis C Virus

HIV: Human Immunodeficiency Virus

LEE: Liver Enzyme Elevation

LPV: Lopinavir

NNRTI: Non-Nucleoside Reverse-Transcriptase Inhibitor

NRTI: Nucleoside Reverse-Transcriptase Inhibitors

NVP: Nevirapine

PI: Protease Inhibitor

RTV: Ritonavir

TDF: Tenofovir-Disoproxil-Fumarate

TE: Transaminase Elevations

ULN: Upper Limit of Normal

UNAIDS: United Nations Program on HIV/AIDS

WHO: World Health Organization

ZMH: Zewditu Memorial Hospital

LIST OF TABLES

LIST OF FIGURES

1. INTRODUCTION

1.1. Background of HIV AIDS.

Human Immunodeficiency Virus (HIV) is a virus that leads to Acquired Immunodeficiency Syndrome (AIDS) (UNAIDS, 2000). There are two major families of HIV, of which HIV-1 is the major cause of AIDS. Although, the second retrovirus, HIV-2, is rare in other part of the country it is concentrated in west Africa (Dipro, 2014; Ramachandran & Shanmughavel, 2014). AIDS is developed when lenti-viruses (a family of mammalian retroviruses) evolved to establish chronic persistent infection with gradual onset of clinical symptoms (Flexner, 2006).

The retroviruses are transmitted primarily by sexual contacts, contact with infected blood or blood products through sharing of injection equipment or receipt of contaminated blood products, and prenatal transmission (Aberg *et al*., 2009). The risk of infection and transmission is high with the cases of sexually transmitted diseases and male un-circumcision (Public Health Agency of Canada, 2012).

In order to control and monitor HIV epidemic, HIV disease staging and classification systems are important tools. Two main clinical staging systems are: world health organization (WHO) disease staging system and center of disease control and prevention (CDC) classification system in HIV infection. The CDC's classification system is frequently adopted in developed countries. Since the WHO's staging system does not require laboratory tests, it is suited to the resource limited settings (AETC, 2014).

The symptoms of AIDS range from asymptomatic to influenza like illness or a mononucleosis-like illness. The symptoms vary between the individuals, duration, severity and developed opportunistic infections or stages. The most common symptoms include fever, large tender lymph nodes, throat inflammation, a rash, headache, and/or sores of the mouth and genitals. Even though non-specific in nature, gastrointestinal symptoms such as nausea, vomiting or diarrhea may occur within one or two weeks of infection (Hemasri *et al*., 2016).

Although a variety of manual and automated test methods are available, the preferred method for diagnosing of HIV infection is an enzyme-linked immune-sorbent assay (ELISA). ELISA is of-

ten performed on automated systems, so that large numbers of samples can be tested safely and economically. Even though an indirect immune-fluorescence assay is available, western blot is the most commonly used confirmatory test in HIV infection (Buttò *et al*., 2010).

1.1.1. Epidemiology of HIV/AIDS

HIV infection /AIDS is a global pandemic and remains to be major global public health problems. According to the report of United Nations Program on HIV/AIDS (UNAIDS), from the start of HIV epidemic, about 74.9 million people have been infected with HIV, of which about 32 million people died due to AIDS related disease worldwide. Particularly, in 2018, 37.9 million people were living with HIV of which 36.2 were adults (UNAIDS, 2019).

In 2018, 23.3 million peoples living with HIV accessed ant-retroviral therapy (ART) worldwide. Of which, 62% were adults aged 15 years and older. The increment is as high as 7.7 million [6.8 million–8.0 million] since 2010 (UNAIDS, 2019).

The Sub-Sahara African region keeps being the center of people living with HIV, with the estimation of 66%, which indicates HIV cases concentrated to low and middle income countries. Even though, reduction in incidence of HIV infection is as high as 30% since 2010, the east and southern Africa share about 19.6 million (53.1%) of people from Sub-Sahara region (UNAIDS, 2017).

According to HIV related estimates and projections for Ethiopia, the series increment of total HIV positive populations were 715404, 718500 and 722248 in 2015, 2016 and 2017 respectively. In addition to these, the total new infection were 27104, 27288 and 22827 within the mentioned consecutive years, of which majority of the reported people were adults (Ethiopian Puplic Health Institute, 2017).

1.1.2. Treatment option for HIV AIDS

Highly active antiretroviral therapy (HAART) results in profound suppression on the incidence of opportunistic infections as well as improved morbidity and mortality among HIV patients. According to WHO guideline, the HAART should be a combination of three drugs or more, fixed or loose combination. Generally the first-line ART for adults should consist of two nucleoside reverse-transcriptase inhibitors (NRTIs) plus a non-nucleoside reverse-transcriptase inhibi-

tor (NNRTI) or an integrase inhibitor, of which TDF + 3TC (or FTC) + EFV as a fixed-dose combination is recommended as the preferred option to initiate ART (WHO, 2016).

Now days, HAART coverage has improved dramatically in low- and middle-income countries. Similarly, since 2005, the access to HAART started on massive scale in Ethiopia (Yirdaw & Hattingh, 2015). The Ethiopian government, in collaboration with its key development partners, has been developing and implementing national strategies that adhere to global directions and practices within the country (Central Statistical Agency (CSA) [Ethiopia] and ICF, 2016).

The introduction of protease inhibitors (PIs) in late 1995 changed the medical management of HIV dramatically and marked the beginning of the HAART. Since that time, the use of combination ART has been associated with impressive clinical benefits, including increases in immune function, decreases in the incidence of opportunistic infections, and increased survival. But, the optimum success of HAART has been tempered by the issue of anti-retroviral (ARV) associated adverse reactions or toxicity, particularly, liver-related toxicities due to the ART regimen including some boosted PIs regimens (Goicoechea & Best, 2007; Sulkowski, 2003).

Currently several PIs are globally available of which boosted lopinavir/ritonavir (LPV/r) and atazanavir/ritonavir (ATV/r) are widely available in Ethiopia. Although low proportion of patients are treated with PIs based regimens, but when there is treatment failure or severe side effects of first line, a second line regimens which contains PIs is recommended by Ethiopian guideline (FMHACA, 2014).

1.2. Background on liver injury

Liver is a vital organ that regulates maintenance, performance and homeostasis of the body. In addition to this, it is also the main organ of drug metabolism (Yang *et al.,* 2017). Moreover the major functions of the liver are to metabolize essential diet (carbohydrate, protein and fat), detoxification, secretion of bile and storage of vitamins in our body (Pandit, Sachdeva, & Bafna, 2012).

Majority of liver cells are hepatocytes, which represent 60 % of the liver‘s cells and about 80 % of the liver‘s total cell mass. Most of the liver‘s functions like that of synthetic and metabolic capabilities depend on the work of hepatocytes (Jevas, 2017).

Hepatitis is an inflammation of liver cells characterized by the presence of inflammatory cells in the tissue of the organ. The condition can be mild as self-limiting or can progress to fibrosis and chronic cirrhosis. Even though, jaundice, anorexia and malaise often appear, hepatitis may occur without or with limited symptoms. Hepatitis can be acute or chronic depending on the duration of inflammation. Hepatitis is said to be acute when it lasts less than six months and chronic when it persists longer. Hepatitis is frequently caused by a group of viruses. Toxins, including medications can also be responsible for injury of liver cells (Robin *et al.,* 2012).

1.2.1. Drug induced liver injury

Drug-Induced Liver Injury (DILI) is the inflammation of the liver cells caused by exposure to a drug or medications, and it is associated with different levels of organ dysfunction (Marrone *et al.,* 2017; Yang *et al.,* 2017). In such cases, either the drug itself or its metabolic products may be responsible during the course of treatment (Yang *et al.,* 2017).

DILI is not always easy to diagnose, but it is a frequent differential diagnosis in patients with acute liver injury without obvious etiology. In addition to the exclusion of competing etiologies, the essential element in the diagnostic process is the previous information about hepatotoxic nature of the agents (Björnsson, 2016).

DILI is a major problem not only for affected patients and their health care professionals, but also it is a serious problem for the pharmaceutical industry and regulatory bodies, as most drugs fail in clinical trials and the most common factor that compels regulatory actions on drugs approval (Watkins & Seeff, 2006).

Although DILI is rare in the general population, but it is the most common cause of acute liver failure and has been the leading cause of withdrawal of drugs from the market. Worldwide, it represents about 10% of acute liver failure and 40 - 50% of all cases of liver injury (Ponte *et al,* 2017).

Even if overall DILI is a rare event, it is responsible for a high percentage of hospital admission for jaundice, and remains the first cause of acute liver failure (ALF) and ALF-related liver transplantation in the US (Marrone *et al.,* 2017). The exact epidemiology of DILI is hard to assess due to difficulty in diagnosis and signaling issues.

All classes of anti-retroviral drugs have been reported to cause liver toxicity (Mira *et al.*, 2006). Study on HIV clinical management showed that NNRTIs were most frequently associated with liver injury, followed by the PIs, and then NRTIs (Puoti *et al.*, 2009). The incidence of severe hepatotoxicity is greater in HIV infected patients with hepatitis C virus (HCV) and/or hepatitis B virus (HBV) co-infections than without co-infection (Brinker *et al.*, 2000; Savès *et al.*, 2000). Advanced liver fibrosis stage has been associated with a higher risk of developing severe liver injury in patients co-infected with HCV and receiving NNRTIs (Mira *et al.*, 2006).

1.3. Statements of problems

DILI is a major type of liver damage and various types of drugs are responsible for it. Now days, about 1,000 drugs, toxins, and herbs have been reported to cause liver injury, of which 75% of the idiosyncratic drug reactions result in liver transplantation or death (Busardò & Grieco, 2017). In addition to this drug induced liver injury is responsible for 5% of all hospital admissions and 50% of all acute liver failures (Pandit *et al*., 2012).

The big access to HAART has transformed HIV from untreatable condition into a chronic manageable disease. However, use of ART and other co-medications in HIV infection resulted in an increment in serious adverse drug reaction (ADR), some of which are life-threatening conditions (Gudina *et al*., 2017).

In limited income settings, ADRs related to HAART are among the major challenges and resulted in discontinuing the medication and non-adherence. The challenges are more severe with those who work with a limited formulary (Murphy *et al.*, 2007). Therefore ADRs are the most relevant factors that influence the success of treatment, particularly in low income countries like Ethiopia.

Liver toxicity increases cost by generating: medical visits, work-up exams, and frequent hospital admissions (Nu, 2006). In addition, liver toxicity may complicate some conditions. For instance it can exacerbate the progression of HCV-related liver disease to cirrhosis, which is a leading cause of morbidity and mortality among HIV-infected patients (Mira *et al.*, 2006).
HAART related liver injury became more evident after the introduction of ART of high activity, which initially contains PIs as combined regimens. However, some studies reported as a full-dose ritonavir (RTV) has been found to be more hepatotoxic, but lack of the evidence to prove

the higher potential for liver toxicity of this particular family of drugs as other studies did not confirm it (Nu, 2006). In addition to this, the safety of low dose boosted PIs based regimens is not known in low income countries like Ethiopia within this class of ART.

The incidence of liver injury or liver enzyme elevation (LEE) and symptomatic hepatitis are common among HIV-infected patients receiving combination ART, such as nevirapine (NVP) and RTV boosted PIs (Murphy *et al*., 2007). Additionally, HBV and HCV co-infection, and the use of concurrent anti-tuberculosis or other hepatotoxic drugs increase the risk of hepatotoxicity (Murphy *et al*., 2007). But further study is needed in order to identify the more related risk factors in developing countries like Ethiopia.

More importantly, the magnitude of the problem between developed and developing world significantly differs due to various conditions. Patients in developing world, particularly in Sub-Saharan Africa, are late presenter with advanced disease state, occurrence of opportunistic infections and co-morbid medical conditions, like malnutrition that have made the problem even worse. Additionally, lack of frequent laboratory monitoring results in failure or delay in diagnosing specific toxicities and enhance the progression of the toxicity (Gudina *et al.,* 2017).

1.4. Literature review

1.4.1. Anatomy of liver

The liver is the largest internal organ and accounts for about 2% to 3% of average body weight. It`s divided in to two parts, known as the right and left lobes in which the right one is much bigger than the left and settled in the right upper quadrant of the abdominal cavity, beneath the right diaphragm and protected by the ribs bones (Sherif *et al.,* 2010).

Depending on its structure, the liver can be divided into five tissue systems: vascular system, hepatocytes and hepatic lobule, hepatic sinusoidal cells, biliary system, and stroma. Furthermore it is composed of many different cell types which can be generalized into parenchymal cells (hepatocytes) and non-parenchymal cells (Ishibashi *et al.*, 2014).

The basic functional unit of the liver is the liver lobule. The structures found in a liver lobule include: Plates of hepatocytes which forms the bulk of the lobule, portal triads at each corner of hexagon , central vein, liver sinusoids that run from the central vein to the portal triads, hepatic

macrophages (Kupffer cells) and bile canaliculi formed between walls of adjacent hepatocytes (Figure 1) (Ozougwu, 2017).

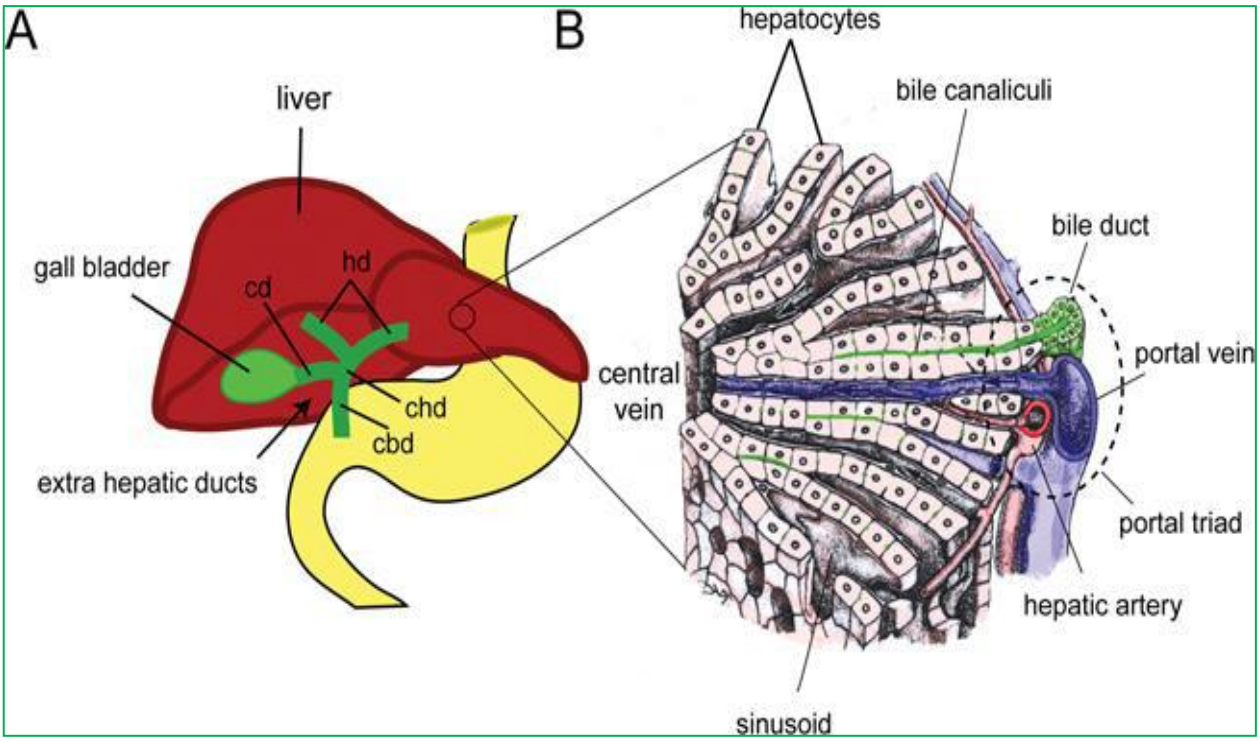

Figure 1: Cellular architecture of the liver.
Cd=cystic duct: **cbd=** common bile duct: **hd=** hepatic duct: **chd=** common hepatic duct. (Adopted from: (Ozougwu, 2017)).

1.4.2. Mechanisms of protease inhibitors induced liver injury

The exact mechanism how a variety of ARV drugs including PIs cause hepatic damage remains unknown (Rockstro *et al.*, 2002; Bruno *et al.*, 2006; Rockstroh and Vogel, 2007). Although the exact mechanism remains unknown, all ARV drugs can exert direct toxic effects on the hepatocyte depending on dose. The effect is severe whenever drug-drug interactions have not been noticed, wrong dosing schedule has been used, or a preexisting chronic liver disease or end-stage liver disease that can impair clearance and cause or exacerbate the condition (K Rockstroh & Vogel, 2007).

Hepatocellular injury is indicated by rises in aminotransferase (AT) activities in serum reflecting release of alanine or aspartate aminotransferase (ALT or AST) from injured liver cells. However, in some cases the drug's potential for severe DILI, may not be related to degree of hepatocellular injury. The drug is clearly related to hepatocellular injury, whenever it reduces the liver's func-

tional ability to clear bilirubin from the plasma or to synthesize prothrombin and other coagulation factors that cause severe DILI (FDA, 2009).

The potential hepatotoxins to cause hepatocellular injury, indicated by release of AT enzymes from liver cells, mostly, when there is more than double of ALT (Aithal *et al.,* 2011). However, the condition is not such significant as hepatocelluar injury, many drugs cause cholestasis pattern of injury, in which level of alkaline phosphatase (ALP) increased significantly compared to that of ALT and it is less likely to lead to death or transplant (FDA, 2009).

1.4.3. Protease enzyme inhibitors based regimens induced liver injury

Although the inclusion of PIs in therapeutic regimens has a significant impact on treatment success, this class has been associated with liver toxicity in some individuals, particularly, who are co-infected with hepatitis viruses (Bruno *et al.,* 2006). Incidence of hepatotoxicity from registration trials of various PIs ranged from 1%to 9.5%, of which, few patients had serious liver-related outcomes (Kress, 2005).

According to HIV nucleic acid amplification testing randomized controlled trials in Thailand, the incidence of severe hepatotoxicity was particularly low within PIs group and comparable to that in individuals receiving two NRTI alone (Law *et al.,* 2003).

In comparison with other drugs in its class, full-dose ritonavir has consistently been shown to be more hepatotoxic (John *et al.*, 1999; Antonio *et al.*, 2002; Sulkowski *et al.*, 2002; Wit *et al.*, 2002; Sulkowski, 2003; Murphy *et al.*, 2007). The use of low-dose ritonavir for pharmacokinetic boosting of other PIs drugs appears to be safe compared with high dose of ritonavir (Soriano *et al.*, 2008).

Prospective, cohort analysis indicated that LPV/r is not associated with a significantly increased risk of hepatotoxity among HCV-infected and uninfected patients compared with an alternative PI-based regimen, nelfinavir (Sulkowski *et al.,* 2004).

According to randomized, controlled multicenter trial in Netherlands and Belgium, although clinically relevant LEE during antiretroviral combination therapy is more frequently observed among HIV infected patients with concurrent chronic HBV, the incidence of LEE was 9% in patients who were treated with ART (Gisolf *et al.,* 2000).

In a study that assessed liver toxicity after initiation of treatment with LPV containing regimen the total cumulative incidence of severe liver toxicity linked to LPV/r use at 12 months was 4% (4/99), however, among HCV-positive patients, the cumulative incidence of LPV-associated liver toxicity was 8% (4/51), which was significantly greater than among HCV-negative individuals (González-requena *et al.*, 2004).

According to the retrospective study at Duke university medical center; on the incidence of severe hepatotoxicity related to antiretroviral therapy in HIV/HCV co-infected patients, although, the incidence of severe hepatotoxicity (grades 3 or 4) associated with any PIs or NNRTIs-based regimen were 10.7% (N = 6), no cases of severe hepatotoxicity were observed with LPV/r or ATV/r, which had the greatest months of exposure among PIs (Heil *et al.*, 2010).

A cohort study conducted in Spain showed the incidence of severe TE associated with ATV/r containing regimens is low in HIV infected patients with chronic viral hepatitis, as only twelve (6%) patients developed a grade 3–4 TE during follow-up. The incidence of grade ≥3 TEs was 8% (95% CI: 4–14) per 100 persons-year. In addition to this, the frequency of toxic hepatitis in patients with viral liver cirrhosis receiving such combinations is similar to that observed in subjects without cirrhosis (Pineda *et al.*, 2008).

According to concise review of different clinical trials, indinavir and ATV were associated with increases in un-conjugated bilirubin in 6%–40% of patients. However, the condition is not related to signs of hepatocellular injury, such as increases in serum liver enzyme levels. Bilirubin level returned to normal after the discontinuation of the drug in all subjects (Sulkowski, 2004).

Study conducted in Canada on hepatotoxicity associated with antiretroviral therapy containing dual versus single protease inhibitors, in individuals co-infected with HCV, therapy was discontinued for 3 of 27 dual-PIs–treated individuals because of the occurrence of tender hepatomegaly while nine of 39 single-PI–treated subjects discontinued therapy as a result of symptomatic hepatomegaly (5 patients) and malaise associated with acute transaminitis (4 patients) (Cooper *et al.*, 2002).

One of the largest cohorts of HIV-HCV co-infected patients that assessed risk associated with anti-retroviral treatment regimens containing single-PI, multiple-PIs and NNRTIs drugs on naïve

and experienced patients, from the naïve arm patients incidence of grade 4 hepatotoxicity was 4.13 per 100 persons-year (corresponding to a total of 5 events), with 0 per 100 persons-year in single PI group, 12.49 per 100 p-yrs in multiple PIs group. In experienced patients, a total of 58 cases of grade ≥3 hepatotoxicity were observed (6 on single-PI, 21 on multiple-PIs) (Torti *et al*., 2005).

2. OBJECTIVES OF THE STUDY

2.1. General objective

To evaluate protease inhibitors based anti-retroviral regimens induced liver injury at Zewditu Memorial Hospital.

2.2. Specific objectives

- ⇨ To determine the incidence of drug induced liver injury after initiation of PIs based anti-retroviral regimens.
- ⇨ To assess the severity of liver injury due to PIs based anti-retroviral regimens.
- ⇨ To determine the patterns of liver injury associated with PIs based anti-retroviral regimens.
- ⇨ To assess time dependent liver injury due to PIs based anti-retroviral regimens.
- ⇨ To identify the risk factors associated with liver injury in patients treated with PIs based anti-retroviral regimens.

3. METHODS

3.1. Study area and period

This study was conducted at Zewditu memorial hospital (ZMH), in Addis Ababa. Addis Ababa, the capital of Ethiopia, which is the largest city in the country located in the central part of Ethiopia. Addis Ababa has 10 sub-cities with 13 public hospitals distributed throughout ten sub cities. These public hospitals are administered under federal ministries (5 hospitals), Addis Ababa health bureau (6 hospitals), federal police (1 hospital) and army (1 hospital). Among the public hospitals, Zewditu memorial hospital, operating under Addis Ababa health bureau was selected as study site purposely, as it provides service to large retroviral infected patients, particularly, those who rely on second line ART regimens. It serves 446 adult patients who are currently on second line regimens (PI based regimens). The average incidence rate of 12 patients per month, that shifts to second line. The study was conducted at ART clinic of ZMH from March 1, 2018 to December 30, 2018. Patients were followed for 6 months after recruitment.

3.2. Study design

Observational cohort prospective study was used.

3.3. Source population

All patients who were HIV positive and had follow up at ZMH during the recruitment period.

3.4. Study population

All patients who were HIV positive and had follow up at ZMH and candidate for PI based regimens or on PI based regimens at most for three years, and fulfilling inclusion criteria during the recruitment period.

3.5. Eligibility criteria

3.5.1. Inclusion criteria

The inclusion criteria include:-

- Patients ≥18 years.
- HIV positive patients who started PI based regimens from March 1, 2015, to June 30, 2018.

3.5.2. Exclusion criteria

The exclusion criteria were those who were:-

- ► Not willing to participate in the study.
- ► On prior PI based anti-retroviral treatment for more than three years.

3.6. Sample size determination and sampling technique

- ➢ **Sample size determination**

To determine the required sample size single population proportion formula was employed;

Hence,

$n= (Z\alpha/2)^2pq/d^2$

Where:

n = Sample size

P = Proportion : 9 % rate of liver toxicity according to previous study on PI induce liver toxicity P=9% (Sulkowski *et al.,* 2004).

q = 1-p

d = Desired degree of precision.

Z= Standard normal value at the level of 95% confidence level.

n= (1.96)2(0.09X0.91)/ (0.05)2 =126, by adding 10% contingency, n=139.

- ➢ **Sampling techniques**

Convenience sampling technique was employed. HIV positive patients who will start PI and PI experienced in the last 3 years of study period were recruited after having informed consent.

3.7. Study variables

- ➢ **Independent variables**
  - ✓ Socio-demographic characteristics
  - ✓ Disease related variables: Baseline WHO stage, Base line CD4, Base line viral load,
  - ✓ Co morbidity, Co medication
  - ✓ Types of previous treatment
  - ✓ Social habit: Smoking, drinking alcohol
  - ✓ Family history
- ➢ **Dependent Variable**
  - ✓ Clinical outcome of liver toxicity (signs and symptoms)
  - ✓ Laboratory tests for LEE

3.8. Assessments

4.8.1 General assessment

Patients were undergone treatment and assessments in accordance with the standard practice. PI naïve patients were assessed on baseline, 4th week, 8th week, 16th week and 24th week and experienced patients were assessed on 1st visit (week 0), 2nd visit (8th week) and 3rd visit (24th week). On each visit data collection tools with socio-demographic, clinical and laboratory evaluation data were completed during routine follow-up evaluation for six months.

3.8.2. Assessment for performance

The diagnosis of liver injury was considered when abnormalities of liver-related biochemical tests are identified. Laboratory tests and clinical presentations were used to measure markers of liver injury and identify liver-related disease progress.

3.9. Statistical analysis

Data were analyzed using the Statistical Package for Social Sciences v.23.0 (SPSS 23.0) software. Descriptive parameters were expressed as percentages and frequencies, continuous variables were expressed as mean and standard deviation (SD). X^2(chi-square)/independent t-test statistics were used to test the association between variables. Binary logistic regression was used to determine predictive factors for liver injury. P-value of less 0.05 was considered as statistically significant.

3.10. Operational definitions and liver enzyme elevation severity grades

According to AIDS Clinical Trials Group (ACTG) criteria, the severity grade of LEE is described as follow:

Grade 1: 1.25–2.5 ULN,

Grade 2: 2.6–5.0 ULN

Grade 3: 5.1–10 ULN

Grade 4: >10 ULN

The ULN of ALT and AST were 40 U/L and for ALP 250 U/L, for both men and women. AST may be used instead of ALT only when the latter is unavailable.

To avoid selection bias favoring the inclusion of patients with other risk factors of liver toxicity and patients with elevated pre-treatment serum AST, ALT and ALP levels (higher than ULN) were classified on the basis of mean baseline value rather than ULN.

According to national institute on alcohol abuse and alcoholism (NIAAA) on alcohol intake:-
***No alcohol intake*:** For those patients who never had a habit of drinking alcohol
***Moderate alcohol intake*:** Drinking as up to 4 alcoholic drinks for men and 3 for women in a single day and a maximum of 14 drinks for men and 7 drinks for women per week.
***Heavy alcohol intake*:** drinking 5 or more alcoholic drinks for males or 4 or more for females at the same time or within couple of hours on 5 or more days in the past month.

Patterns of liver injury were based on R-value defined as;
R= (ALT/ULN)/(ALP/ULN)
Hepatocellular type of injury = $R \geq 5$
Mixed type of injury = $2<R<5$
Cholestatic type of injury = $R \leq 2$

3.11. Data collection tools and procedure

Guidelines of Council for international organization of medical science (CIOMS) and AIDS clinical trial groups were used to develop annex, the data collection tool. Both patients' interview and laboratory values were used. Data were collected by two trained nurses.

3.12. Data quality assurance

The collected data were reviewed every day and checked by the investigator for completeness and consistency of response. For the participants who missed their appointments were reminded using their phone number that was registered on their 1st visit.

3.13. Ethical consideration

Ethical approval was obtained from ethics review committee of school of pharmacy and institutional review board of college of health sciences, Addis Ababa university (Protocol No.ERB/SOP/05/10/2018). Participation was solely voluntary and had full right to get the treatment and follow up even if they refused to participate. Confidentiality of the participants was kept by giving the code instead of righting their names.

4. RESULTS

4.1. Socio-demographic data of study participants at ZMH.

Among the study participants, 88(62%) were female and 49 (34.5%) completed primary school. The mean age and body mass index (BMI) of study participants were 42.11 years and 21.81 Kg/m^2 respectively. Fifty nine (41.5%) and 67 (47.2%) study participants were in the range of 35-44 years old and 18-23.99 Kg/m^2 BMI respectively. Table 1 summarizes the socio-demographic characteristics of the study participants.

Table 1: Socio-demographic data of study participants at ZMH, N=142.

Variables	Category	Frequency	Percent	Mean ± SD
Sex	M	54	38.0	
	F	88	62.0	
Age	18-24	4	2.8	42.11 ± 10.21
	25-34	25	17.6	
	35-44	59	41.5	
	45-54	31	21.8	
	>54	23	16.2	
BMI	<18	15	10.6	21.81 ± 4.29
	18-23.99	67	47.2	
	24-29	43	30.3	
	>29	17	12.0	
Level of education	No formal schooling	24	16.9	
	Primary school	49	34.5	
	Secondary school	45	31.7	
	College/University	24	16.9	

4.2. Baseline characteristics of study participants.

The mean CD4 count, and viral load were 145.31 cells/mm^3 and 223664.01 copies/ml respectively. Among the prior regimens, TDF/3TC/EFV accounts (46.5%, n=66), while ABC/3TC/ATV/r was the major (55.6%, n=79) PI based combination of current drugs. Among the study participants, 70 (49.3%), 58 (40.8%) and 101 (71.1%) took herbal remedy, other drugs (other than ART and cotrimoxazole) and cotri-moxazole prophylaxis therapy respectively. Dama-kassie (*Ocimum lamifolium*) was the most commonly used herbal remedy; while amoxicillin was the main co-administered drug in the past three months of recruitment period. Among the study patients, 21 (14.8%) had at least one co- morbidity condition, of which majority of them had kidney problems. Most patients, 93 (65.5%) were categorized under WHO stage 1. The habits of drinking alcohol and smoking cigarettes were very rare as only 1 (0.7%) and 3 (2.1%) had habit of smoking and heavy alcohol drinking, respectively. Majority of the patients 107 (75.4%) were PI based treatment experienced prior to the recruitment. The mean baseline of AST, ALT and ALP were 31.18, 27.80 and 267.85 respectively. Baseline characteristics of the study participants were summarized in Table 2.

Table 2: Baseline characteristics of study participants at ZMH, N=142.

Baseline variables		Value n (%)
WHO staging	Stage 1	93 (65.5)
	Stage 2	15 (10.6)
	Stage 3	20 (14.1)
	Stage 4	14 (9.9)
CD4 count (mean ± SD)		145.31 ± 126.95
Viral load count (mean ± SD)		223664.01± 518505.11
Previous 1st lines	D4t/3TC/NVP	1 (0.7)
	AZT/3TC/NVP	31 (21.8)
	AZT/3TC/EFV	17 (12)
	TDF/3TC/EFV	66 (46.5)
	TDF/3TC/NVP	27 (19)
Current 2nd lines taken	AZT/3TC/LPV/r	2 (1.4)
	AZT/3TC/ATV/r	13 (9.2)
	TDF/3TC/LPV/r	2 (1.4)
	TDF/3TC/ATV/r	40 (28.2)
	ABC/3TC/ATV/r	79 (55.6)
	ABC/3TC/LPV/r	6 (4.2)
Herbal medicine used	Yes	70 (49.1)
	No	72 (50.7)

Cotrimoxazole prophylactic therapy	Yes	101 (71.1)
	No	41 (28.9)
Use of other drugs	Yes	58 (40.8)
	No	84 (59.2)
History of other medical condition	Yes	21 (14.8)
	No	121 (85.2)
History of alcohol intake	Never	129 (90.8)
	Moderate	10 (7)
	Heavy	3 (2.1)
History of cigarette smoking	Yes	1 (0.7)
	No	141 (99.3)
History of PI	Naïve	35 (24.6)
	Experienced	107 (75.4)
AST (mean ± SD)		31.18 ± 48.27
ALT (mean ± SD)		27.80 ± 57.58
ALP (mean ± SD)		267.85 ± 296.81

D4t: Stavudine, **3TC**: Lamivudine, **NVP**: Nevirapine, **AZT**: Zidovudine, **TDF**: Tenofovir-Disoproxil-Fumarate, **EFV**:Efavirenz, **ABC**: Abacavir, **LPV/r**: Lopinavir/ritonavir and **ATV/r**: Atazanavir/ritonavir

4.3. Follow-up.

A total of 162 participants were recruited during the recruitment study period. Among them, 20 participants were excluded from the study, of which, 14 patients withdrew informed consent, 3 patients died and 3 patients discontinued ART with non-hepatic events during the study period. Finally, 142 participants were included in the analysis, of which 107 (75.4%) were PI experienced and 125 (88%) had normal liver enzyme at baseline (Figure 2).

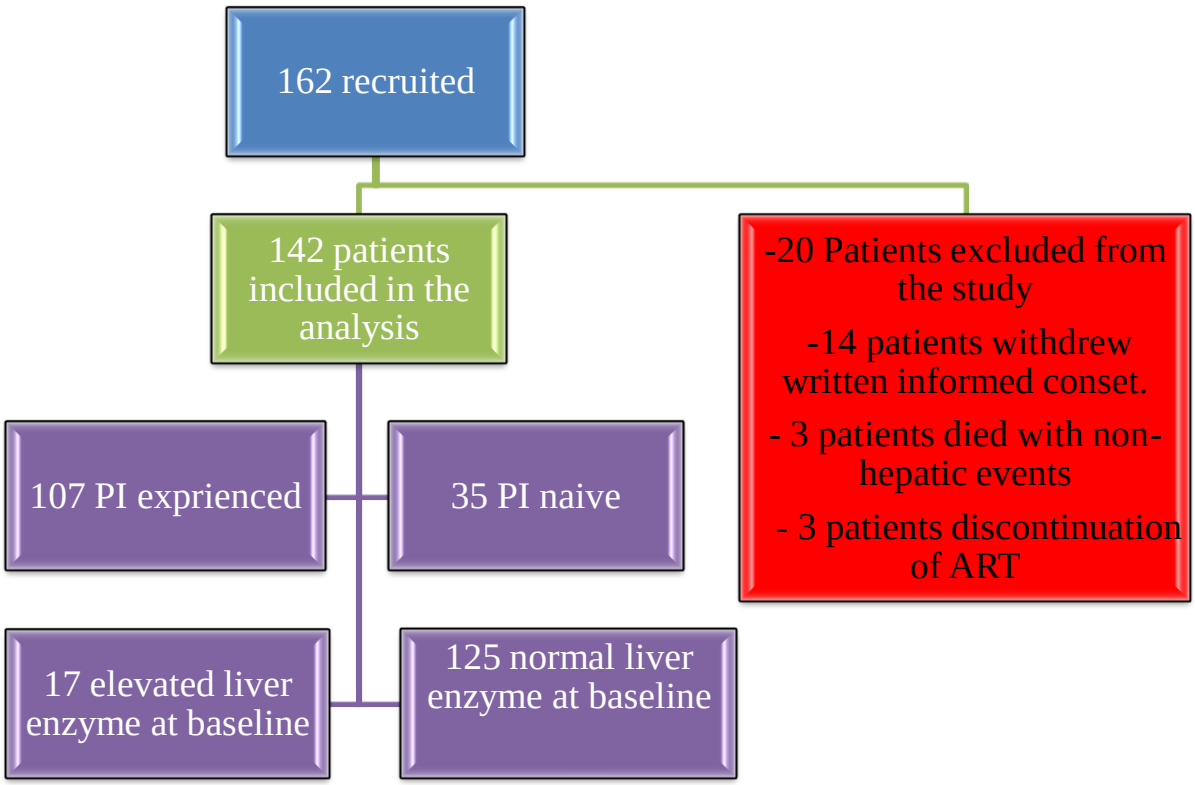

Figure 2: Flow chart of patients' composition and disposition during the study period.

4.4. Incidence of liver injury.

Out of 142 study participants, overall liver injury of any grade was observed in 25 (17.6%) participants. Incidence of liver injury was higher in patients with elevated baseline liver enzyme 10 (58.8%) than those with normal baseline liver enzyme 15 (12%). Among 35 PI based naïve and 107 PI based experienced participants, 3 (8.6%) and 22 (20.6%) developed liver injury during the study period, respectively. From PI naïve who developed liver injury, 2 (66.7%) participants had elevated baseline liver enzyme, while 1 (33.3%) participant had normal baseline liver enzyme. Furthermore, among PI experienced participants who developed liver injury, 8 (36.4%) and 14 (63.6%) participants had elevated and normal baseline liver enzyme respectively. Liver injury in PI based naïve and experienced participants and their inter comparison based on the status of liver enzyme at baseline were summarized as Figure 3.

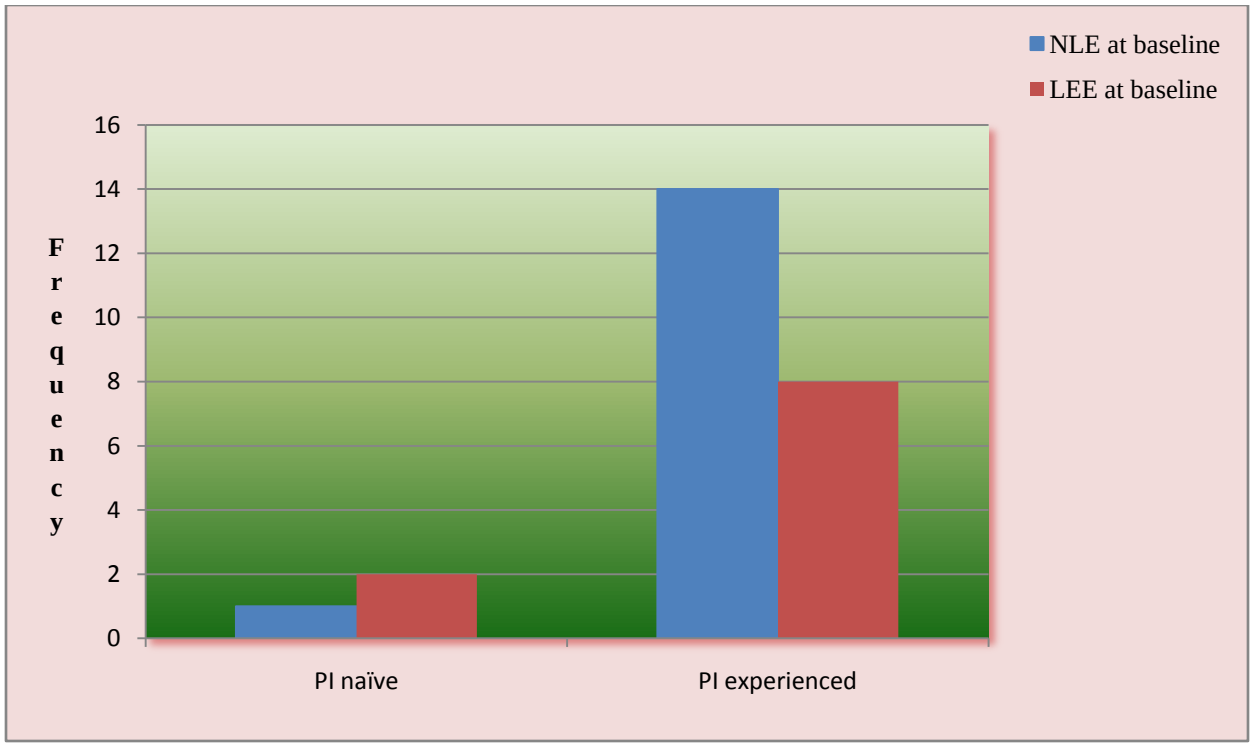

Figure 3: Incidence of liver injury based on PIs history and liver enzyme at baseline.
NLE=normal liver enzyme: **LEE**=liver enzyme elevated (≥1.25 ULN): **PI**= protease inhibitor.

4.5. Severity grade of liver injury.

From the total of 25 participants who developed liver injury, none of the patients developed grade-4 liver injury, whereas the majority of them developed grade 1. Overall incidence of grade 1, 2 and 3 were 16 (64%), 6 (24%) and 3 (12%) respectively (Figure 4). Severity grade varied between PI naïve and experienced patients, of whom 15 (68.2%), 5 (22.7%) and 2 (9.1%) PI experienced and 1 (33.3%), 1 (33.3%) and 1 (33.3%) PI naïve partiipants developed grade 1, grade 2 and grade 3 liver injury, respectively. In addition to this, 9 (60%), 5 (33.3%) and 1 (6.7%) participants with normal baseline liver enzyme and 7 (70%), 1 (10%) and 2 (20%) participants with elevated baseline liver enzyme developed grade1, 2 and 3 liver injury, respectively. The comparison of severity grade in terms of PI history and status of liver enzyme at baseline is illustrated in Table 3.

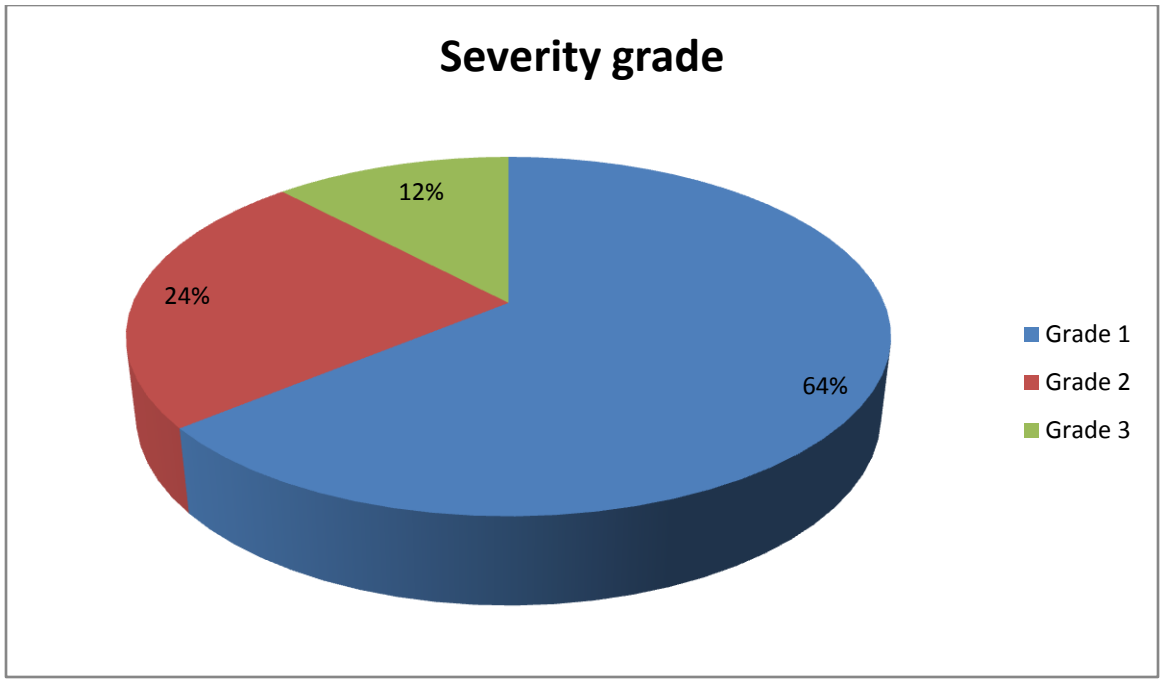

Figure 4: The overall severity grade among the study participants.

Table 3: The severity grade in comparison with PI history and status of liver enzyme at baseline among study participants at ZMH.

Characteristics		Severity grade		
		Grade 1	**Grade 2**	**Grade 3**
PI history	Naïve (n=3)	1 (33.3%)	1 (33.3%)	1 (33.3%)
	Experienced (n=22)	15 (68.2%)	5 (22.7%)	2 (9.1%)
	Total (n=25)	16 (64%)	6 (24%)	3 (12%)
Status of liver enzyme at baseline	Normal (n=15)	9 (60%)	5 (33.3%)	1 (6.7%)
	Elevated (n=10)	7 (70%)	1 (10%)	2 (20%)
	Total (n=25)	16 (64%)	6 (24%)	3 (12%)

4.6. Proportion of signs and symptoms with liver enzyme elevation and severity grade.

Pruritus, jaundice and vomiting were significantly associated with liver enzyme elevation. Vomiting and pruritus were associated with grade 1 and grade 3, liver injury respectively, while jaundice was common in both grade 2 and 3, liver injury. Even though, hepatomegaly and hepatic tenderness were rare parameters and not significantly associated with overall LEE, but significantly associated with grade 3, liver injury. Among PI naïve participants, nausea and vomiting were common with both grade 1 and 2, liver injury. In addition to this, pruritus was associated with grade 2, and jaundice was common in both grade 2 and 3, liver injury, while hepatomegaly and hepatic tenderness were associated with grade 3, liver injury for PI naïve participants. Vo-

miting was significantly associated with both grade 1 and 3, liver injury while jaundice was common with grade 2 liver injury in PI experienced participants. Moreover, fever, pruritus and nausea were significantly associated with grade 3, liver injury within this group of participants (Table 4).

Table 4: Proportion of signs and symptoms *with* liver enzyme elevation and severity grade.

Signs and symp-toms	Overall liver injury of any grade		Grade of liver in-jury	History of PI				Total liver injury within grade of injury	
				Naïve		Experienced			
	n (%)	P-Value		n (%)	P-Value	n (%)	P-Value	n (%)	P-Value
Fatigue (N=62)	9 (36)	0.506	1	0 (0.0)	0.244	3 (20.0)	0.090	3 (18.8)	0.228
			2	1 (100)	0.224	3 (60.0)	0.409	4 (66.7)	0.264
			3	0 (0.0)	0.244	2 (100)	0.061	2 (66.7)	0.418
Fever (N=28)	7 (28)	0.272	1	0 (0.0)	0.468	1 (6.7)	0.294	1 (6.3)	0.196
			2	1 (100)	0.081	2 (40.0)	0.255	3 (50.0)	0.089
			3	1 (100)	0.081	2 (100)	0.009	2 (66.7)	0.073
Abdominal pain (N=28)	2 (8)	0.164	1	0 (0.0)	0.576	1 (6.7)	0.183	1 (6.3)	0.196
			2	0 (0.0)	0.576	0 (0.0)	0.115	0 (0.0)	1.000
			3	0 (0.0)	0.576	1 (50.0)	0.370	1 (33.3)	0.575
Dark urine (N=12)	0 (0)	0.126	1	0 (0.0)	0.670	0 (0.0)	0.354	0 (0.0)	0.361
			2	0 (0.0)	0.670	0 (0.0)	0.343	0 (0.0)	1.000
			3	0 (0.0)	0.670	0 (0.0)	0.551	0 (0.0)	0.464
Pruritus (N=17)	7 (28)	0.013	1	0 (0.0)	0.670	4 (26.7)	0.107	4 (25.0)	0.103
			2	1 (100)	0.022	0 (0.0)	0.230	1 (16.7)	0.542
			3	0 (0.0)	0.670	2 (100)	0.004	2 (66.7)	0.024
Rash (N=44)	12 (48)	0.057	1	1 (100)	0.171	7 (46.7)	0.119	8 (50.0)	0.092
			2	1 (100)	0.171	2 (40.0)	0.555	3 (50.0)	0.322
			3	0 (0.0)	0.308	1 (50.0)	0.508	1 (33.3)	0.930
Poor appetite (N=39)	5 (20)	0.462	1	0 (0.0)	0.620	3 (20.0)	0.376	3 (18.8)	0.557
			2	0 (0.0)	0.620	1 (20.0)	0.518	1 (16.7)	0.525
			3	0 (0.0)	0.620	1 (50.0)	0.610	1 (33.3)	0.821
Nausea (N=25)	5 (20)	0.774	1	1 (100)	0.043	0 (0.0)	0.068	1 (6.3)	0.305
			2	1 (100)	0.043	1 (20.0)	0.939	2 (33.3)	0.342

			3	0 (0.0)	0.576	2 (100)	0.009	2 (66.7)	0.057
Vomiting (N=19)	10 (40)	0.000	1	1 (100)	0.022	6 (40.0)	0.010	7 (43.8)	0.001
			2	1 (100)	0.022	0 (0.0)	0.197	1 (16.7)	0.585
			3	0 (0.0)	0.670	2 (100)	0.005	2 (66.7)	0.057
Hepatomegaly (N=1)	1 (4)	0.061	1	0 (0.0)	0.808	-	-	0 (0.0)	0.624
			2	0 (0.0)	0.808	-	-	0 (0.0)	0.768
			3	1 (100)	0.003	-	-	1 (33.3)	0.004
Hepatic tenderness (N=2)	1 (8)	0.292	1	0 (0.0)	0.808	0 (0.0)	0.582	0 (0.0)	0.488
			2	0 (0.0)	0.808	0 (0.0)	0.756	0 (0.0)	0.677
			3	1 (100)	0.003	0 (0.0)	0.846	1 (33.3)	0.021
Jaundice (N=19)	8 (32)	0.006	1	0 (0.0)	0.730	1 (6.7)	0.457	1 (6.3)	0.696
			2	1 (100)	0.012	4 (80.0)	0.001	5 (100)	0.000
			3	1 (100)	0.012	1 (50.0)	0.257	2 (66.7)	0.031

n=Frequency

4.7. Protease inhibitors Based regimens induced liver injury.

The frequency distribution of PIs based regimens was ABC/3TC/ATV/r (79 participants), TDF/3TC/ATV/r (40 participants), AZT/3TC/ATV/r (13 participants), ABC/3TC/LPV/r (6 participants), AZT/3TC/LPV/r (2 participants) and TDF/3TC/LPV/r (2 participants), of whom, 13, 9, 1, 1, 1, 0 participants developed liver injury, respectively. Among the study participants, who took ABC/3TC/ATV/r, 8 patients developed grade-1 liver injury, while 4 of them developed grade 2, liver injury. Furthermore, the severe liver injury of grade 3, was developed by a single patient in each of ABC/3TC/ATV/r, TDF/3TC/ATV/r and ABC/3TC/LPV/r regimens. Figure 5 shows the liver injury profile with different PI regimens.

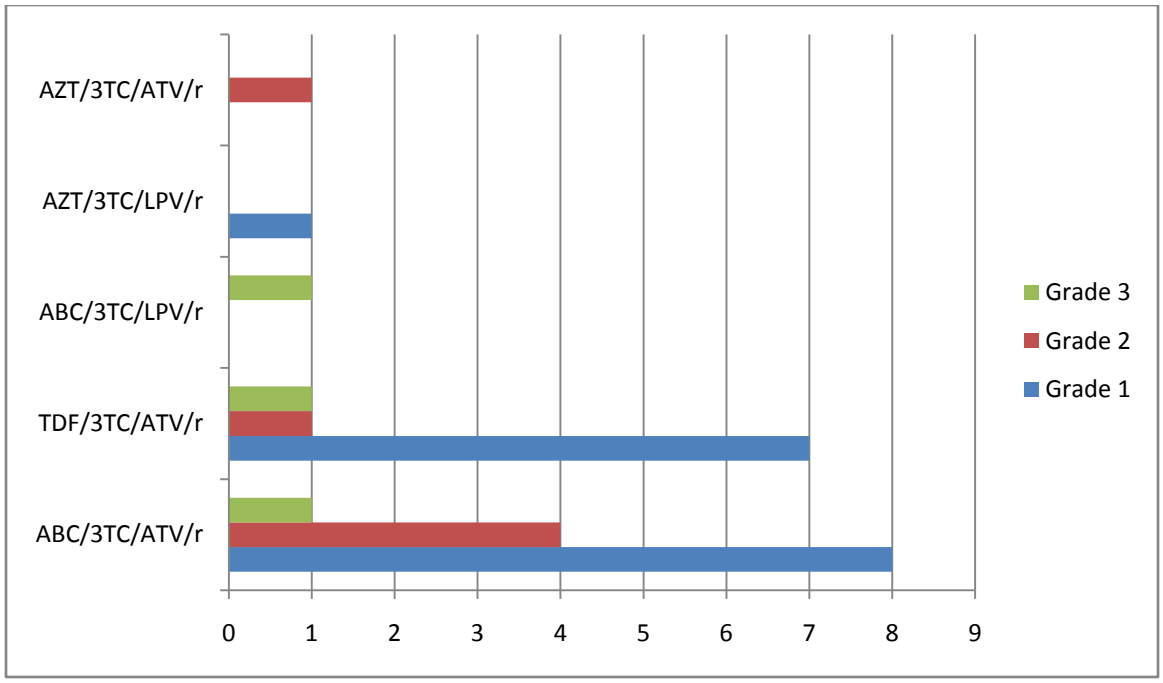

Figure 5: Liver injury profiles of participants with different PIs based regimens.

4.8. Patterns of liver injury.

Among the total of 25 participants who developed liver injury, majority of them 21 (84%) developed cholestatic pattern of liver injury. Few patients, 3 and 1 developed mixed and hepatocellular types of liver injury respectively. Even though, the hepatocellular type of liver injury was uncommon in this study, the single case of hepatocellualar liver injury was a severe case of grade 3, liver injury. The patterns of liver injury with their severity grade are summarized in Figure 6.

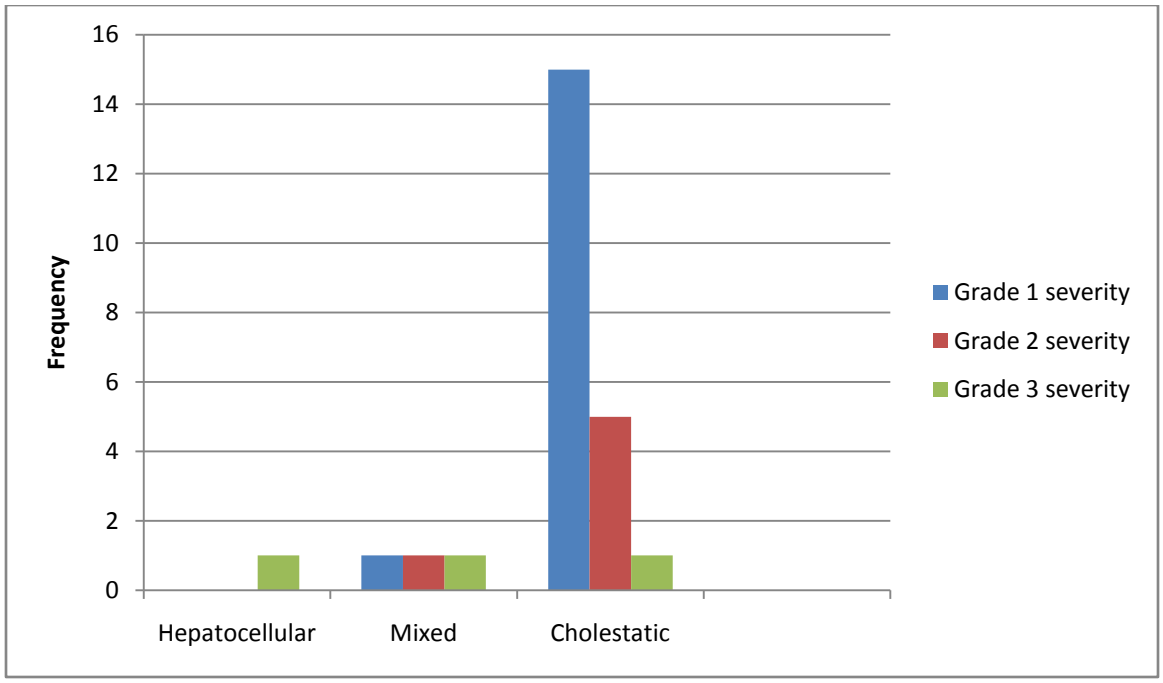

Figure 6: Patterns of liver toxicity among participants with different severity grade.

4.9. Time dependent liver injury.

The onset of time in which liver injury developed within PIs based regimens various. From the study participants, 35, 35, 36, 36, 7, 26, and 73 were within 0-4, 5-8, 9-16, 17-24, 25-48, 49-96 and 97-144 duration of time in weeks respectively, since the start of PI based regimens. The incidence of liver injury was high (57.1%, 4/7), within 25-48 weeks from the starting time of PI based regimens. Furthermore, among study participants, (5.7%, 2/35), (2.9%, 1/35), (0%, 0/36), (2.8%, 1/36), (38.5%; 10/26) and (9.6%, 7/73) developed liver injury within 0-4, 5-8, 9-16, 17-24, 48-96 and 97-144, weeks respectively. The grade 3 severity was developed by 2 and 1 participants in 0-4 and 49-96 weeks respectively. Figure 7: illustrates the time dependent liver injury (Figure 7).

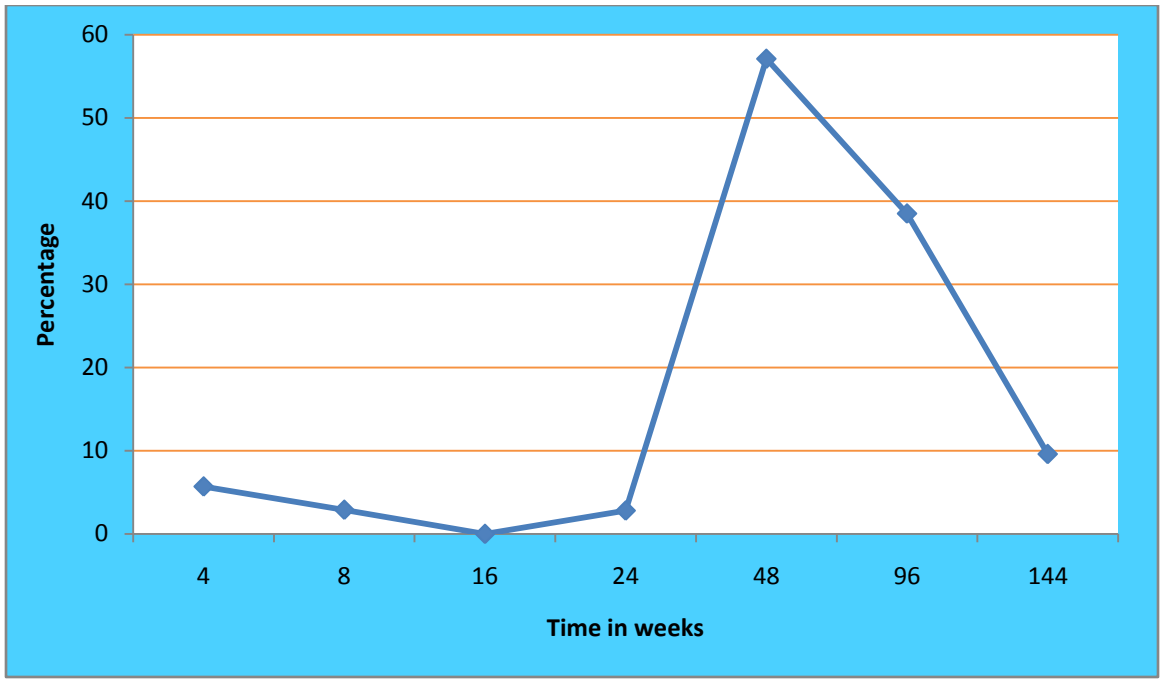

Figure 7: Time dependent liver injury.

4.10. Risk factors associated with liver injury.

The baselines characteristics of patients were analyzed with chi-square/independent t-test and binary logistic regression. In chi-square test ($\chi 2$) categorical variables are compared with each other based on their effect on liver injury. Accordingly cotrimoxazole prophylactic therapy (P=0.032) and previous first line (P=0.004) were significantly associated with liver injury. Continues variables were analyzed by independent t-test, of which, elevated baseline of AST (P=0.011) and ALT (P=0.007) were associated with liver injury. Those variables which were significant (P<0.05) in chi-square/independent t-test were included in multivariate logistic regression to determine their adjusted odds ratio. In this multivariate analysis cotrimoxazole prophylactic therapy (p=0.022, AOR=0.302, CI=0.109-0.839) and previous first line on AZT/3TC/NVP (P=0.000, AOR =0.144, CI=0.051-0.408) were significantly associated with liver injury (Table 5).

Continues variables; age, BMI, CD4 and VL were analyzed with both independent t-test and chi-square test by recoding to categorical variables. Age was not associated with liver injury in continues (P=0.563) and categorical (P=0.119) variable. Moreover, it had no relationships (P=0.527) with liver injury, with broad range age category (<55 and ≥55 years old) of study participants.

Similar to categorical form (Table 5), the baseline continues variables of BMI (P=0.063), CD4 (P=0.713) and VL (P=0.132) were not associated with liver injury. More importantly, BMI was not associated with liver injury during 2nd (P=0.964), 3rd (P=0.916) and 4th (P=0.825) visits.

In multivariate logistic regression few baseline parameters of the study participants were associated with signs and symptoms they developed during the study period. Hence, under weight (<18 BMI) (P=0.035, AOR 0.886, CI=0.793-0.991), elevation of AST (P=0.017, AOR=0.934, CI=0.883-0.988) and WHO stage-1 (P=0.034, AOR=14.051, CI=1.113-14.737) were significantly associated with fever, poor appetite and nausea respectively, and the rest signs and symptoms did not have association with baseline parameters of the study participants.

Table 5: Risk factors associated with liver injury.

Variables		P-value (t-test/χ2)	Multivariate Logistic Regression		
			P -Value	AOR	95% CI for AOR
Sex	Male Female	0.266	NS	-	
Age	18-24	0.119	NS		
	25-34		NS		
	35-44		NS		
	45-54		NS		
	>54		NS		
Level of education	No formal schooling	0.648	NS		
	Primary school		NS		
	Secondary school		NS		
	College / university		NS		
BMI	<18	0.161	NS		
	18-23.99		NS		
	24-29		NS		
	>29		NS		
Prior HAART used	D4t/3TC/NVP	0.004	NS		
	AZT/3TC/NVP		0.000	0.144	0.051-0.408
	AZT/3TC/EFV		NS		
	TDF/3TC/EFV		NS		
	TDF/3TC/NVP		NS		
Current PI based regimens	AZT/3TC/LPV/r	0.604	NS		
	AZT/3TC/ATV/r		NS		

	TDF/3TC/LPV/r		NS		
	TDF/3TC/ATV/r		NS		
	ABC/3TC/ATV/r		NS		
	ABC/3TC/LPV/r		NS		
PI history	Naive Experienced	0.129	NS		
CD_4	<200		NS		
	200-400	0.619	NS		
	>400		NS		
Viral load	<1000		NS		
	1000-10000	0.640	NS		
	>10000		NS		
WHO stage	Stage 1		NS		
	Stage 2	0.304	NS		
	Stage 3		NS		
	Stage 4		NS		
Herbal remedy use	Yes No	0.275	NS		
Use of other medication	Yes No	0.823	NS		
CPT	Yes No	0.032	0.022	0.302	0.109-0.839
Prior history of liver problem	Yes No	0.543	NS		
History of other medical condition	Yes No	0.533	NS		
History of alcohol consumption	Never	0.245	NS		
	Moderate	0.689	NS		
	Heavy	0.080	NS		
AST at baseline		0.011	NS		
ALT at baseline		0.007	NS		
ALP at baseline		0.163	NS		

AOR=Adjusted Odds Ratio: **CI**= Confidence interval: **CPT**=Cotrimoxazole Prophylactic Therapy: **NS**=Non-Significant

4.11. Correlations of parameters in patients with and without liver enzyme elevation for PI naïve and experienced participants.

Chi-square test and independent t-test compared the correlation of characteristics of participants with and without LEE for both PI naïve and experienced patients. Accordingly, sex and cotrimoxazole prophylactic therapy were significantly difference between LEE and without LEE during 3rd and 4th visits respectively, for PI naïve participants. The liver enzyme tests (AST, ALT and ALP) were significantly difference between PI naïve patients who did and did not develop LEE during 2nd, 3rd and 4th visits (Table 6). WHO stage and cotrimoxazole prophlaxis therapy

were significantly difference between PI experienced participants with and without LEE, during 1st visit. Previous 1st line during 3rd visit and liver enzyme tests (AST, ALT and ALP) in all visits (1st, 2nd and 3rd) were statistically difference among PI experienced patients (Table 7).

Table 6: Comparison of parameters between participants developing LEE and those not developing LEE in the subgroup PI naive patients.

Characteristics		2nd visit		3rd visit		4th visit		5th visit	
		Pts with LEE, n (%)	Pts without LEE, n (%)	Pts with LEE, n (%)	Pts with-out LEE, n (%)	Pts with LEE, n (%)	Pts without LEE, n (%)	Pts with LEE, n (%)	Pts without LEE, n (%)
Sex	Male	1 (7.7)	12 (92.3)	0 (0)	13 (100)	0 (0)	13 (100)	0 (0)	13 (100)
	Female	5 (22.7)	17 (77.3)	4 (18.2)	18 (81.8)	2 (9.1)	20 (90.9)	1 (4.5)	21 (95.5)
	P-value	0.231		0.045		0.165		0.331	
Age (mean ± SD)		42 ±10.37	39.83 ± 9.42	41.75 ± 4.35	40 ± 9.96	42 ± 5.66	40.09 ± 9.69	46	40.03 ± 9.55
	P-value	0.650		0.733		0.786		0.542	
BMI (mean ± SD)		22.4 ± 2.81	21.63 ± 3.16	22.26 ± 3	21.7 ± 3.13	22.63 ± 0.85	21.71 ± 3.16	23.23	21.72 ± 3.11
	P-value	0.567		0.739		0.687		0.635	
Pre-vious ART	AZT/3TC/NVP	2 (28.6)	5 (71.4)	1 (14.3)	6 (85.7)	1 (14.3)	6 (85.7)	0(0)	7 (100)
	AZT/3TC/EFV	0 (0)	4 (100)	0 (0)	4 (100)	0 (0)	4 (100)	0 (0)	4 (100)
	TDF/3TC/EFV	3 (18.8)	13 (81.2)	3 (18.8)	13 (81.2)	1 (6.3)	15 (93.7)	1 (6.3)	15 (93.7)
	TDF/3TC/NVP	1 (12.5)	7 (87.5)	0 (0)	8 (100)	0 (0)	8 (100)	0 (0)	8 (100)
	P-value	0.527		0.297		0.550		0.659	
Cur-rent ART	AZT/3TC/ATV/r	2 (22.2)	7 (77.8)	2 (22.2)	7 (77.8)	1 (11.1)	8 (88.9)	1 (11.1)	8 (88.9)
	TDF/3TC/LPV/r	0 (0)	1 (100)	0 (0)	1 (100)	0 (0)	1 (100)	0 (0)	1 (100)
	TDF/3TC/ATV/r	2 (18.2)	9 (81.8)	1 (9.1)	10 (90.9)	1 (9.1)	10 (90.9)	0 (0)	11 (100)

	ABC/3TC/ATV/r	2 (15.4)	11 (84.6)	1 (7.7)	12 (92.3)	0 (0)	13 (100)	0 (0)	13 (100)
	ABC/3TC/LPV/r	0 (0)	1 (100)	0 (0)	1 (100)	0 (0)	1 (100)	0 (0)	1 (100)
	P-value	0.918		0.811		0.671		0.591	
CD4 (mean ± SD)		151 ± 91.16	156.6 ± 78.45	87.5 ± 92.63	157.32 ± 76.95	22	157.13 ± 75.18	22	157.13 ± 75.18
	P-value	0.991		0.237		0.092		0.092	
VL (mean ± SD)		208051.67 ± 164275.37	334561.73 ± 820018.08	278268.25 ± 287170.76	312316..46 ± 278268.25	344335.5 ± 180513.76	304615.27 ± 756198.13	471978	301358.89 ± 741706.31
	P-value	0.505		0.933		0.942		0.823	
WHO stage	Stage 1	5 (16.7)	25 (83.3)	3 (10.0)	27 (90.0)	1 (3.3)	29 (96.7)	1 (3.3)	29 (96.7)
	Stage 2	0 (0)	1 (100)	0 (0)	1 (100)	0 (0)	1 (100)	0 (0)	1 (100)
	Stage 3	1 (25)	3 (75)	1 (25)	3 (75)	1 (25)	3 (75)	0 (0)	4 (100)
	P-value	0.764		0.646		0.356		0.855	
CPT	Yes	4 (16.7)	20 (83.3)	2 (8.3)	22 (91.7)	0 (0)	24 (100)	0 (0)	24 (100)
	No	2 (18.2)	9 (81.8)	2 (18.2)	9 (81.8)	2 (18.2)	9 (81.8)	1 (9.1)	10 (90.9)
	P-value	0.912		0.410		0.027		0.123	
AST (mean ± SD)		68 ± 105.91	22.24 ± 6.37	43.25 ± 30.97	21.13 ± 6.85	42 ± 35.36	19.76 ± 6.03	15	17.32 ± 4.46
	P-value	0.020		0.001		0.001		0.611	
ALT (mean ± SD)		77.83 ± 101.93	17.41 ± 7.69	38 ± 31.97	18.81 ± 8.77	42.5 ± 38.89	16.18 ± 5.8	13	15.09 ± 5.18
	P-value	0.002		0.008		0.000		0.694	
ALP (mean ± SD)		318.83 ± 122.17	187.86 ± 62.72	290.5 ± 142.1	152.26 ± 73.39	242.5 ± 221.32	121.15 ± 54.5	399	119.71 ± 62.51
	P-value	0.000		0.003		0.017		0.000	

Pt= patient: **CPT**=Cotrimoxazole prophylactic therapy: **LEE**=liver enzyme elevated (≥1.25 ULN): **SD**=Standard deviation: **n**= Frequency: **VL**=Viral load

Table 7: Comparison of parameters between participants developing LEE and those not developing LEE in the subgroup PI experienced patients.

Characteristics		1st visit			2nd visit			3rd visit		
		Pts. with LEE n(%)	Pts. without LEE n (%)	P-value	Pts. with LEE n(%)	Pts. without LEE n (%)	P-value	Pts. with LEE n(%)	Pts. without LEE n (%)	P-value
Sex	Male	7 (17.1)	34 (82.9)	0.570	7 (17.1)	34 (82.9)	0.384	5 (12.2)	36 (87.8)	0.745
	Female	8 (12.1)	58 (87.9)		7 (10.6)	59 (89.4)		6 (9.1)	60 (90.9)	
Age (mean ± SD)		42.8 ± 10.48	42.78 ± 10.48	0.977	39.43 ± 7.66	42.23 ± 10.71	0.205	41.64 ± 4.46	42.85 ± 10.89	0.154
BMI(mean ± SD)		19.84 ± 3.52	22.15 ± 4.72	0.073	19.89 ± 4.17	22.12 ± 4.65	0.093	19.94 ± 3.01	22.04 ± 4.75	0.154
Previous ART	D4t/3TC/NVP	0 (0)	1(100)	0.167	0 (0)	1(100)	0.748	0 (0)	1(100)	0.015
	AZT/3TC/NVP	6 (25)	18 (75)		5 (20.8)	19 (79.2)		6 (25)	18 (75)	
	AZT/3TC/EFV	0 (0)	13 (100)		1 (7.7)	12 (92.3)		1 (7.7)	12 (92.3)	
	TDF/3TC/EFV	7 (14)	43 (86)		6 (12)	44 (88)		4 (8)	46 (92)	
	TDF/3TC/NVP	2 (10.5)	17 (89.5)		2 (10.5)	17 (89.5)		0 (0)	1 (100)	
Current ART	AZT/3TC/LPV/r	2 (100)	0 (0)	0.079	0 (0)	2 (100)	0.763	1 (50)	1 (50)	0.464
	AZT/3TC/ATV/r	0 (0)	4 (100)		0 (0)	4 (100)		0 (0)	4 (100)	
	TDF/3TC/LPV/r	0 (0)	1 (100)		0 (0)	1 (100)		0 (0)	1 (100)	
	TDF/3TC/ATV/r	3 (10.3)	26 (89.7)		3 (10.3)	26 (89.7)		4 (13.8)	25 (86.2)	
	ABC/3TC/ATV/r	9 (13.6)	57 (86.4)		10 (15.2)	56 (84.8)		6 (9.1)	60 (90.9)	
	ABC/3TC/LPV/r	1 (20)	4 (80)		1 (20)	4 (80)		0 (0)	5 (100)	
CD4 (mean ± SD)		146.4 ± 116.54	143.48 ± 139.41	0.939	139.31 ± 176.07	144.54 ± 130.41	0.897	100.82 ± 88.04	148.94 ± 139.88	0.268

VL (mean ± SD)		106494.92 ± 206943.75	211764.68 ± 456731.62	0.439	47131.36 ± 59241.71	218611.3 ± 461369.61	0.224	60994.22 ± 106318.61	212907.33 ± 456204.97	0.324
WHO stage	Stage 1	7 (11.1)	56 (88.9)	0.028	7 (11.1)	56 (88.9)	0.382	3 (4.8)	60 (95.2)	0.152
	Stage 2	0 (0)	14 (100)		1 (7.1)	13 (92.9)		2 (14.3)	12 (85.7)	
	Stage 3	3 (18.8)	13 (81.3)		2 (12.5)	14 (87.5)		3 (18.8)	13 (81.3)	
	Stage 4	5 (35.7)	9 (64.3)		4 (28.6)	10 (71.4)		3 (21.4)	11(78.6)	
CPT	Yes	6 (7.9)	70 (92.1)	0.011	10 (13.2)	66 (86.8)	0.972	7 (9.2)	69 (90.8)	0.726
	No	9 (29)	22 (71)		4 (12.9)	27 (87.1)		4 (12.9)	27 (87.1)	
AST (mean ± SD)		55.67 ± 47.74	19.95 ± 5.95	0.000	45.79 ± 35.18	20.28 ± 6.15	0.000	45.82 ± 20.74	19.98 ± 6.04	0.000
ALT (mean ± SD)		77.27 ± 73.39	17.78 ± 7.54	0.000	58.57 ± 39.38	15.97 ± 7.29	0.000	59.82 ± 29.29	16.89 ± 7.22	0.000
ALP (mean ± SD)		324.47 ± 182.95	194.36 ± 54.41	0.000	427.21 ±295.63	179.62 ±65.11	0.000	463.27 ± 341.25	134.13 ± 64.43	0.000

Pt= patient: **CPT**=Cotrimoxazole prophylactic therapy: **LEE**=liver enzyme elevated (≥1.25 ULN): **SD**=Standard deviation: **n**= Frequency: **VL**=Viral load

5. DISCUSSION

To date, there are scarce data on safety of liver associated with PIs-based ART regimens in clinical practice with prospective cohort study particularly in resource limited settings. We, therefore, performed an observational prospective study to evaluate the incidence, pattern, severity, onset of time and risk factors of liver injury due to PI based ART regimens on PI based naïve and experienced HIV-1 patients. Our research findings showed that majority of the patients develop grade 1 and cholestatic pattern of liver injury. Even though, hepatocellular is uncommon, a single hepatocellular liver injury pattern was associated with grade 3 liver injury. The incidence of liver injury was high within 25-48 weeks of duration. Cotrimoxazole prophylactic therapy and previous 1st line on AZT/3TC/NVP were independent risk factors of liver injury.

Hepatocellular pattern of hepatotoxicity is generally associated with marked elevation in the serum level of ALT, AST, or both, while cholestatic hepatotoxicity is characterized by marked elevation of serum alkaline phosphatase levels, usually with development of pruritus and jaundice and in mixed pattern of hepatotoxicity, neither ALT/AST nor ALP elevations are clearly predominant (Andrade *et al.*, 2007). In our study, 84% of liver injury induced study participants, developed cholestasis pattern of liver injury. Drugs that are excreted by the liver into bile and drugs that inhibit bile acid transport processes are prone to elicit cholestatic liver injury. As a result, boosted ATV and LPV inhibit bile salt export pump and can cause accumulation of bile acids (Griffin *et al.*, 2013; Mcrae *et al.*, 2010).

Unlike other first line HAART studied in Uganda in which only jaundice was significantly associated to liver enzyme elevation (Kalyesubula *et al.*, 2011),our results showed Jaundice and pruritus were related with severe liver enzyme elevation (grade 3), in line with the clinical practice of drug induced hepatotoxicity (Andrade *et al.*, 2007). Moreover, hepatomegaly and hepatic tenderness were associated with grade-3 liver injury, but related with single case that cannot able to generalize.

Jaundiced patients with a hepatocellular pattern of liver injury are prone to severe hepatotoxicity and about 10% rate of liver related death (Aithal *et al.*, 2011). Similarly, in our study hepatocellular developed patient, with severe grade of liver injury (grade-3) had jaundice, hepatomegaly and hepatic tenderness.

Although the introduction of HAART significantly improved the life expectancy of patients with HIV, morbidity and mortality has been observed in some patients that can be attributed to discontinuation of HAART resulting from liver toxicities (Shirish *et al.,* 2010), We, however, found that none of the patients, discontinued the therapy due to liver injury. This is due to, in our study participants, no grade 4 liver injury was observed, but Ethiopian guideline recommended discontinuation of therapy for grade 4 liver injury (Minstry of Health, 2017).

In contrast to the severe toxicity of first line ART, observed in the previous multi-center retrospective and prospective cohort study (Gudina *et al.,* 2017), our findings showed that the majority were grade 1 liver injury.

Even though the study did not include PIs-based regimens, another study done in the same setting reported 20.1% and 22% developed liver enzyme abnormalities in HAART experienced and naive patients, respectively (Shifera *et al.,* 2016) ,which was higher compared with our study. It implied PIs-based ART regimens had less liver injury than other ART regimens.

ART associated liver toxicities depend on the type of ART regimens. Several studies reported that full-dose of ritonavir (regimen when ritonavir is not used as a booster) has consistently shown to be more hepatotoxic (John *et al*., 1999; Antonio *et al.*, 2002; Sulkowski *et al.,* 2002; Wit *et al.,* 2002; Sulkowski, 2003; Murphy *et al.,* 2007). The use of low-dose ritonavir for pharmacokinetic boosting of other PI drugs appears to be safe compared to high dose ritonavir (Soriano *et al.,* 2008). Some studies reported that ritonavir was the most hepatotoxic antiretroviral drug next to nevirapine (Antonio *et al.,* 2002). We, however, did not do comparative study among different PIs based regimens, as all combination of PI based regimens had lower dose of ritonavir only and the available PIs were limited in our setting.

Systemic review indicated that different studies focus on individual drug of PI class to identify the more toxic, but none of the studies has been able to prove the higher potential for liver toxicity of PI family of drugs (Nu, 2006). In addition to this, liver toxicities of the PI based regimens varied across the world between clinical setting and clinical trials with different risk factors (Bruno *et al.,* 2006; Nu, 2006; Sulkowski, 2003)**.** In our case, the distribution of the study participants on PI based regimens had huge ranges that cannot be suitable for inter comparison, but LPV/r containing regimens showed slightly more liver toxic effect during the study period.

Unlike our study, clinical trial in Thailand, reported that none of the patients who took boosted (protease inhibitor/low-dose ritonavir) containing regimens developed severe hepatotoxicity from 50 patients (Law *et al.*, 2003). This may be due to the difference in number of participants. small sample size may not be able to demonstrate the desired difference, or estimate the frequency of the event of interest with acceptable precision (Jeovany *et al.,* 2014). Therefore, number of participants was greater and severe liver injury was observed in our study.

A retrospective study indicated that the use of LPV/r and ATV/r were safer compared with other PIs and NNRTIs. Thus, the incidence of severe hepatotoxicity (grades 3 or 4) associated with any PI or NNRTI-based regimen was 10.7% (N = 6), but no cases of severe hepatotoxicity were observed with LPV/r or ATV/r, which had the longest duration of exposure among PIs (Heil *et al.,* 2010). In our study insignificant severe liver toxicity was reported in 3 cases out of 142 participants. This may be due to different research design and sample size, as retrospective method of research design may have incomplete clinical and biochemical data from chart review and small sample size patients were used by Heil *et al*.

Sulkowski *et al.* (2004), reported that incidence of severe liver toxicity due to LPV/r was 9%, which is greater, compared with our study. This can be because of the co-morbid condition with chronic hepatitis, as several study reported HIV/HCV co-morbid increase the risk of liver injury (Gisolf *et al.*, 2000; González-requena *et al.*, 2004; Molina *et al.*, 2005; Vispo *et al.*, 2013).

Gonzalez-Requena *et al*. (2004) showed the incidence of severe liver toxicity linked to LPV/r was 4%. This finding was slightly more frequent compared with our study, which might be attributed to co-morbid with HCV. Similar to our study, the incidence of severe liver toxicity was; however, lower in HCV-negative patients.

Elevation of transaminase associated with ATV/r containing regimens was lower compared to the one observed in a study done by Pineda *et al.*, (2008) which may be attributed to co-morbidity of chronic viral hepatitis.

Unlike our study, retrospective chart review in Spain showed that most of transaminase elevated patients developed pure cytolysis (n=62) and only 6 patients developed mixed pattern (cytolysis and cholestasis) of hepatotoxicity (Marina Nunaz *et al.,* 2001). Cholestatic pattern of liver injury

was by far higher than the other type in our study. The variation mainly depends on the combination of the drugs used, as their mechanism of pathogenesis and roots of excretion of the drugs can determine the type of liver toxicities.

In line with our study, the study from a resource limited setting also reported that the incidence of severe hepatotoxicity within three months of first line antiretroviral therapy was low. As our study included PIs experienced patients, the incidence of liver injury was high within 25-48 weeks of duration. Although the mechanism of time dependent PIs induced liver injury is not clearly known, it may be related to the modification of PY450 cytochrome enzyme (Antonio Aceti *et al*., 2002).

Our study indicated that there was a gap in identifying the risk factors, as there were a limited number with majority of co-factors which is unable to generalize, but cotromoxazole prophylactic therapy and previous 1st line on AZT/3TC/NVP were significantly associated with liver injury. This is supported by studies that have found cotrimoxazole causes liver injury (Salaheldin A. and Johnson S., 2008) and NVP was the most drug induced liver injury from ART (Antonio Aceti *et al.*, 2002).

In contrast to our study, several studies reported that chronic viral hepatitis, HCV and/or HBV co infection had significantly associated with the development of liver toxicities (Brinker *et al.*, 2000; Gisolf *et al.*, 2000; González-requena *et al.*, 2004; Molina *et al.*, 2005; Pineda *et al.*, 2008; Savès *et al.*, 2000; Vispo *et al.*, 2013), and retrospective study reported that alcohol consumption and others were independent risk factors for the development of liver toxicity (Marina Nunaz *et al.*, 2001).

6. LIMITATION OF THE STUDY

As the sample size of this study was small it was difficult to address risk factors like co-morbidity. Even though, several PIs are available currently worldwide, but according to the current national guideline, ritonavir boosted atazanavir and lopinavir are the only PIs available in Ethiopia. Thus, the study could not assess all PIs regimens induced liver injury. Moreover, the two PI based regimens were not applied in equal extent that made it difficult to compare each other.

7. CONCLUSION

Overall, liver injury of any grade was observed in 25 (17.6%) participants, while only 3 (2.1%) participants developed grade-3 liver injury. None of the participants developed grade-4 liver injury, and no death related to liver toxicity during the study period. Majority of liver injury induced participants 21 (84%), developed cholestatic type of liver injury. The incidence of liver injury was high within 25-48 weeks of duration. The risk of developing liver enzyme elevation was high among participants who took cotrimoxazole prophylactic therapy and those who were on AZT/3TC/NVP, previous first line drugs. The use of PIs based ART regimens, therefore, seems to be tolerable with respect to liver injury.

8. RECOMMENDATION

- The regular follow up of liver function tests and sign and symptoms of liver toxicity is important in timely detection of liver toxicities in HIV positive patients particular with PIs-based regimen.

- Future studies that can address the mechanism of PIs-induced liver injury is very important by studying the individual class of ART, then after identifying which pathogenesis is more dominant in combination of ART drugs that contains PIs-based regimens.

- Prospective studies on patients with long term follow up would be required to answer the exact onset of liver toxicity with common cycle of visiting.

- Moreover, further study need to be conducted with large sample size to identify factors that associate with liver injury in PIs-based ART regimens.

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ASSESSMENT TOOLS

Code_____ Date_____

Addis Ababa University, School of Pharmacy, Department of Pharmacology and Clinical Pharmacy

Evaluation of protease inhibitors based anti-retroviral regimen induced liver injury.

For closed ended questions, encircle on the letter. For open ended questions, write your response in the space provided

DEMOGRAPHIC DATA

1. Age (yrs): _____
2. Sex: A. Male B. Female
3. If female, pregnant status: A. pregnant B. non-pregnant
4. Body weight (Kg): _____
5. Height (Cm): _____

PREVIOUS AND CURRENT ART REGIMENS

6. Previous ART regimens with duration:_____

_____

7. Current ARTregimens:_____

CURRENT STATUS OF THE DISEASE

8. CD4 count (cells/mm3):_____
9. HIV viral load (copies/ml): _____
10. WHO Stage: A. stage 1 B. stage 2 C. stage 3 D. stage 4

PAST AND CONCURRENT MEDICATION Hx

11. Herbal medicine use: A. yes B. no

If yes identify with duration:_____

_____

12. Prior use of drugs (other than ART) in the past 3 months with a suggestive duration of time:

_____

_____

13. Concomitant drug(s) use with a suggestive duration of time and strength:

_____

_____

PAST AND CONCURRENT MEDICAL Hx

14. Prior history of a liver problem: A. yes B. no

If yes identify with duration:_____

_____

15. Anti-HCV (hepatitis C virus test): A. positive B. negative

16. HBsAg (hepatitis B surface antigen): A. positive B. negative

17. Acute viral hepatitis due to HAV (IgM anti-HAV): A. positive B. negative

18. Co-infection with tuberculosis(TB): A. present B. absent

19.Other/s:_____

_____

OTHER CHARACTERISTICS

20. History of alcohol intake: A. never B. moderate C. heavy

21. History of cigarette smoking: A. yes B. no if yes quantify(packet/day)_____

22. Family Hx to drug induced liver diseases: A. yes B. no

CLINICAL AND LABORATORY CHARACTERISTICS

In the table below make "X" for clinical presentation and write the exact numerical values for liver enzyme tests on the space provided.

Clinical presentation				**Liver enzyme tests**	
Fatigue		Poor appetite			
Fever		Nausea		ALT	
Abdominal pain		Vomiting			
Dark urine		Hepatomegaly		AST	
Rash		Hepatic tenderness			
Pruritus		Jaundice		ALP	
If others:					

Amharic version of Assessment tools

የአማርኛ መጠይቅ

ኮድ_____ የመጡበት ቀን----------

ለሚከተሉት ጥያቄዎች መልስ ሲሰጡ ምርጫ ከሆነ በፊደሉ ላይ ያክብቡ፣ ለደረቅ ጥያቄዎች በተሰጥዎ ባዶ ቦታ ላይ ይጻፉ።

የግል ሁኔታ መረጃ

1. እድሜ ----------
2. ፆታ ሀ. ወንድ ለ. ሴት
3. ሴት ከሆኑ የእርግዝና ሁኔታ ሀ. እርጉዝ ለ. አላረገዝኩም
4. የሰውነት ክብደት (ኪ..ግ) ----------
5. ቁመት (ሴ.ሜ) ----------

የቀድሞ እና የአሁኑ ፀረ ኤች አይቪ ኤድስ መድሃኒቶች

6. የቀድሞ ፀረ ኤች አይቪ ኤድስ መድሃኒቶች አይነት እና ለምን ያህል ግዜ እንደወሰዱ---
   ----
7. አሁን እያወሰዱ ያሉት ፀረ ኤች አይቪ ኤድስ መድሃኒቶች----------

የበሽታው ሁኔታ

8. የሲዲፎር ሕዋሳቶች ቁጥር (ሕዋሳት/ሚ-ሚ^3) ----------
9. የኤች አይቪ ቫይረስ ቁጥር (ብዛት/ሚ.ል) ----------
10. የአለም ጤና ድርጅት የበሽታው ደረጃ
    ሀ. ደረጃ-1 ለ. ደረጃ-2 ሐ. ደረጃ-3 መ. ደረጃ-4

<u>የቀድሞ እና አሁን የሚወሰድ መድሃኒቶች ከ ፀረ ኤች አይቪ ኤድስ መድሃኒቶች ውጪ</u>

11.ባሕላዊ ቅጠላቅጠል ተጠቅመው ያውቃሉ? ሀ. አዎ ለ. አላውቅም

ከተጠቀሙ አይነቱን፣ መቼ እና ለምን ያህል ግዜ እንደ ተጠቀሙ ያብራሩ----------

12.ባለፉት 3 ወራት ውስጥ ተጨማሪ መድሐኒት ተጠቅመው ያውቃሉ?

ሀ. አዎ ለ. አላዉቅም

ከተጠቀሙ መጠኑ እና ለምን ያህል ግዜ እንደ ተጠቀሙ ለማብራራት ይሞክሩ----------

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13. በአሁኑ ግዜ ከ ፀረ ኤች አይቪ ኤድስ መድሃኒቶች ጋር ሌላ መድሐኒት ይጠቀማሉ?

ከተጠቀሙ መጠኑ እና ለምን ያህል ግዜ እንደ ተጠቀሙ ለማብራራት ይሞክሩ----------

<u>የቀድሞ እና የአሁኑ የበሽታ ሁኔታ ከኤች አይቪ ኤድስ ውጭ</u>

14.ከዚህበፊት የጉበት ችግር ነበረብዎት? ሀ. አዎ ለ. የለብኝም

ካነበረብዎት አይነቱን እና ግዜውን ይገለጹ፡፡ ----------

15.የሄፓታይተስ “ሲ” ቫይረስ ዓለብዎት? ሀ አዎ ለ. የለብኝም

16.የሄፓታይተስ “ቢ” ቫይረስ ዓለብዎት? ሀ. አዎ ለ. የለብኝም

17.የሄፓታይተስ “ኤ” ቫይረስ ዓለብዎት? ሀ. አዎ ለ. የለብኝም

18.የቲቢ በሽታ ዓለብዎ? ሀ. አዎ ለ. የለብኝም

19.ሌላ ካለ ----------

<u>ሌሎች ሁኔታዎች</u>

20.የመጠጥ (አልኮል) አወሳሰድ ሁኔታ ሀ. በጭራሽ ለ. በመጠኑ ሐ. በብዛት

21.ሲጋራ ያጨሳሉ ሀ. አዎ ለ. አላጨስም

ከአጨሱ ምንያህል? (ፓኬት/ቀን) ----------

22.በመድሃኒት የመጣ የጉበት ችግሮች በቤተሰብዎ ውስጥ ነበረ? ሀ. አዎ ለ. አልነበረም

የክሊኒካል እና የላቦራቶሪ ሁኔታዎች

ከተዘረዘሩት አማራጮች፣ ምልክቱ ከአለ በፊለፊቱ ባለው ሳጥን ውስጥ የኤክስ (x) ምልክት ያድርጉ፡፡

የጉበት ህመም ክሊኒካል እና አካላዊ ምልክቶች				የጉበት ኢንዛይሞች	
የድካም ስሜት		የምግብ ፍላጎት መቀነስ			
ትኩሳት		ማቅለሽለሽ		ኤ ኤል ቲ	
የሆድ ሕመም		ማስመለስ			
የሽንት ቀለም መጥቆር		የጉበት ማበጥ		ኤ ኤስ ቲ	
የቆዳ ማቃጠል ስሜት		የጉበት ቴንደርነስ		ኤ ኤል ፒ	
የማሳከክ ስሜት		ዐይን ብጫ መሆን			
ሌላከለለ					