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SeroPrevalence of Hepatitis C Virus among HIV Infected Individuals and Comparison of Basic Laboratory and Clinical Parameters at ART clinics of Tikur Anbessa Specialized and Zewditu Memorial Hospital, Addis Ababa, Ethiopia.

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Dedication

This thesis is dedicated to my beloved husband, Ato Teklabrham Woldeyesuse, who has always encouraged me to pursue my dreams.

List of Figure

Page

List of Abbreviations

Ab	Antibody
Ag	Antigen
AIDS	Acquired Immunodeficiency Syndrome
ALP	Alkaline phosphatase
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
BMI	Body mass index
CD	Cluster of Designation / Differentiation
CDC	Center For Disease Control
CI	Confidence Interval
CSA	Central Statistical Agency
EIA	Enzyme Immunoassay
ELISA	Enzyme Linked Immunosorbent Assay
ER	Endoplasmic Reticulum
HAART	Highly Active Anti-Retroviral Therapy
HAV	Hepatitis A Virus
HBV	Hepatitis B Virus
HCC	Hepatocellular Carcinoma
HCT	Hematocrit
HCV	Hepatitis C Virus
Hgb	Hemoglobin
HIV	Human Immunodeficiency Virus
IDU	Intravenous/Injection Drug Users
IFN	Interferon
Ig	Immunoglobulin
IL	Interleukin
IP	Infection prevention
LY	Lymphocytes

NEU	Neutrophil
PBMCS	Peripheral Blood Mononuclear Cells
PEP	Post-Exposure Prophylaxis
PLTS	Platelets
SPSS	Statistical Package for the Social Sciences
STD/STI	Sexually Transmitted Disease/Infection
SVR	sustained virological response
TNF	Tumor Necrosis Factor
UNAIDS	United Nation Program on HIV/AIDS
VCT	Voluntary Counseling and Testing
WBC	White blood cell
WHO	World Health Organization

Operational definition

Mono infection: It means that person who is infected with HIV only in this study.

Co infection: subjects who are infected with HIV and HCV based on the evidence we have in this study.

ART naïve patients: Patients who did not start antiretroviral drugs at all after knowing their HIV status.

HCV infection: Infection as evidenced by the presence of anti HCV antibody using enzyme immunoassay methods.

Hematological tests : Test that include Total white blood cell count, Hematocrit, hemoglobin, Neutrophil count, Lymphocyte count , Platelet count,

Abstract

Background: Hepatitis C virus (HCV) infection is common in Human Immunodeficiency virus (HIV)-infected persons as both may have common mode of transmission. HIV and HCV co infection is a major global health concern. However, data on co-infection are limited in Ethiopia.

Objective: The aim of this study was to determine sero-prevalence of hepatitis C virus among HIV infected individuals and comparison of basic laboratory and clinical parameters at ART clinics of Tikur Anbessa Specialized and Zewditu Memorial Hospital, Addis Ababa, Ethiopia**.**

Methods: A hospital based cross-sectional survey was carried out on HIV follow up attendees from July to November, 2013. An interviewer-administered questionnaire was used to collect data on demographic information, and risk factors associated with HCV infection and clinical, common hematological ,CD_4 T cell count and LFT profiles were also collected using pretested structured checklists .AntiHCV antibodies was detected using enzyme immunoassay test kit (EIA-ANTI-HCV) according to manufacturer instruction. We used Epiï Info version 3.5.1 and SPSS version 21 statistical package for data entry and analysis.

Results: All consecutive attendants recruited in the study (n=282), were HIV follow up cases. There were more females 207(73.4%) than males 75 (26.6%). The sero-prevalence of HCV was 5.3% (15/282, 95% CI= 3% - 8%). There was no significant difference in HCV seroprevalence with age and sex ($p > 0.05$). Hospital admission was found to be a possible risk factor for HCV infection ($p<0.05$). Majority of HCV infected patients were functionally active and had normal BMI. About 3.7 % of HCV infected subjects were belongs to WHO stage II. Mean WBC, Hgb, HCT, Plateletes,Lymphocyte, Neutrophile, CD4 T cells count , ALT, AST and ALP for HIV/HCV co-infected and only HIV infected persons found to be {9,09 ± 10.05*,13.3 ±1.6, 40.7 ± 4.8, 287.7 ± 72.1*, 2.1 ± 1.1*, 3. 6 ± 1.9*,415.5 ± 219.2, 29.47 ± 30.8, 40.07 ± 39.4, 273.2 ± 120.8}, {7.38 ± 9.039*,14.3 ± 5.149, 42.07 ± 4.94, 291.9 ± 103.56*, 2.43 ± 0.96*, 3.7 ± 1.8*, 451.29 ± 243.2, 25.4 ± 14.6, 26.1 ± 12.1, 254.58 ± 96.82}, respectively. About 5 % of co infected patients are taking ART. There is statistically significant difference in mean AST level for HCV co infected and HIV monoinfected groups ($p < 0.001$).

Conclusion: The seroprevalence of HCV infection in HIV positive cases is found to be moderately high. Hence it is appropriate to screen all HIV patients for HCV. It is also appropriate to give health education concerning transmission, risk factors and prevention of HCV infection.

*** should be multiplied with 10^3**

Keywords: HIV, HCV, Co-infection, ALT, AST, ALP

1. Introduction

1.1. Background

Hepatitis is a general term applied for inflammation of the liver and can be caused by several mechanisms, including infectious agents. Viral hepatitis can be caused by a variety of different viruses such as hepatitis A, B, C, D and E. Since the development of jaundice is a characteristic feature of liver disease and not just viral hepatitis, a correct diagnosis can only be made by testing patientsô sera for the presence of specific anti-viral anti-bodies. In 1989 the virus responsible for most transfusion-associated non-A non-B hepatitis, and was identified and cloned, and named hepatitis C virus (HCV) [1, 2].

HCV is classified as the member of the Hepacivirus in the Flaviviridae family. It measures 30 to 60 nm in diameter, with a positive-sense RNA genome and is enveloped. The genome of HCV encodes 10 proteins including 2 glycoproteins (E1, E2). It is categorized into six major genotypes, many subtypes and about 100 different strains based on the genomic sequence heterogeneity. The variability is distributed throughout the genome. The genes coding for the envelope E1 and E2 glycoproteins are the most variable. Amino acid changes may alter the antigenic properties of the proteins, thus allowing the virus to escape neutralizing antibodies [3, 4].

The replication cycle of the virus is likely to enter the cell through an interaction with a specific cell surface receptor molecule, there is strong evidence that E2 binds specifically to CD81, a cell surface molecule that is widely expressed in many different tissues including the liver. After attachment, penetration, and uptake into the cellular endosomes, the viral RNA directing the cap-independent translation of the viral polyprotein. Viral translation occurs in association with the rough endoplasmic reticulum (ER), and segments of polyprotein are secreted in to the lumen of ER as it under goes a series of co- translational proteiolytic cleavages. The core protein remains within the cytoplasm, likely anchored to the ER membrane at its carboxyl terminus, where as E1 and E2 are secreted into the lumen of the ER and become heavily glycosylated. As the core protein is immunogenic, antibody is typically present in infected individuals. The genomic RNA is packaged into new viral particles, which likely are extruded into the ER leading to the release of the virus via the vascular secretary pathway [5, 6].

Globally, an estimated 130-170 million persons (2%-3% of the worldôs population) are living with (HCV) infection. This infection, particularly in its chronic form, is associated with sizable morbidity and mortality. More than 350 000 deaths are attributed to HCV infection each year, most of which are caused by liver cirrhosis and Hepatocellular carcinoma (HCC). An estimated 27% of cirrhosis and 25% of HCC can be attributed to HCV worldwide, and disease rates can be even more substantial in countries with a high burden of infection. For example, in Japan, up to 90% of all reported cases of HCC are caused by HCV infection. Available data indicate that infection with HCV varies considerably by country and region [7, 8].

However, the true burden of disease is not well known in many countries, because capacity is limited for collecting epidemiologic data. In contrast, the mode of transmission is fairly well defined and most often involves exposure to contaminated needles or syringes, although the means by which this exposure occurs differs by country. Whereas HCV transmission in developing countries frequently results from exposure to infected blood and blood products in healthcare and community settings, HCV infections in most developed countries are associated with injection drug use (i.e., personal behavior typically of an illicit nature) [7,8].

The virus causes swelling and eventually scarring of the liver, called cirrhosis. This scarring of the liver can reduce the liverôs ability to function after long-term HCV infection. This damage develops slowly over many years. HCV is transmitted when the blood of someone who has HCV directly enters the bloodstream of another individual. Common ways that this can happen include: Sharing infected needles (or other equipment), sharing personal items that may have come into contact with blood (e.g. razors, nail clippers, toothbrushes, or glucose monitors), unsterilized tattoo or piercing equipment or unprotected sex with someone who has HCV [3].

An estimated 20% to 40% of individuals with acute HCV infection spontaneously clear the virus with out treatment, but the rest develop chronic hepatitis C long-lasting more than six months. The likelihood of chronic infection is higher for people with HIV. Sixty to seventy percent of persons newly infected with HCV typically are usually asymptomatic or have a mild clinical illness. HCV RNA can be detected in blood within 1-3 weeks after exposure. The average time from exposure to antibody to HCV (anti-HCV) seroconversion is 8-9 weeks, and anti-HCV can

be detected in >97% of persons by 6 months after exposure. Chronic HCV infection develops in 70%-85% of HCV-infected persons; 60%-70% of chronically infected persons have evidence of active liver disease. The majority of infected persons might not be aware of their infection because they are not clinically ill [9].

However, HCV infected persons serve as a source of transmission to others and are at risk for chronic liver disease or other HCV-related chronic diseases decades after infection. Approximately two-thirds of people with chronic hepatitis C experience some degree of disease progression, developing necro-inflammation (hepatocyte inflammation and death), liver fibrosis (buildup of fibrous scar tissue), or liver steatosis (fat accumulation). According to different reports, about 10% to 25% of people with chronic hepatitis C progress to severe liver disease, including cirrhosis (replacement of normal liver cells with nonfunctional scar tissue), hepatocellular carcinoma (a form of primary liver cancer) and end-stage liver disease. Progression to severe disease typically takes ten to 40 years, and many people have no symptoms until they reach advanced stages [10, 11]. HCV is also associated with development of hematological diseases ranging from immune thrombocytopenia to lymphoma [12].

Human immunodeficiency virus (HIV) is a retrovirus of the Lentiviridae family. This positive strand RNA virus infects specific cell populations of the immune system through its receptor specificity. At present, more than 33 million people are infected with HIV. HIV infection is characterized by an acute and a chronic phase, possibly leading to AIDS. Immediately after infection the viral load increases with exponential growth kinetics and CD4+ T cells rapidly decline [13, 14].

HIV /AIDS remained a global pandemic. Sub-Saharan Africa, including Ethiopian, remains the region most heavily affected by the virus, accounting for 68% of people living with HIV and 70% of new infections in 2010. Nonetheless, progresses in the prevention of the pandemic have been shown in the past decade. Ethiopia remained one of the highly affected countries with current estimated HIV/AIDS national prevalence of 1.5% among the adult population [15-17].

Because of their similar routs of transmission HIV/HCV co-infection is common. Both viruses can be transmitted through exposure to contaminated blood, sexual intercourse and from mother to child. HCV is more transmissible through per-coetaneous blood exposure compared to HIV. In contrast HIV is more transmissible through sexual intercourse and from mother to child compared to HCV. HCV infection increases the complications in persons who are co-infected with HIV [18].

Co-infection with the HCV presents many challenges to the effective management of HIV. An estimated 35 % of HIV-infected patients also infected with HCV. HIV has a negative impact on the natural history of HCV, including a higher rate of viral persistence, increased viral load, and more rapid progression to fibrosis, end-stage liver disease (ESLD) and death.. Whether HCV has a negative impact on HIV disease progression continues to be debated. Without treatment these patients progress to cirrhosis three times faster than HCV mono-infected patients leading to other negative outcomes of HCV infection including end-stage liver disease, hepatic cell carcinoma and death. Liver disease is one of the leading non- AIDS related causes of death in HIV patients. Thrombocytopenia is characterized by low blood platelet count; it has been linked to HIV and HCV infections. Severe cases of thrombocytopenia can lead to intestinal bleeding, intracranial bleeding, and/or death [19].

The mechanism of increased HCV replication and accelerated fibrosis leading to liver mortality in HIV/ HCV-co infected patients are the generalized immune suppression resulting from the loss of CD4 T cells, an intra hepatic interaction between the viruses or their gene products (on hepatocyte or other hepatic cells), and an indirect effect on the liver secondary to HIV infection of other organs. HIV-induced immune suppression may be a major factor. T-cell responses against HCV play an essential role in preventing progression from acute infection to chronicity. In HIV-infected patients that develop acute hepatitis C, HCV specific T-cell responses are markedly diminished a finding consistent with a higher rate of progression to chronicity in these patients. In chronic hepatitis C, T-cell responses are generally weak, co-infected patients appear to have even weaker CD4 and CD8 responses and these responses are not restored, even after CD4 cell counts recover in response to HAART [20].

How HCV may affect the course of HIV infection among co-infected individuals is not well known even if it was suggested that HCV co-infection is able to increase immune activation and to sensitize CD4+ T-cells towards apoptosis in the absence of HIV therapy. In fact, it was demonstrated that although HCV alone did not increase CD4+ T-cell apoptosis, HIV/ HCV co-infection disproportionately increased the rates of apoptosis in CD4+ T-cells, compared to HIV mono-infected controls. HCV co-infection seems to be able to sensitize CD4+ T cells towards apoptosis in the absence of HIV therapy, as this effect is rapidly lost under HAART. On the other hand there are many evidences that the simultaneous presence of HIV infection accelerates the liver damage from HCV favoring the evolution to cirrhosis in co-infected patients. HIV increasing of TNF alpha liver production and of HCV replication in peripheral blood lymphomonocytes is the mechanisms at the basis of this phenomenon [21].

Despite extensive data support the evidence that HIV/HCV co-infected individualôs progress more rapidly to serious liver disease than HCV mono-infected subjects, the mechanisms by which HIV can accelerate the HCV liver damage has not been clarified yet. They may include direct viral effects or immunologic alterations such as immune activation and apoptosis. Immune activation may induce cytokines, which increase liver inflammation and fibrosis. Accumulation of HIV specific cytotoxic CD8+ T cells in the liver can produce TNF-Ǔ, which is associated with fibrosis. In addition, HCV is not only hepatotropic, but can replicate in PBMCs (peripheral blood mononuclear cells) and in native human macrophages in vitro. After HIV infection, the replication of HCV from PBMC cultures of mono-infected subjects can increase by 1 to 2 logs, compared to HIV uninfected controls [2].

1.2. Statement of the problem

Hepatitis C is primarily a blood-borne or parentally transmitted infection. The epidemiology of HCV infection in the community among the western world has changed dramatically over the past two decades, primarily a results of the identification of non-A, non-B hepatitis as the major cause of transfusion associated hepatitis, the development of screening tests for blood and blood products for transfusion to eliminate HCV from the blood supply [22,23]. As many as 2 to 4 million persons may be chronically infected in the United States, 5 to 10 million in Europe, and about 12 million in India and most do not know they are infected. About 150 000 new cases occur annually in the US and in Western Europe, and about 350 000 in Japan. Of these, about 25% are symptomatic, but 60 to 80% may progress to chronic liver disease, and 20% of these develop cirrhosis. About 5%-7% of patients may ultimately die of the consequences of the infection. Most European countries report a prevalence of HCV in the general population of between 0.5 and 2%.The WHO (1997) estimates that 3% of the world's population is chronically infected with HCV and that there are more than 170 million chronic carriers who are at risk of developing liver cirrhosis and/or liver cancer [24]. In the US, 15%ï 30% of HIV-infected individuals are co-infected with HCV [25].

Morbidity and mortality from infection with HCV in HIV-positive patients are increasing and have become a major challenge in the management of such patients. In some studies 12% of deaths of patients with HIV infection were due to liver disease, but others have found that end-stage liver disease underlies more than 45% of in-hospital deaths in HIV-infected individuals in more developed countries. Owing to shared routes of transmission, an estimated 30% of HIV-positive individuals are co-infected with HCV in the USA and Europe [26].

It is also found that a consistent evidence of high HCV prevalence in many countries of Africa. In general, the overall prevalence of HCV in Sub-Saharan Africa is estimated to be 3%. Even though the prevalence of HCV infection varies throughout the world, the highest number of infection ranging from 6% to 28% in Egypt [24]. In Gambia it was reported as 0.6%. In Nigeria, prevalence rates ranging from 4.5% to 10.3% have been reported in various studies [25-27].

There is only limited number of studies conducted in Ethiopia on the prevalence of HCV and its co-infection with HIV. A prevalence of antibody to HCV (anti-HCV) in healthy adult Ethiopian blood donors was found to be 1.4% [28].

HCV prevalence was 0.9% for the total population and 1.3% for adult over 15 years of age in a study conducted on two blood banks in Addis Ababa. However, there is limited information on HCV infection among HIV-infected patients in the country. Liver diseases are common in Africa and account for high morbidity and mortality. Hospital based analysis indicate that acute viral hepatitis, chronic hepatitis, cirrhosis and HCC are responsible for at least 12% of medical admissions and over 20% of hospital mortality in many parts of Africa. In Ethiopia 12% of medical admission and 31% of mortality in medical wards is due to liver disease [29]. Generally, the HIV/HCV co-infection status of HIV infected Ethiopians has not been well documented.

By now many studies indicated that HCV is an opportunistic infection in HIV infected persons and known to be a risk factor for highly active anti-retroviral therapy (HAART) related hepatotoxicity. Actually liver diseases represent the second leading, and in some cases, preventable cause of death in HIV positive patients [2]. So, it is important to screen this population.

Despite these effects, HIV infected individuals in Ethiopia are taking anti-retroviral drugs. To ensure the optimal clinical managements of HIV/ HCV co infection it is important to know the immunologic (CD4+ T-lymphocyte count), hematologic (WBC, hemoglobin, Hematocrit, platelet counts, lymphocyte, and Neutrophil levels), liver enzyme (Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST),Alkaline phosphatase (ALP) changes and Clinical description, (WHO stage, Primary HIV infection, clinical stage 1, clinical stage 2, Clinical stage 3, Clinical stage 4 [30] Functional status, an individual's ability to perform normal daily activities required to meet basic needs, fulfill usual roles, and maintain health and well-being [31].

1.3. Significance of the study

Because of the shared routes of transmission, co-infection with HCV and HIV is common and is of increasing clinical relevance. Co-infection with HIV leads to an increased risk of chronic HCV infection, increased HCV loads, and an increased risk of progression to several pathologic hepatic damage mainly cirrhosis. Studies also showed that rate of fibrosis progression are increased among HIV/HCV co-infection than HCV mono-infections [2].

Since, HIV/HCV co-infection is important disease in the Ethiopian context as few studies showed that both are comparatively prevalent and are becoming a concern of the public health [22]. Treatment administration of HIV is usually based on hematologic and immunologic profile of patients. Thus, studies on these parameters together with clinical description is significant for accurate and effective treatment out comes.

It follows that the clinician needs to have a good idea of certain parameters of liver function in HIV/HCV co-infected patients before and during the treatment. This study is designed to know the seroprevalence of HCV and to assess and compare the CD_4 T cell count, hematologic liver enzyme, and clinical profile of HIV/ HCV co-infected and only HIV-infected patients at ART clinics of Tikur Anbessa and Zewditu Memorial Hospital.

Therefore, assessing and comparing of the various CD4+ T-lymphocyte count, hematological (WBC, hemoglobin, Hematocrit, platelet counts, lymphocyte, and Neutrophil levels), liver enzyme such as ALT, AST,ALP and clinical parameters of HCV/HIV co-infected and those only HIV-infected study participants were provide relevant information for proper patient managements. This also will provide useful information on how to choose the appropriate therapeuticoptions.

2. Literature Review

The study conducted in USA in 2013 to determine the Progression of Platelet Counts in Treatment Naïve HIV/HCV Co-Infection the result should that among 929 HIV/HCV co infected and 3558 HIV mono infected with mean follow up time of 1.2 years. The result should that HIV/HCV co infected participants had on average a slighter lower platelet count at baseline (234,040 vs. 242780/micro lit; p- value = 0.004),mean and a more rapid mean reduction per year (7230 vs.3580/ micro.lit; p- value < o.oo1)after adjusting for age, sex baseline CD4 count. In general in treatment naïve participants HIV/HCV co infections associated with a more rapid decline in platelet count compared with HIV mono infection [19].

A study conducted in 2012 in Italy to determine HIV/HCV co-infection: epidemiology, pathogenesis and therapeutic implications. The result showed that HIV increasing of TNF alpha liver production and of HCV replication in peripheral blood lymphomonocytes is the mechanisms at the basis of this phenomenon. HAART had a positive effect on HIV/HCV co-infection; otherwise it does not appear to fully correct the adverse effect of HIV infection on HCV-related outcomes. Traditional treatment with pegilated interferon plus ribavirin have low rates of sustained virological response in co-infected patients especially if infected with HCV genotype 1, and better results were often obtained in patients in which the use of antiretroviral treatment was avoided to reduce the occurrence of adverse effects. [2].

A mini review conducted in 2009 in Maryland to assess Co infection with HCV and HIV: Virological, Immunological, and Clinical Outcomes. Some studies suggest that HAART actually decreases liver-related mortality and that increased levels of CD4 cells after treatment were associated with improved liver survival. However, in most HAART era studies, CD4 cell count, and either on-treatment or at baseline, did not affect the liver associated mortality of co-infected patients. Most cases of liver-associated mortality in co-infected patients were due to end-stage liver disease or Hepatocellular carcinoma. Thus, the increased mortality of co-infected patients over that of HCV-monoinfected ones reflects accelerated progression of chronic liver disease [20].

A study conducted in France from 1995-8 to determine liver Fibrosis Progression in HIV/HCV Co infected Patients. The result showed that the prevalence of patients with a liver fibrosis score was higher in HIV-infected patients (60%) than in HIV-negative control patients (47%).

Moderate and severe necro-inflammatory liver activity was found in 54% and 30% of HIV-infected patients and HIV negative control patients, respectively [32].

A study conducted in 2010 to determine HCV co-infection in HIV positive population in British Columbia, Canada. The result showed that of 4,598 HIV cases with personal identifiers, 3,219 (70%) were linked to the combined HCV database, 1,700 (53%) of these were anti-HCV positive. HCV was diagnosed first in 52% of co-infected cases. HIV and HCV were diagnosed within a two week window in 26% of cases. Among individuals who were diagnosed with HIV infection at baseline, subsequent diagnoses of HCV infection was independently associated with: i) intravenous drug use (IDU) in males and females [33].

A study conducted in India in2009 to determine prevalence of hepatitis c virus (HCV) co infection in HIV infected individuals in south India and characterization of HCV genotypes the results showed that a total of 1487 HIV-infected serum samples were screened for HCV infection, of which, a 1443 (97.04%) were negative and 45 (3.02%) were co infected. HIV/HCV co-infection was predominant in the age group 41-50 years (51.1%)[34].

A study conducted in 2011 to assess selected biochemical and hematological abnormalities in Nigerians with human immunodeficiency virus and hepatitis C virus co-infection. The result showed that one hundred and eighty HIV-infected patients and 180 control subjects participated in the study. The seroprevalence of anti-HCV antibodies in the HIV-infected patients and control subjects were 6.7% and 4.4%, respectively. Serum total bilirubin, conjugated bilirubin, and alkaline phosphatase were significantly higher in the HIV/HCV co infected patients compared with their HCV monoinfected counterparts. The mean hemoglobin, white cell count, platelet count, and CD4+ T lymphocyte count were significantly lower in the HIV/HCV co infected patients than the HCV monoinfected control group .[35].

A study conducted in October 2003 to February 2004, Screening for hepatitis C among HIV positive patients at Mulago Hospital in Uganda. The result showed that one hundred and twenty two HIV positive patients were enrolled into the study with a mean age of 33.9 years. There were more females 81 (66.4%) than males. Only 4 patients had anti-HCV, giving an estimated HCV prevalence of 3.3% [36].

A study conducted in 2013 in South Africa to determine treatment outcomes of Treatment-Naive Hepatitis C Patients Co-Infected with HIV: A Systematic Review and Meta-Analysis of Observational Cohorts. The result showed that 40 studies were included for review, providing outcomes on 5339 patients from 17 countries. The pooled proportion of patients achieving sustained virological response (SVR) was 38%. Significantly poorer outcomes were observed for patients infected with HCV genotypes 1 or 4 (pooled SVR 24.5%), compared to genotypes 2 or 3 (pooled SVR 59.8%). The pooled proportion of patients who discontinued treatment due to drug toxicities (reported by 33 studies) was low, at 4.3% (3.3-5.3%). Defaulting from treatment, reported by 33 studies, was also low (5.1%, 3.5-6.6%), as was on-treatment mortality studies, 0.1% (0-0.2%) [37].

A study conducted in 2013 in Nairobi, Kenya on the Prevalence of hepatitis B and C viral co-infections among HIV-1 infected individuals the Results showed In a total of three (300) hundred infected individuals consisting of 129 (43%) males and 171 (57%) females 15.3% (46/300) were HIV-1 co-infected with either HBV or HCV or both, 10.3% (31/300) with HIV-1 and HCV and 6% (18/300) with HIV-1 and HBV infections. However, only three individuals (1%) were co infected with the three viruses (HIV/HBV/HCV) [38].

A study conducted in 2011 to determine hepatitis C virus and human immunodeficiency virus (HIV) co-infection among attendants of voluntary counseling and testing center and HIV follow up clinics of Mekelle hospital, Mekelle, North Ethiopia. The result showed that Out of a total of 300 consecutive attendants, the overall anti-HCV prevalence was 6.0%. There were no significant differences in HCV sero-prevalence among the different categories of age and sex. Of the 174 persons with HIV, 16 (9.2%) cases had antibodies to HCV, where as among 126 HIV negative subjects, 2 (1.58%) were HCV sero-positive. Accordingly, there was a significant difference in sero-positivity of HCV between HIV positive and HIV negative participants. No apparent risk factor that caused HCV infection was inferred from this study. This study showed a significant percentage of HCV infection in HIV positive cases [23].

A study conducted in 2011 at University of Gondar Teaching Hospital, Northwest Ethiopia to asses HBV and HCV seroprevalence and their correlation with CD4 cells and liver enzymes among HIV positive individuals the result showed that, Among 400 study participants, the

overall prevalence of HIV-viral hepatitis co-infection was 42(11.7%). The prevalence of HIV-HBV, HIV/HCV and HIV/HBV/HCV co-infections were 20(5.6%), 18(5.0%) and 4(1.1%) respectively. Study participants who had HIV-HBV, HIV/HCV and HIV/HBV/HCV co-infection have relatively raised mean liver enzyme levels (ALT, AST and ALP) than HIV mono-infected once. Individuals with HIV-HBV, HIV-HCV and HIV-HBVHCV co-infection also had a lower mean CD4 levels than HIV mono-infected study participants. The mean CD_4 value in males was lower than females [39].

A study conducted to assess Prevalence and risk factors of Hepatitis C among individuals presenting to HIV testing centres, Hawassa city, Southern Ethiopia. The result showed that of the 402 HIV-positive and 404 HIV-negative individuals approached during the study period, 2 HIV-positive and 4 HIV-negative participants were excluded due to insufficient blood volume and refusal, respectively. Two-hundred (50%) HIV-infected participants were receiving ART. The prevalence of anti-HCV positivity was 10.5% in the HIV- infected individuals compared with 6% in the HIV negative group. HCV-RNA was detected in 9.1% of anti-HCV positive samples and rates were comparable between HIV- infected and HIV- non-infected individuals. There was no significant difference in odds of HCV infection in participants with and without HCV risk factors in either HIV sero-group [40].

A study conducted in 2012 on the Assessment of hepatitis B virus and hepatitis C virus infections and associated risk factors in HIV infected patients at Debretabor hospital,South Gondar, Northwest Ethiopia , the result showed that From a total of 395 HIV infected patients, 234 (59.2%) were females and 161 (40.8%) males with mean age of 36.31 (±9.91)years. The prevalence HBsAg and Anti HCV antibody was 6.1%and 1.3%.respectively [22].

A study conducted in 2013 to assess the impact of hepatitis c virus co-infection on HIV patients before and after highly active antiretroviral therapy: an immunological and clinical chemistry observation, At Yekatit-12 and Zenbaba General Hospitals, Addis Ababa, Ethiopia. The result showed that the prevalence of HIV/HCV co-infection in this study was 6.5%. Both HCV/HIV co-infected and HIV mono-infected under HAART groups showed CD4+ recovery (343 vs. 426) [41].

3. Objectives

3.1 General objective

To determine ñSeroprevalence of Hepatitis C Virus among HIV Infected Individuals and Comparison Of basic laboratory and clinical Parameters at Tikur Anbessa Specialized and Zewditu Memorial Hospital, Addis Ababa, Ethiopiaò.

3.2 Specific Objectives

- To determine seroprevalence of HCV among HIV-infected patients at ART clinics of Tikur Anbessa Specialized and Zewditu Memorial Hospital.
- To assess the CD4 T cell count among HIV-infected and HIV/HCV co-infected participants at ART clinics of Tikur Anbessa Specialized and Zewditu Memorial Hospital.
- To assess the hematological profiles among HIV-infected and HIV/HCV co-infected participants at ART clinics of Tikur Anbessa Specialized and Zewditu Memorial Hospital.
- To assess liver enzyme profile among HIV-infected and HIV/HCV co-infected participants at ART clinics of Tikur Anbessa Specialized and Zewditu Memorial Hospital.
- To assess the clinical profiles among HIV-infected and HIV/HCV co-infected participants at ART clinics of Tikur Anbessa Specialized and Zewditu Memorial Hospital.

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4. Materials and Method

4.1. Study Design and Period

A cross-sectional study was conducted on HIV-infected patients who were attending ART clinics of Tikur Anbessa Specialized and Zewditu Memorial Hospitals, Addis Ababa, Ethiopia from July to November 2013.

4.2. Study Area

We selected Tikur Anbessa Specialized Hospital (TASH) and Zewditu Memorials Hospital (ZMH) as they are convenient study areas for our sample collection in terms of various reasons such as efficient time usage, financial issues, and the required number of samples could be collected in a reasonably short period of time.

Tikur Anbessa specialized hospital (TASH) is located in the nationôs capital Addis Ababa, is Ethiopiaôs central general public hospital. In 1998 Tikur Anbessa, which is also the main referral hospital in the country was given to Addis Ababa University (AAU) by the Ministry of Health for the faculty as a main teaching hospital. The faculty is the oldest and the leading among the health training institutions in the country, staffed with the most senior specialists. It provides a tertiary level referral treatment and is open 24 hours for emergency services. The hospital is administered by Addis Ababa University and is the largest and oldest teaching hospital among all in Ethiopia providing teaching for about 300 medical students and 350 Residents every year. It offers diagnosis and treatment for approximately 370,000- 400,000 patients a year. The hospital has 800 beds, with 130 specialists, 50 non-teaching doctors. The emergency department sees around 80,000 patients a year.

Zewditu Memorials Hospital (ZMH) is a hospital in central Addis Ababa, Ethiopia. It is Ethiopia's leading hospital in the treatment of ART patients and currently treats over 6,000 patients each month. CDC-Ethiopia helped launch Ethiopiaôs first ART program at Zewditu in July 2003, and in March 2005 it received technical assistance from Johns Hopkins Universityôs (JHU) TSEHAI Program. Zewditu became the largest HIV clinic in Ethiopia, with 14,000 patients in its care. Since, ART programs have been initiated in other hospitals around the

country, relieving pressure on the hospital. The hospital also deals with palliative care, HIV counseling and testing, STI services and Post-exposure prophylaxis (PEP) services.

4.3. Population

4.3.1. Source Population

All adult HIV positive patients attending ART clinics of Tikur Anbessa Specialized and Zewditu Memorial Hospitals were source populations.

4.3.2. Study Subjects

All Volunteers adult HIV patients attending ART clinics at Tikur Anbessa Specialized and Zewditu Memorial Hospitals, who gave blood sample for HCV testing during the study period.

4.4. Inclusion and Exclusion Criteria

4.4.1. Inclusion Criteria

- Adult HIV-infected patients (age Ó18), who was attending ART clinics at Tikur Anbessa Specialized and Zewditu Memorial Hospitals during the study period were included in this study.
- HIV-infected individuals at ART who were signed over the consent form were provided in this study.

4.4.2. Exclusion Criteria

- Patients clinically diagnosed with chronic disease (Diabetic, TB)

4.5. Study Variables

4.5.1. Dependent Variables

- Sero-prevalence of HCV infected patients.
- $CD4^+$ T-lymphocyte count, hematological (WBC, hemoglobin, Hematocrit, platelet counts, lymphocyte, and Neutrophile levels), liver function test (ALT, AST and ALP) and clinical profile of study subjects.

4.5.2. Independent Variables

- Socio-demographic characteristics (i.e., age and sex)
- Behavioral risks (i.e., history of STI, abortion and multiple sex partners);
- Parentral risks (i.e., history of drug injection, dental procedure, catheterization, surgery, blood transfusion and hospitalization); and
- Community acquired risks (i.e., ear/nose piercing, circumcision, dental extraction, tattooing.

4.6. Sample Size Determination and Sampling Technique

4.6.1 Sample Size Determination

Sample size was calculated on the basis of a previous HIV/HCV prevalence study in Addis Ababa, Ethiopia which reported prevalence of 6.5% (Taye S, Lakew M, 2013)[41] by applying the following statistical formula [42].

$$N = (Z_{\breve{U}2})^{2*} P (1-P)/d^2$$

Where: N is the number of sample size, $Z_{\breve{U}2}$ is the 95% confidence interval (1.96),d (delta) is the degree of freedom (0.05), p is the proportion to be used on estimation (6.5%);. Since prevalence is < 10, d=p/2, i.e. 0.065/2=0.0325

$$N= \frac{1.96^{2*}0.065(1-0.065)}{0.0325^2} = 221$$

Hence, based on the above formula we are supposed to enroll 221 HIV-infected participants. However, considering a 10% contingency, the total number of participants who was enrolled in this study was 243 HIV-infected patients. However we have collected a total of 310 study subjects with an intention to include more ART naïve patients.

4.6.2. Sampling Technique

We have used a convenient sampling strategy until the target sample size is achieved.

4.7. Data Collection

Socio-demographic information, hematologic, immunologic, liver function test and the clinical features of each study participant was collected using structured and pre tested data extraction sheet and questionnaire by trained nurses. $CD4^+$ T- cells count, common hematological tests including WBC, hemoglobin, Hematocrit, platelet counts, lymphocyte, and Neutrophile count and WHO clinical stage were collected using the data extraction sheet from each patient card and charts . Socio-demographic information, risk factor such as Behavioral risks (i.e. history of STI, abortion and multiple sex partners) ; Parentral risks (i.e. history of drug injection, dental procedure, catheterization, surgery, blood transfusion and hospitalization) ; and Community acquired risks (i.e. ear / nose piercing , circumcision , dental extraction, tattooing) were collected using structured questionnaire for each participant (Annexes III).

4.8. Laboratory Diagnosis of HCV

4.8.1 Specimen Collection and Handling

Blood samples were collected for HCV screening from ART clinic follow-up patients by professional laboratory personnel. We used Serum for the screening of HCV. Only clear non-Hemolyzed specimens were used. All the necessary precautions were taken during handling of blood or serum samples. The specimens were frozen at -20°C until analysis was done and repeated freeze thaw was minimal.

4.8.2. Anti-HCV Antibodies Detection

The diagnosis & identification of HCV infection is was based on the detection of antibodies to recombinant HCV peptides (EIA-ANTI-HCV, LINER chemicals S.L, SPAIN, Barcelona). Briefly, multiple epitopes of HCV proteins (Core, NS3, NS4 and NS5) were bound to the micro titer wells. When antibodies to HCV are present in the test sample it reacts with recombinant proteins and attach to the solid-phase. Non-reactive antibodies are removed with the wash buffer. Human IgGs bound to the antigen are reacted with horseradish-anti-human IgGs peroxidase conjugate and visualized by subsequent reactions with a chromogenic substrate. Positive sample generates a medium to dark blue color. No color or very pale blue color indicates a negative reaction. The intensity of the reaction was photo metrically determined at 450nm and 630 nm. Finally result was interpreted as positive and negative based on the value of cutoff (Test kit insert).

4.9. Flowchart showing patient recruitment and data collection

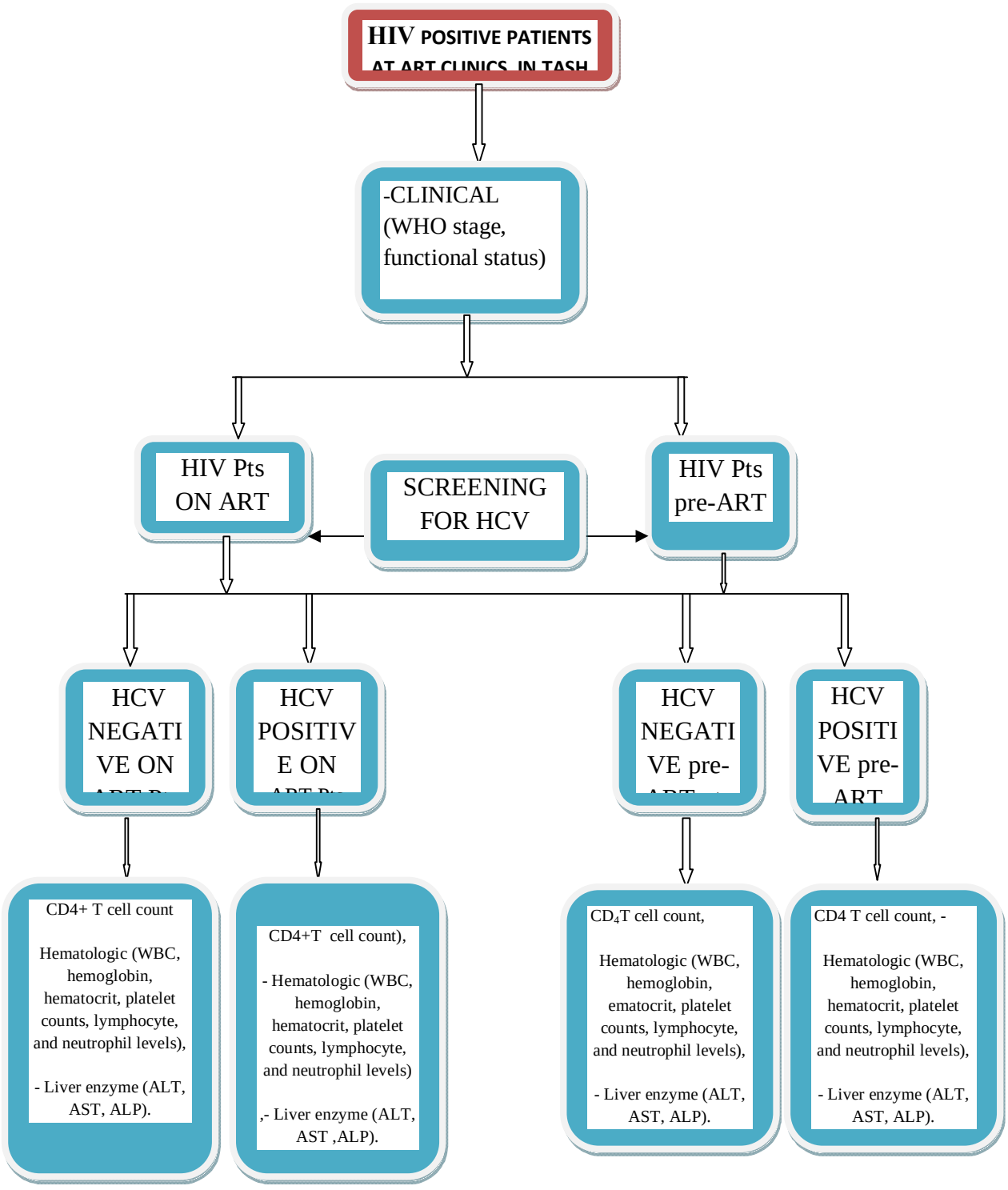

Figure1. Flowchart of patient recruitment and data collection

4.10. Data Quality Assurance

4.10. Quality Assurance

The pre-analytical, analytical and post-analytical phases were controlled throughout the processes of the study. The standard operational procedures were strictly followed for the quality control issues. Hepatitis C kits were checked by using known anti-HCV antibody positive and negative control samples. We used pretested questionnaires and all the data was coded, entered, cleaned, before analysis in EPI-INFO version 3.5.1 computer soft ware package. Data analysis was done using SPSS version 21 software package.

4.11. Dissemination of the result

After conducting the research, the results of the study were communicated to concerned bodies. The result presented to the school of medical laboratory science, college of health science, AAU and other concerned bodies. The results of the study will also be communicated through publication.

4.12. Ethical Consideration

The study was conducted after obtaining ethical clearance from the ethical and research review committee of Department of Medical Laboratory Science, Addis Ababa University, College of Medicine and Health Sciences and from Addis Ababa City Administration Health Bureau. Informed consent was also obtained from each study participants. In addition, we were request for ethical clearance and supportive letter to the study areas from Addis Ababa City Administration Health Bureau. During the study period, participants were provided with brief information about the purposes/objectives of this study. All information had been kept confidential. HCV result was communicated to the clinician in charge in both hospitals for appropriate management.

4.13. Project Management

Cross checking and data cleaning was done and missing information was obtained by going back to the questionnaire. All laboratory and clinical data was recorded on appropriate record during the study period and the data was also be stored in a CD, flash, and other secondary storage devices as a backup. The log book and CD was stored in lockable shelves and only the investigator had an access to the files.

4. 14.Data management and Analysis

All the data was coded, double entered, cleaned, before analysis in EPI-INFO version 3.5.1 computer soft ware package. Frequency running was used to explore and find out inconsistent and incomplete data. The Epi Info data file was then exported into SPSS version 21 software package for further statistical analysis. Descriptive and bivariate analyses for study variables were conducted. The significance of any differences was assessed using chi-square analysis and Fisherôs exact test. P-value < 0.05 was used as statistically significant.

5. Results

5.1. Socio demographic characteristics of study subjects

In this study a total 310 HIV positive patients were enrolled and 282 study subjects had complete data. The remaining 28 study subjects were excluded due to incomplete information and/or sample hemolysis.

The results of socio-demographic characteristics of the study populations were displayed in table 5.1. It was found that 73.4 % (207 /282) of the study participants were females and 75 (26.6) were male. Male to female ratio was 1: 2.76. Mean age of the study population was 40.74 ± 9.50, Larger proportion of the study population (44.3 %, N=282) belongs to age group of 30-39 years followed by house wives (20.9%, N=282). Regarding the occupational status, 34.8 % of them were governmental or nongovernmental workers. Moreover, 136 patients (48.8%) are married and 146 (51.8 %) have completed their high school (Table 5.1)

Table 5.1: Socio-demographic factors of study subjects, Tikur Anbessa and Zewditu memorial hospital, July-November 2013 (n=282)

Characteristics	Number	Percent
Sex		
Male	75	26.6
Female	207	73.4
Age range		
18-29	16	5.7
30-40	125	44.3
40-49	90	31.9
>50	51	18.1
Occupation		
Driver	12	4.3
Merchant	20	7.1
Gov and non-gov	98	34.8
Student	6	2.1
Farmer	1	0.4
Day labor	14	5.0
House wife	59	20.9
No work	46	16.3
No clear occupation stated	26	9.2
Marital status		
Married	136	48.8

Single	37	13.1
Divorced	42	14.9
Widowed	67	23.8
Education		
Illiterate	22	7.8
Writing & reading	22	7.8
Elementary	56	19.9
High school	146	51.8
Collage and above	36	12.8

5.2. Seroprevalence of HCV among study subjects

The overall seroprevalence of HCV among the study subjects was found to be 5.3 % (N=282). Seroprevalence of HCV among female is 3.9 % (11/282) and while in male it was 1.42 % (N=282). Study subjects with age of 40 and above have the highest HCV prevalence of 3.54 % (10 /282). Seroprevalence of HCV was found to be high among government and nongovernment workers accounting 2.12 % (6/282) and married subjects had a prevalence of 2.48 % (7/282) and 3.9 % of seroprevalence found in subjects who completed high schools (Table 5.2.

Table 5.2: HCV sero -status by socio-demographic factors of study subjects, Tikur Anbessa and Zewditu memorial hospital, July-November 2013 (n=282)

Characteristics	HCV Test result		Total (N,%)	Fisher 's Exact test/X^2	P-Value
	Positive (N,%)	Negative (N,%)			
Sex					
Male	4 (1.4)	71 (25.1)	75 (26.6)		
Female	11 (3.9)	196 (69.4)	207 (73.4)		1.000
Total	15 (5.3)	267 (94.5)	282 (100)		
Age Range					
18-29	1 (0.3)	15 (5.3)	16 (5.7)		
30-39	4 (1.4)	121 (42.8)	125 (44.3)	3.189	0..363
40-49	5 (1.8)	85 (30.1)	90 (31.9)		
>50	5 (1.8)	46 (16.3)	51 (18.1)		
Total	15 (5.3)	267 (94.5)	282 (100)		
Residence					
Rural	0 (0.0)	1 (0.4)	1 (0.4)		
Urban	15 (5.3)	266 (94.7)	281 (99.6)		1.000
Total	15 (5.3)	267 (94.7)	282 (100)		
Occupation					
Driver	0 (0)	12(4.2)	12 (4.2)		
Merchant	0 (0)	20 (7.1)	20 (7.1)		
Gov and NGO	6 (2.1)	92 (32.6)	98 (34.7)		
Student	0 (0)	6 (2.1)	6 (2.2)		

Farmer	0 (0)	1 (0.4)	1 (0.4)	4.802	0.779
Daily labor	2 (0.7)	12 (4.2)	14 (4.9)		
House wife	3 (1.1)	56 (19.8)	59 (20.9)		
No work	3 (1.1)	43 (15.2)	46 (16.4)		
Other	1 (0.4)	25 (8.9)	26 (9.2)		
Total	15 (5.3)	267 (94.7)	282 (100)		
Religion					
Muslim	1 (5.3)	18 (6.4)	19 (6.7)		
Christian	14 (4.9)	249 (88.1)	263 (93.3)		1.000
Total	15 (5.3)	267(94.7)	282 (100)		
Marital status					
Marriage	7 (2.5)	129 (45.7)	136 (48.3)	1.148	0.765
Single	1 (0.4)	36 (12.7)	37 (13.1)		
Divorce	2 (0.7)	40 (14.2)	42(14.9)		
Widow	5 (1.2)	62 (21.9)	67 (23.7)		
Total	15 (5.3)	267 (94.7)	282 (100)		
Education					
illiterate	0 (0)	22 (7.8)	22 (7.8)		
Writing reading	0 (0)	22 (7.8)	22 (7.8)	4.238	0.375
elementary	2 (0.71)	54 (19.1)	56 (19.8)		
high school	11 (3.9)	135 (44.8)	146 (51.7)		
Collage above	2 (0.71)	34 (12.0)	36 (12.8)		
Total	15(5.3)	267(94.7)	282(100)		

5.3. HCV risk factors among study subjects

We have tried to analyze the possible risk factors for HCV acquisition among the study subjects. Seroprevalence of HCV among subjects with ear piercing practice is found to be 3.9 % compared with 1.4 % among non exposed subjects. However, there was no statically significance difference between the two groups ($p > 0.05$).

Similarly, seroprevalence of HCV was common among study subjects who practice tattooing on gum (2.8 3 %), dental extraction at health facility (2.83 %), experience in curcumsciation (4.6%), hospital admission (4.6%) and delivery at health facility (3.5 %). However, except hospital admission, other exposure of study subjects to most risk factors are not associated significant (p value >0.05). Likewise almost all HCV infections found among subjects who perceived not using condom during sexual practice (p value > 0.05) (Table 5.3).

Table 5.3: Risk factors for HCV of study subjects, Tikur Anbessa specialized and Zewditu memorial hospital, July-November 2013 (n=282)

Characteristics	HCV Test result		Total (N, %)	Fisher's Exact test/ X^2	p-value
	Positive (N,%)	Negative (N,%))			
Ear piercing					
Yes	11 (3.9)	198 (70.2)	209 (74.0)		
No	4 (1.4)	69 (94.5)	73 (25.8)	0.005	0.943
Total	15 (5.3)	267 (94.7)	282 (100)		
Nose piercing					
Yes	0 (0)	4 (1.4)	4 (1.4)		
No	15 (5.3)	263 (93.2)	278 (98.4)	0.228	0.633
Total	15 (5.3)	267 (94.7)	282 (100)		
Tattooing on body					
Yes	4 (1.4)	49 (17.4)	53 (18.8)		
No	11 (3.9)	218 (77.3)	229 (81.2)	0.643	0.423
Total	15 (5.3)	267 (94.7)	282 (100)		
Tattooing on gum					
Yes	8 (2.8)	102 (36.1)	110 (38.9)		
No	7 (2.5)	165 (58.4)	172 (60.1)	1.367	0.242
Total	15 (5.3)	267 (94.7)	282 (100)		
Dental extraction at home					
Yes	1 (0.4)	14 (4.9)	15 (5.3)		
No	14 (4.9)	253 (89.7)	267 (94.7)	0.057	0.811
Total	15 (5.3)	267 (94.7)	282 (100)		
Dental extraction at health facility					
Yes	8 (2.8)	131 (46.4)	139 (49.2)		
No	7 (2.5)	136 (48.2)	143 (50.7)	0.104	0.748
Total	15 (5.3)	267 (94.7)	282 (100)		

Circumcision					
Yes	13 (4.6)	232 (82.1)	245 (86.7)		
No	2 (0.71)	35 (12.4)	37 (13.1)	0.001	0.980
Total	15 (5.3)	267 (94.7)	282 (100)		
Abortion					
Yes	3 (1.1)	95 (33.6)	98 (47.3)		
No	8 (2.8)	101 (35.8)	109 (52.7)	1.875	0.392
Total	11 (3.9)	196 (69.4)	207 (100)		
Hospital admission					
Yes	13 (4.6)	142 (50.3)	155 (54.9)		
No	2 (0.71)	125 (44.3)	127 (45.0)	6.432	0.011
Total	15 (5.3)	267 (94.7)	282 (100)		
Surgical procedure					
Yes	8 (2.8)	96 (34.0)	104 (36.8)		
No	7 (2.5)	171 (60.5)	178 (63.0)	1.843	0.175
Total	15 (5.3)	267 (94.7)	282 (100)		
Blood transfusion					
Yes	3 (1.1)	47 (16.7)	50 (17.7)		
No	12 (4.2)	220 (77.9)	232 (82.1)	0.056	0.813
Total	15 (5.3)	267 (94.7)	282 (100)		
Frequent alcohol consumption					
Yes	3 (6.5)	43 (93.5)	46 (16.3)		
No	12 (5.1)	224 (94.9)	236 (83.5)	0.158	0.691
Total	15 (5.3)	267 (94.7)	282 (100)		
STI exposure					
Yes	**4 (1.4)**	**94 (33.3)**	**98 (34.7)**		
No	**11 (3.9)**	**173 (61.2)**	**184 (65.1)**	**0.457**	**0.499**
Total	**15 (5.3)**	**267 (94.7)**	**282 (100)**		
Frequent use of sharp materials like blade & other					
Yes	1 (0.4)	37 (13.1)	38 (13.5)		
No	14 (5.0)	230 (81.4)	244 (86.4)	0.630	0.427
Total	15 (5.3)	267 (94.7)	282 (100)		

Place of delivery					
Home	0 (0)	25 (8.9)	25 (8.9)		
Health inst.	10 (3.5)	148 (52.4)	158 (60.0)		
Home & health	1 (0.4)	15 (5.3)	16 (5.7)	2.202	0.699
No birth	0 (0)	8 (2.8)	8 (2.8)		
Total	11 (4.0)	196 (69.4)	207 (100)		
Condom use.					
Yes	0 (0)	37 (13.1)	37 (13.1)		
No	15 (5.3)	230 (81.4)	245 (86.7)	2.393	0.122
Total	15 (5.3)	267 (94.7)	282 (100)		
Use of Injection drugs treatment of diseases					
Yes	0 (0.0)	36 (12.7)	36 (12.7)		
No	15 (5.3)	231 (81.8)	246 (87.1)	2.318	0.128
Total	15 (5.3)	267 (94.7)	282 (100)		

5.4. Common Hematological and CD4 T cells profiles with HCV status of study subjects

Mean total white blood cell count of the study subjects found to be 7470 ± 9.1 / mm^3. The mean CD4 T cell count of study subjects is found to be 410 ± 241.8 / micro liters of blood. The seroprevalence of HCV is found to be high with subjects with normal WBC, Hgb, HCT, Platelet count, Neutrophil count and lymphocyte count. On the other hand the seroprevalence of HCV is high, 3.5 % (10/ 282) among study subjects with CD4 T cell count below 500 / µl of blood sample. However, there is no statically significant difference between CD_4 T cell count, hematological profiles and HCV sero status (p value > 0.05) (Table 5.4.).

Table 5 4: The status of HCV by Hematological and CD4 T cell count of study subjects, Tikur Anbessa specialized and Zewditu memorial hospital, July-November 2013 (n=282)

Characteristics	HCV Test result		Total (N,%)	Fisher' Exact/X^2	p-Value
	Positive (N, %)	Negative (N,%)			
White blood cell/ µl					
<4.0	2 (0.71)	30 (10.6)	32 (11.3)		
4-10.5	11 (4.0)	227 (80.4)	238 (84.3)	3.335	0.187
>10.5	2 (0.71)	10 (3.5)	12 (4.2)		
Total	15 (5.3)	267 (94.7)	282 (100)		
Hemoglobin gm/dl					
<11.5	3 (1.1)	16 (5.7)	19 (6.7)		
11.5-15	10 (3.5)	193 (68.3)	203 (71.9)	4.668	0.0.097
>15	2 (0.71)	58 (20.5)	60 (21.2)		
Total	15 (5.3)	267 (94.7)	282 (100)		
Hematocrit %					
<34	2 (0.71)	11(4.0)	13(4.5)		
34-44	10 (3.5)	165 (58.4)	175(60.4)	3.514	0.173
>44	3 (1.1)	91 (96.8)	94(32.4)		
Total	15 (5.3)	267 (94.7)	282(100)		
Platelets/µl					
<140	1 (0.4)	11 (3.9)	12 (4.2)		
140-415	14 (4.9)	227 (80.1)	241 (85.3)	1.961	0.375
>415	0 (0.0)	29 (10.1)	29 (10.1)		
Total	15 (5,3)	267 (94.7)	282 (100)		
Lymphocytes /µl					
<0.6	0 (0.0)	1 (0.4)	1 (0.4)		
0.6-4.1	14 (5.0)	259 (91.7)	273 (96.6)	0.895	0.639
>4.1	1 (0.4)	7 (2.5)	8 (2.8)		
Total	15 (5.3)	267 (94.7)	282 (100)		

Neutrophil					
<2	4 (1.4)	52 (18.4)	56 (19.8)		
2-7.8	10 (3.5)	208 (73.6)	218 (77.1)	1.421	0.491
>7.8	1 (0.4)	7 (2.5)	8 (2.8)		
Total	15 (5.3)	267 (94.7)	282 (100)		
$CD_{4\ cells/mm^3}$					
<500	10 (3.5)	171 (60.5)	181 (64.1)		
500-1300	5 (1.8)	95 (33.6)	100 (35.4)	0.092	0.955
>1300	0 (0.0)	1 (0.4)	1 (0.4)		
Total	15 (5.3)	267 (94.7)	282 (100)		

5.5. Liver function profile of study subjects with HCV sero-status

Higher seroprevalence of HCV is found among study subjects with normal serum AST and ALT levels 4.96% (14 /282) each for ALT and AST respectively. On the other hand all HCV sero-positive subjects had higher alkaline phosphates enzyme level (> 150 u/l). However, there is no statistically significant difference between HCV sero-status and liver function enzymes (p value > 0.05) (Table 5.5.).

Table 5.5. Seroprevalence of liver enzymes (AST, ALT, ALP) profiles of study subjects , Tikur Anbesa speclised hospital and Zewditu Memorial Hospital, July-Nov, 2013 (n=282)

Characteristics	HCV Test result		Total (N,%)	Fisher' Exact /X^2	P-Value
	Positive (N, %)	Negative (N,%)			
ALT					
0-50	14 (5.0)	255 (90.1)	269 (95.2)		
>50	1 (0.4)	12 (4.2)	13 (4.6)	0.516	
Total	15 (5.3)	267 (94.7)	282 (100)		
AST					
0-60	14 (5.0)	266 (94.1)	280 (99.1)		0.104
>60	1 (0.4)	1 (0.4)	2 (0.71)		
Total	15 (5.3)	267 (94.7)	282 (100)		
ALP					
40-150	0 (0.0)	10 (3.5)	10 (3.5)	1.000	
>150	15 (5.3)	257 (91.1)	272 (96.3)		
Total	15 (5.3)	267 (94.7)	282 (100)		

5.6. HCV sero status of study subjects with clinical parameters

The result given in table 5.6 has given the clinical parameters and their association with HCV. The highest prevalence is found in WHO stage II 7(2.4). All HCV infected patients are functionally working (5.3%). In terms of clinical symptoms, 9 patients (3.2 %) do not have any clinical symptoms. About 3.5 % (10/282) study subjects have BMI of 18.5-24.9. HCV seroprevalence reported in 13 subjects accounting 4.6 % of the total HCV prevalence however ,there is no statistically significant difference between ART users and naïve patients .similarly 2.1 % of study subjects are HCV positive and has been using ART for 5-10 years.

Table 5.6: HCV sero-status by clinical parameters of study subjects, Tikur Anbessa and Zewditu memorial hospital, July-November 2013 (n=282)

Characteristics	HCV Test result		Total (N,%)	Fisher' Exact /X^2	P-Value
	Positive (N, %)	Negative (N,%)			
WHO staging					
I	2 (0.71)	97 (34.3)	99 (35.0)		
II	7 (2.8)	98 (34.7)	105 (37.1)	5.871	0.118
III	3 (1.1)	54 (19.1)	57 (20.1)		
IV	3 (1.1)	18 (6.3)	21 (7.4)		
Total	15 (5.3)	267 (94.7)	282 (100)		
Functional status					
Working	15 (5.3)	265 (93.8)	280 (99.1)		
Ambulatory	0 (0.0)	2 (0.71)	2 (0.71)	0.113	0.737
Bedridden	0 (0.0)	0 (0.0)	0 (0.0)		
Total	15 (5.3)	267 (94.7)	282 (100)		
BMI					
<18	1 (0.4)	21 (7.4)	22 (7.9)		
18.5-24.9	10 (3.5)	165 (58.4)	175 (62.0)	0.646	0.724
25-29.9	3 (1.1)	63 (22.3)	66 (23.4)		
>30	1 (0.4)	18 (6.4)	19 (6.7)		
Total	15 (5.3)	267 (94.7)	282 (100)		
ART drug started					
Yes	13 (4.6)	250 (88.5)	263 (93.1)		
No	2 (0.7)	17 (6.0)	19 (6.7)	0.883	0.347
Total	15(5.3)	267 (94.7)	282 (100)		
ART how long					
1month-5yrs	4(1.4)	40 (14.1)	40 (14.1)		
5.1yrs-10yrs	6(2.1)	136 (48.1)	142 (50.5)	12.427	0.572
>10yrs	3(1.1)	88 (31.2)	91 (32.2)		
Total	13(4.6)	264(95.3)	277 (100)		

5.7. Comparison of hematologic, CD4 T cells and Liver function tests between HIV HCV co-infected and HIV mono-infected subjects.

Mean White blood cell count and liver enzymes of HIV HCV co infected patients is higher than patients with HIV infected only (9090 / mm^3 ± 10.05 vs. 7380 ± 9.04; 29.47 ± 30.8 vs 25.36 ±14.2, 40.07 ±39.4 vs26.1±12.06; 273.2 ±120.8 vs. 254 .58 ±96.9 for WBC, ALT, AST and ALP respectively). There is statistically significant difference between mean AST level between HIV-HCV co infected patients and HIV monoinfected subjects ($p < 0.05$) (Table 5.7)

Table 5.7. Mean distribution of Common hematologic, CD4 T cells and liver function enzymes among HIV HCV Co infected and HIV mono infected study subjects , Tikur Anbessa and Zewditu memorial hospital, July-November 2013 (n =282)

Characteristics	Mean Parameters		P-value	
	HIV/ HCV positive (n=15)	HIV Monoinfected (n=267)		
White blood cell	9.09 ± 10.05*	7.38 ± 9.039*	0.478	NS
Hemoglobin	13.3 ±1.6	14.27 ± 5.149	0.470	NS
Hematocrit	40.7 ± 4.8	42.07 ± 4.94	0.288	NS
Platelets	287.67 ± 72.1*	291.9 ± 103.56*	0.876	NS
Lymphocytes	2.147 ± 1.1*	2.426 ± 0.96*	0.278	NS
Neutrophile	3. 633 ± 1.9*	3.697 ± 1.8*	0.896	NS
$CD_{4\ T\ cell}$	415.53 ± 219.2	451.29 ± 243.2	0.578	NS
Alanine Aminotransferase	29.47 ± 30.8	25.36 ± 14.6	0.327	NS
Aspartate Aminotransferase	40.07 ± 39.4	26.1 ± 12.1	0.001	S
Alkaline phosphatase	273.2 ± 120.8	254.58 ± 96.829	0.475	NS

S: significant; NS: Non significant * should be multiplied with 10^3

6. Discussion

Viral hepatitis such as HCV is one of the major health problems in the general population and in HIV infected individuals in particular. With the increased access to antiretroviral therapy in resource limited settings, people living with HIV/AIDS will continue to live longer. However, morbidity and mortality due to co-infections with other microbes like HCV infection current problem and co morbid for HIV patients . Hence it is important to have sufficient information concerning HCV seroprevalence among HIV positive patients that could guide policy makers and concerned bodies to plan appropriate intervention in co-infected individuals at local conditions.

The seroprevalence of HCV in this study 5.3 % (15/ 282) is comparable with previous study conducted in Ethiopia such as University of Gondar Teaching Hospital, Northwest Ethiopia among HIV positive individuals the overall prevalence of HIV/HCV was **5.0% (18/400)** [42]; in Addis Ababa, Ethiopia , 6.5 % seroprevalence of HCV among HIV infected patients has been reported at Yekatit 12 and Zenbaba General Hospitals[44] **,** 6.7 % anti HCV seroprevalence from HIV infected Nigerian patients and 4.4. % HCV seroprevalence from controlled population (n= 180 each group) [37]., 5.7 % at Kigali Ruanda [43].

Lower HCV seroprevalence of 0.062 % (4/4361) had been reported in Gondar, Ethiopia and 0.2 % (10/ 6063) reported in Jimma, Ethiopia among blood donors . The large sample size they used, use of retrospective data in both towns, being the study subjects were blood donors and difference in sociodemographic nature of the study subjects contribute the difference in seroprevalence [44.] Lower prevalence of HCV among HIV patients has also reported in Nigeria 0.7 % (3/404) [45].

Lower HCV prevalence than our findings has been reported among HIV positive patients at Mulago Hospital in Uganda of 3.3 % (4/122). , with a mean age of 33.9 years. There were more females 81 (66.4%) than males. [38]; 3.02 % (43/ 1443 HIV patients) of prevalence reported in India in 2009 and In South India 3.02 % (45 /1487 HIV-infected patients) and co infection was predominant in the age group 41-50 years (51.1%) [35].

It is also found that a consistent evidence of high HCV prevalence in many countries of Africa. In general, the overall prevalence of HCV in Sub-Saharan Africa is estimated to be 3%. Even though the prevalence of HCV infection varies throughout the world, the highest number of infection ranging from 6% to 28% in Egypt [24]. In Gambia it was reported as 0.6%. In Nigeria, prevalence rates ranging from 4.5% to 10.3% have been reported in various studies [25-27].

Worldwide, the overall prevalence of HCV is estimated as 3%. Approximately 4 to 5 million persons are co-infected with HIV. In the US and Western Europe, among HIV-infected persons, HCV prevalence is 72% to 95% among injection drug users (IDU), 1% to 12% in men who have sex with men (MSM), and 9% to 27% in heterosexuals. Similarly [46] reviewed that Overall it is estimated that around 25% of all European HIV patients have concomitant hepatitis C virus (HCV) co-infection However, it is difficult to compare our findings with this review as we concentrate only HIV patients from ART clinic and limited data we have [47].

Slightly higher prevalence of HCV (7.5 %) was reported among attendants of voluntary counseling and testing center, and HIV follow up clinics at Tikur Anbessa Hospital, Addis Ababa, Ethiopia [22].

Relatively higher seroprevalence of HCV 10.5 % (n= 200) among HIV infected patients has been reported in Hawassa city, Southern Ethiopia. While HCV prevalence among HIV negative patients was only 6 % ($p = 0.002$) [42]. The difference is sample size (282 vs. 200) and study population may contribute this discrepancy in seroprevalence of this study with them.

The prevalence of HCV among HIV infected patients in Kenya showed 10.3 % (31/300). Of the study subjects 129 (43%) males and 171 (57%) were females. A higher seroprevalence of HCV 9.2 % (16/174) has been reported in Mekelle, North Ethiopia among attendants of voluntary counseling and testing center and HIV follow up clinics in 2011 and the overall HCV prevalence was 6 % (18/ 300) in the total groups[23]. In British Columbia, Canada HCV seroprevalence of 52 % had been reported [33].

Higher prevalence of HCV 33.8 % (27/ 80) has reported in Iranian HIV patients in Mazandaran province. Of the samples, 66 (82.5%) were males and 14 (17.5%) were females with the mean age of 37 years [48]. The prevalence of HCV infection in sampled men who were HIV-1 positive was 8.70%, whereas it was 2.18% for those who were HIV-1 negative among the conscripts of young Thai men [49]. These findings are higher than our finding even if their population is entirely men and age distribution is homogenous most study subjects had history of unsafe injection uses.

A community-wide survey of subjects 40 years old who had undergone a preventive health examination in A-Lein Township, Kaohsiung County, Taiwan revealed that prevalence of anti-HCV antibody was 17.4 % (n= 1690). However, the study subjects are general population were their HIV status is not known [50]. In Tamil Nadu, India seroprevalence of HIV HCV coinfcetion was 1.17 % (6/512) [51], in Badalona, Spain, HCV prevalence of 52 % has been reported [52].

Most of study subjects in this study had normal AST and ALT levels and it is not surprising to have higher HCV seroprevalence with this group of subjects. On the other hand all HCV positive subjects had abnormal ALP levels. Even if our sample size is comparable to Nigerian study (n= 180) there is no significant difference between HCV sero-status and ALP While in Nigerian study, ALP level is significantly higher for HIV-HCV co infected patients than HCV mono infected patients. though, we do not have control group / HCV monoinfected group to appreciate the difference properly [35].In Gondar , Ethiopia ,Study participants had relatively raised mean liver enzyme levels (ALT, AST and ALP) than HIV mono-infected once and lower mean CD4 levels than HIV mono-infected study participants [39].

In Addis Ababa, Ethiopia Taye and Lakew in 2013 reported that 75% of co-infected groups had higher mean ALT and AST levels than above the upper limit of normal reference range [41]. But, we did not consider mean patterns of liver function tests for different ART visit in our case that could explain the difference. On the other hand we have seen significant difference between mean AST levels between HIV HCV co infected subjects and HIV infected alone. Here the presence of double infection may have severe effect / co morbid on liver and AST level could be higher. We need to further follow the two groups with larger sample size to look the effect in

better evidence (Table 5.7). HCV HIV co-infected patients in Tamil Nadu India had mean AST level of 56.78 ± 4.401 IU/ liters which is similar with our findings [50] HCV sero-prevalence among females subjects in this study is higher than male subjects (3.9 % vs. 1.41 %) and majority of HCV seropositive subjects (3.54 %) belongs to age of 40 years and above. However, there is no significant difference in HCV seroposotivity with age and sex. This finding is similar with previous study conducted in Mekelle, North Ethiopian study. [23]. The mean age of our study subjects 40.74 ± 9.50 years is slightly higher than similar study conducted in Mulago , Uganda that report mean age of 33.9 years. While more females subjects were involved in our study which is similar with Mulago, Ugandan study were 66.4% of the study subjects were females [38]. There was also reported more females were affected by HIV HCV coinfcetion compared to male subjects [51]. Similarly in Indian study HIV /HCV co-infection was predominant in the age group 41-50 years (51.1%) [34]. and more than half of study subjects in 234 (59.2%) were females and 161 (40.8%) males. [22]

Hospital admission of study subjects in this study is strongly associated with HCV seroposotivity (p value, 0.007). While other risk factors are not associated well. Similar report has been observed in Mekelle, Ethiopia study were no apparent risk factor that caused HCV infection was inferred (p >0.05). [23]. Government and nongovernment workers had higher seroprevalence of HCV 2.12 % (6/282) and most HCV infected patients had normal Body mass index which is similar with study conducted in Kigali Ruanda[43]. Higher body mass index and HCV seroposotivity was strongly associated in Spain study however; there is difference in study subjects with our findings [52].

Study conducted in Sao polo Brazil revealed that intravenous drug use, homosexual , receiving blood transfusion were considered as risk factors for transmission of HCV among HIV patients [53]. However, it is difficult to compare this finding with ours as the socio economic conditions, risk behaviors could be varied. The sample size is also small in our case even if there is injection use of drug for treatment purpose , surgical procedure ,alcohol consumption, STI exposure , blood transfusion , condom use, tattooing of gum and body parts, and other risk factors there is no significant association and or inadequate to infer conclusion on the risk factors. HCV infection was independently associated with intravenous drug use in British Colombia [33].

About 59.6 % of study participants in Nigerian HCV/ HIV seroprevalence study were married subjects and 69 .2 % were females [36]. which is almost similar with the current study we have reported.

All HCV infected HIV patients in our study had CD4 T cell count below 500 per micro liters of blood and mean CD4 T cell count for co infected patient is slightly lower than HIV infected patients (415.5 ± 219.2 vs. 455 ± 243.3 respectively, ($p > 0.05$). The mean CD4 count value between the HIV group (243cells/µl) and the HIV/hepatitis group (205cells/µl) was not significantly different in Nigerian study by [54]. However, there is difference in the cutoff CD4 T cells between theirs and ours.

There is no significant difference in terms of all hematologic parameters between HCV infected and non infected HIV patients. This is in contrast with study conducted in Nigeria were the mean hemoglobin, white cell count, platelet count, and CD4+ T lymphocyte count were significantly lower in the HIV/HCV co infected patients than the HCV monoinfected control group [38]. The difference sample population and use of control subjects in Nigerian study may explain this variation. However, all HCV infected subjects in this study have CD4 T cell count below 500/ micro liters of blood samples.

Majority of HIV ï HCV co infected patients in this study had normal platelet count (140000 per micro litters of blood) .Only one individual had thrombocytopenia. It has been reported that HIV HCV co-infected individuals had significantly lower platelet count than normal or monoinfected individuals [19]. The mean platelet count in subjects with anti-HCV (180,000 platelets/ml) was lower than in those with without anti-HCV and HBsAg (234,000 platelets/ml) ($P = .001$). However, the study subjects are general population were their HIV status is not known [52]. In addition the low sample size we used, lower rate of co-infected subjects and use of different reference interval for platelet count limit us to compare our findings with other studies.

Finally in this study7 subjects out of 15 HCV infected one had classified under WHO clinical stage II. While in Rwanda study most HIV patients were classified under WHO stage I and III [45]. Clinical staging could not be specific and variation could occur during evaluation and recording because it could subjective.

7. Limitation of the study

- The clinical, hematological and CD4 T cell count and Liver function profiles of patients are not uniformly registered and recorded and we are unable to have follow-up data.
- HCV RNA by polymerase chain reaction was not done due to unavailability of required technology. It is most sensitive test detects virus as early as 1-2 weeks after exposure. This may have increased the prevalence of HCV in our study as it would allow early diagnosis of these infections before anti-HCV antibodies were detectable in serum.

8. Conclusion

In this study relatively the overall seroprevalence of HCV among HIV infected patients at the study site is moderately medium (5.3 %) with female dominance (3.9 %) and patients with age of 40 and above had the highest prevalence. Married subjects, government and nongovernment workers had the highest HCV seroprevalence and 3.9 % of seropositive subjects have completed high schools.

In this study hospital admission is found to be a possible risk for HCV/ HIV co infections which is significantly associated (P-value=0.007). Other risk factors like ear piercing practice, tattooing on gum and or body, dental extraction at health facility, experience in curcumsciation, delivery at health facility, frequent alcohol consumption, injection drug use for treatment purpose, abortion, surgical procedures, blood transfusion, and STI exposure were common among co-infected patients but it is not statistically different from HIV mono-infected patients.

About 46.6 % of HIV /HCV co-infected patients belong to WHO clinical stage II, all infected patients are functionally active and majority (3.5 %) of co-infected patients had normal BMI and 86.7 % of co infected subjects are on ART.

All mean Hematological and CD4 T cell count of HIV /HCV co infected subjects had lower than HIV infected subjects alone ($P > 0.05$) where co-infection may have suppressive effect on hematopoisis and destruction of cells than single HIV infection. This is also observed as the mean Liver function enzymes are higher for double infected patients than single HIV infection.

9. Recommendations

- Emphasis should be given to screen HIV patients for viral infection including HCV that could influence HAART.
- Large scale study is required with control group along with hematological, immunological and biochemical profiles in a follow up study.
- Health education / information for HIV/ AIDS patients should consider the transmission and prevention of viral diseases such as HCV**.**
- Infection prevention (Ip) directed towards HCV prevention need to be implemented.

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11. Annexes

11.1. Annex I: Patient information sheet

ñHuman Immunodeficiency Virus and Hepatitis C Virus co-infection: Immunological, Hematological and Clinical description on Pre-Anti retroviral therapy patients at Tekur Anbesa and Zewditu Memorial Hospitalò.

Purpose

This study was concerned with HIV and HCV co-infection, two deadly diseases in the world at large and Ethiopia in particular. The diseases, which have similar transmission mechanism, are common in Ethiopia. We are approaching you today to ask you to participate in this study so as to determine the immunologic, hematologic and clinical profile of the disease you have.

Procedures and participation

In this study, we expect to include 243 individuals to participate in the study. Your participation in this research is entirely voluntary. If you are willing to participate in this study, you will undergo the following two procedures: 1) general clinical examination by one of the health workers in our team, 2). Five cc venous bloods will be collected from you for immunological and hematologic analysis will take place.

Risks and discomforts

While you are participating in this study, you are likely to have some risks although the methods employed are routine medical practices. The possible risks are some bleeding during vein-puncture, if any and none of these are deleterious to your well being. Should these occur by accident, we will avail to you the necessary medical assistance.

Benefits

If you participate in this study, you will not get any material or financial benefit. The direct benefit that you can expect is that, if there is any positive finding in laboratory investigation the

result will be communicated to your physician and prescription of treatment and advice will be effected. However, your participation in this study will help us to better understand our objectives.

Confidentiality

The information that we collect from this study will be kept confidential. No one, except the investigator is able to access information about you. Any information related to you, e.g., detailed clinical and laboratory results will have a number on it, instead of your name, which could be recognized only by the researcher. All your files will be kept in a secured place and data will not be revealed to anyone except the principal investigator and your clinician.

Sharing the result

At the end of this study we write a report about the results of the study through publication or any other means. The reports wonǿ bear any information relevant to your personality e.g. your

Right to refuse

Since participation in this study is entirely voluntary, you can refuse to participate in this research. Your refusal to participate in this study will not affect your right to access health care in your region or elsewhere.

Contact Address

If you have any further question and in case of urgency you can contact the principal investigator at any time using the following address:-

Name: - Agerie yigezu (Principal investigator)
Address: - Addis Ababa University Faculty of Medicine Department of medical laboratory science
City: - Addis Ababa
Telephone (mobile):- +251911918983
P.O. Box: 9086 A.A Ethiopia
Email: - agereyz@gmail.com

11.2. Annex II: Amharic Translated Information Sheet

የጥንቱ ዓላማ

ሄፓታተስ ሲ ቫ□ረሰ በ HIV መካከል ክለውን ስርጭት ለማጥናት የታቀ□ ነው።

በጥናቱ ለመሳተፍ

በ□ህ ጥናት መሳተፍ በሙሉ □ቃ□ኛነት ላ□ የተመሰረተ ነው። ስለነሆም በጥናቱ እንዲሳተፉ □ቃ□ኛነት□ን እንጠ□ቃለን።ለመሳተፍ ከ□ቀ□ 5 ሚሊ ሊትር የ□ም ናሙና ከክንድ□ ተወስ የላባራቶሪ ምርመራ □□ረግሎታል። የላባራቶሪ ምርመራውም ሄፓይታስ ሲ ቫይረስ በደምዎ ው ስጥ መኖር አለመኖሩን ማረጋገጥ ይሆናል። የደም ናሙ ናው ም የሚ ወሰደው ንጽህናው በተጠበቀ አዲስ እና በታሸገ መርፌ ና ስሪንጅ ነው።

በጥናቱ ሊከሰቱ የሚችሉ ተክክኛ ችግሮች

5 ሚሊ ሊተር የ□ም ናሙናውን ለመወስድ መርፌ ሲገባ ከሚ□ጥረው የቅጽበት የህመም ሰሜት በስተቀር የጎላ ችግር አክመ□ም ነገር ግን ምኞት ካልተሰማ□ ሀኪም እንዲክ□□ት □□ረጋል።

በጥናቱ በመሳተፍ የሚገኝ ጥቅም

የ□ም ናሙናው የላብራቶሪ ውጤት ችግር ካሳዬ የባለሙክ ምክርና የመድሃኒት ተ□□ዝ □ገ□ ክገኛሉ።

የጥናቱ መረጃ□ች ሚስጢራዊነት

በጥናቱ ውስጥ የተሰበሰቡ ማናቸውም ግላዊ መረጃ□ች ሚስጥራዊነታቸው የተጠበቀ ይሆናል። ከማንነት□ ጋር በቀጥታ ተክክኛነት ክላቸው መረጃ□ች በሙሉ በዋና መርማሪው ሚስጥራዊ በሆነ የመረጃ ጥንክር □ዴ ከተቀየሩ በኋላ ብቻ ለምርምር ሂ□ቱ የሚውሉ □ሆናል።

የጥናቱን ውጤት ስለማሳወቅ

የ□ህ ጥናት ውጤት በተለክዩ የህትመት ውጤቶች የሚቀርቡ ሲሆን □ህ ከማንነት□ ጋር የተክክ□ ምንም አ□ነት መረጃን አክካትትም ። ስለ□ህም የጥናቱን ውጤት በሪፖርት መልክ እናቀርበው □ንድ □ቃ□ኛነት□ን እንጠ□ቃለን።

ከጥናቱ ስለመው□ትና ስለማቋረጥ

□ህ ጥናት በ□ቃ□ኝነት ላ□ የተመሰረተ እን□መሆኑ መጠን በማንኛውም ወቅት በ□ቃድ□ ከጥናቱ መው□ት □ችላሉ። ከጥናቱ ቢወጡም የተለመ□ውን የህክምና □ርዳታ በጤና ተቋም ውስጥ በማንኛውም ጊ□ የማግኘት መብት አለ□ት።

ከጥናቱ ጋር በተክክ□ ማናቸውም ጥክቄ ቢኖር□ በሚከተለው አድራሻ ጥክቄውን ማቅረብ □ችላሉ።

ዋና ተመራማሪ፡- አገሬ □ገ□

አድራሻ፡- በአዲስ አበባ ዩኒቨርሲቲ የህክምና ፋኩሊቲ ላቦራቶሪ ሳ□ንስ ትምህርት ክፍል

ሞባ□ል ስልክ ቁጥር *251 911 918983*

ፖ.ሣ ቁ፡- 9086 አዲስ አበባ ኢትዮጵክ

11.3. Annex III: Consent form (for the patient English and Amharic version)

I, the undersigned, confirm that, as I give consent to participate in the study, it is with a clear understanding of the objectives and conditions of the study and with recognition of my right to withdraw from the study if I change my mind.

Ié é é é é é é é é é do herby give consent to Dr/Mr./Mrs./Missé é é é é é é é .to include me in the proposed research. I have been given the necessary information about the research. I have also been assured that I can withdraw my consent at any time without penalty or loss of benefits. The proposal has been explained to me in the language I understand.

Name of the patient:-_____

Patientôs signature: - _____

Name of Dr/Mr./Mrs./Miss: - _____

Dr/Mr./Mrs./Miss signature: - _____

Date: - _____

Witness: - _____

11.4. Annex IV: Amharic translated consent form

እኔ ስሜ ከታች የ ተገለፀዉ የጥናቱ ተሳታፊ ለመሆን ስወስን የጥናቱን አላማዎች አሰራሮች ና ቅድመሁ

ኔታዎች በ ግ ል ጽ በ መረዳት ና ከጥናቱ ተሳታፊነት ፈቃደኛነቴን በ ማንኛዉም ደረጃ የማንሳት መብቴን

በማረጋጥ ነ ዉ፡ ፡

እ ኔ ---------- በ ጥ ና ቱ ተ ሳ ታ ፊ መሆ ኔ ን በ ፊ ር ማዬ እ ያ ረ ጋ ገ ጥ ሁ ይህንን ስወስን በጥናቱ ሳቢያ ሊከሰቱ የሚችሉ አደጋዎች በሚገባ የተረ ዳ ሁ ና ከ ጥ ና ቱ በ ማን ኛ ዉም ደ ረ ጃ እ ራ ሴ ን ለ መሰ ረ ዝ ብ ወ ስ ን ተ ገ ቢ የ ሆ ኑ ህ ክ ምና ዎ ች ና እ ገ ዛ ዎ ች ሁ ሉ እ ነ ደ ማይ ነ ፍ ጉ ኝ በ ማመን ነ ዉ፡ ፡ እ ነ ዚ ህ መረ ጃ ዎ ች ሁ ሉ በ ሚገ ባ በ ምረ ዳ ዉ ቋ ን ቋ የ ተ ገ ለ ጸ ል ኝ መሆ ኑ ን በ ፊ ር ማዬ አ ረ ጋ ግ ጣለ ሁ ፡ ፡

የ በ ሽ ተ ኛ ዉ ሙሉ ስ ም---------- ፊ ር ማ----------
የ ተ መራ ማሪ ዉ ሙሉ ስ ም፤ ዶ /ር & ኦ ቶ & ወ /ሮ & ወ /ት ----------
ፊ ር ማ----------
የ ምስክር ሙሉ ስም ---------- ፊ ር ማ----------

11.5. Annex V: Questionnaire

Addis Ababa University
Faculty of Medicine
Department of medical laboratory science
(To be translated to Amharic)

For data collectors: please write on the space provided on the answer among choices accordingly

S No	Question	Response
1. 1.1	**Identification** Code	_____(#)
1.2.	Sex	1. Male 2. Female
1.3	Age in years	_____.
2.	**Back ground information**	
2.1	Residence	1. Rural 2. Urban
2.2.	Current occupational status	1. Driver 2. Merchant 3. Government/private employee 4. Student 5. Farmer 6. Daily laborer/house servant 7. Housewife 8. Jobless 9. Others specify ----------.
2.3	Religion	1. Muslim 2. Christian
2.4	Marital status	1. Married 2. Single 3. Divorced 4. Widow
2.5	Educational Status(adult education)	1. Illiterate 2. Can wire and read 3. Elementary school 4. High school 5. College level and above

3. Questions related to HIV and HCV risk factors. Have you have or ever practiced the following?		
3.1.	Ear piercing	1.Yes 2. No
3.2.	Nose piercing	1. Yes 2. No
3.3	Tattooing on body	1. Yes 2. No
3.4	Tattooing on gum	1. Yes 2. No
3.5	Dental extraction at home	1. Yes 2. No
3.6	Dental extraction at health facility	1. Yes 2. No
3.7	Circumcision	1. Yes 2. No
3.8	Abortion	1. Yes 2. No
3.9	Hospital admission	1. Yes 2. No
3.10	Surgical procedure	1. Yes 2. No
3.11	Blood transfusion	1. Yes 2. No
3.12	Frequent alcohol consumption	1. Yes 2. No
3.13	Venous or body piercing for treatment (Traditional treatment check the scar)	1. Yes 2. No
3.14	STI exposure	1. Yes 2. No
3.15	Frequent use of sharp materials like blade, and other similar materials?	1. Yes 2. No
3.16	Delivery at	1. Home 2. Health Institution 3. both

3.17	Condom utilization	1. Yes 2. No
3.18	Use of Injection drugs for treatment of diseases _____.	1. Yes 2. No
3.19	Current clinical symptom you have.	(1. Vomiting 2. Nausea 3. Fever 4. Right upper quadrant pain .5.1&2 6.1&3 7.1&4 8.2&3 9. 2&4 10.3&4) 1. Yes 2. No
3.20	Have you started taking antiretroviral therapy?	1. Yes 2. No If the answer is yes for how long? ------ List the name of the drug:- _____, ----------, ----------

11.6. Annex VI: Amharic translated questionnaire

አዲስ አበባ ዩኒቨርሰቲ
የህክምና ፋኩሊቲ
የህክምና ላቦራቶሪ ሳ□ንስ ትምህርት ክፍል

ለ መረጃ ሰብሳቢ□ች፤ጥክቄውን ከጠየቃችሁ በኋላ መልሱን በተሰጠው ክፍት ቦታ ወ□ም ከተሰጡት አማራጮች አን□ን በማክበብ □ጻፉ::

ተራ ቁ.	ጥክቄ	መልስ
1.	መለክ	
1.1	ኮ ድ	_____(#).
1.2.	ፆታ	1. ወንድ 2. ሴት
1.3.	እድሜ	----------.
2. ጠቅላላ መረጃ)		
2.1	መኖሪክ አካባቢ	1. ገጠር 2. ከተማ
2.2	ሥራ	1. ሹፌር 2. ነጋዴ

		3. የመንግስት ሠራተኛ ወ□ም መንግሥታዊ ክልሆነ ድርጅት ተቀ□ሪ 4. ተማሪ 5. ገበሬ 6. የቀን (የቤት) ሠራተኛ 7. የቤት እመቤት 8. ሥራየሌለው 9 ሌላ (□ግለጹ) ---------- --------.
2.3.	ሃ□ማኖት	1. ሙስሊም 2. ክርስቲክ
2.4.	የጋብቻ ሁኔታ	1. ክገባ / ች 2. ክላገባ / ች 3. የ□ታ / ች 4. የሞተበት / ባት
2.5.	የትምህርት □ረጃ	1. ክልተማረ 2. መፃፍና ማንበብ የሚችል 3. ከ1ኛ □ረጃ 4. 2ኛ□ረጃ 5. ኮሌጅ ና ከ□ክ በላ□

3.	**HIV እና ሄፓታ□ተስ “ሲ ” ን በተመለከተ ጥያቄዎች**	**ከ□ህ በታች ክሉትን በህ□ወትህ / ሽ አድርገህ / ሽ ታውቃለህ ታውቂክለሽ ?**
3.1	**ጆሮ መብሳት**	**1. አ□** **2.አ□□ለም**
3.2	**አፍንጫን መብሳት**	**1. አ□** **2.** **አ□□ለም**
3.3.	**ሰውነት መቀነስ**	**1. አ□** **2. አ□□ለም**
3.4.	**ድድ መነቀስ**	**1. አ□** **2. አ□□ለም**
3.5.	**ቤት ውስጥ ጥርስ ማስነቀል**	**1. አ□** **2. አ□□ለም**
3.6.	**ጤና ድርጅት ጥርስ ማስነቀል**	**1. አ□** **2. አ□□ለም**

3.7.	**ግር□ት**	**1. አ□ 2. አ□□ለም**
3.8.	**ውርጃ**	**1. አ□ 2. አ□□ለም**
3.9.	**ሆስፒትል መተኛት (ለምን)?**	**1. አ□ 2. አ□□ለም**
3.10.	**ማንኛውም አ□ነት ቀ ጥገና**	**1. አ□ 2. አ□□ለም**
3.11.	**□ም መቀበል ?**	**1. አ□ 2. አ□□ለም**
3.12.	**መጠጥ መጠ□ት(በብ□ት) ?**	**1. አ□ 2. አ□□ለም**
3.13.	**ሞኝ ባገኝ መቆረጥ**	**1. አ□ 2. አ□□ለም**
3.14.	**በ አባለ□ር በስታ ተይዘው ያውቃሉ?**	**1. አ□ 2. አ□□ለም**
3.15.	**ስለት ባለው ነገር (ምላጭ) መቆረጥ ልምድ**	**1.አ□ 2. አ□□ለም**

3.16.	**ሲወልዱ?**	**1. ቤት ውስጥ 2. ጤና ድርጅት** **3. ቤት ውስጥ እና ጤና ድርጅት**
3.17.	**ኮን ም ጠቀማሉ ?**	**1. አ 2.. አለም**
3.18.	**ሌላ ሰው በተጠቀመበት መርፌ ና ሲሪጅ ተጠቅመዋል?**(መዳህኒት)	**1. አ 2. አለም**
2.19.	**አሁን** ምን የህመም ስሜት ይሰማዎታል?	ነ. ትውከት 2. ማቅለሸለሸ 3. ትኩሳት 4. በቀኚ ጎን በኩል የህምም ስሚት 5.1,2 6.1,3 7.1,4 8.2,3 9.2,4) 1. አ 2. **አለም**
2.20.	**የHIV መድሃኒት ወስድው ያውቃሉ?**	**1. አ 2. አለም** **መልሰ አ ከሆነ ለስንት ጊ ?.----------. የመዳሕኒቱ ዝርዝር(ስም)** ---

11.7. Annex VII: Laboratory protocol

Enzyme immunoassay for the detection of antibodies against Human Hepatitis Virus Type C (HCV)

PRINCIPLE OF THE ASSAY

The principle of EIA-Anti-HCVò assay is based on an indirect solid-phase enzyme immunoassay with horseradish peroxidase as the marker enzyme. This involves a two-stage reaction. In the first stage, HCV antibody present in the sample binds with HCV recombinant antigens {mixture of structural core-Ag and nonstructural NS3, NS4, NS5} coated on the wells. Unbound sample is removed by washing. In the second stage horseradish peroxidase {HRP}-labeled antibody conjugate {mixture of anti-human IgG and IgM}binds to any human Ig captured on the well in the first stage. Unbound conjugate is removed by washing. After that colourless enzyme substrate {H_2O_2} and chromogen{TMB} are added. The enzyme action of chromogen/substrate produces a coloured solution. Enzyme-chromogen/substrate reaction is terminated with acid {H2SO4}.The colour intensity is directly related to the concentration of HCV antibodies in a patientõs sample.

Materials and Reagents Required

Materials Provided With the Kits

1. HCV antigen coated strip (96- well)
2. Specimen Diluent: 12 ml chemically defined solution containing proteins, Tween 20, and sodium azide in phosphate buffer
3. Positive Control: 1.0ml
4. Negative Control: 1.0ml
5. Enzyme Conjugate: 12 ml Goat anti-human-IgG HRP Conjugate.
6. Substrate Solution A: 6 ml HRP Substrate.
7. Substrate Solution B: 6 ml TMB.
8. Concentration Washing Solution (20X): 40ml phosphate buffered saline solution with the buffer should be diluted 20 times with distilled water before use. Stop Solution: 6 ml 2N sulfuric Acid.

Materials Required but Not Provided

1. Precision pipettes: 0.02, 0.05, 0.10, 0.15, 0.20, and 1.0 ml.
2. Disposable pipette tips.
3. Distilled water.
4. Humidified Box capable of maintaining 37°C
5. Absorbent paper or paper towel.
6. Microtiter plate or strip-well washer
7. Microtiter plate reader.

Storage of Test Kit

Unopened test kits should be stored at 2-8°C upon receipt and the microtiter should be kept in a sealed bag to minimize exposure to damp air. Use up the reagents as soon as possible after the kit is unpacked.

Procedure Blood Sample Collection

1. Introduce yourself and identify the patient (explain the procedure)
2. Wash hands and wear gloves
3. Prepare the equipment (needles, tubes, etc.)
4. Prepare the patient
5. Apply tourniquet (do not leave it on for more than 1 minute)
6. Choose a vein
7. Disinfect the draw site
8. Obtain the required blood
9. Draw Vacutainer tubes in correct order and invert (mix) correctly according to manufacturer instructions
10. Exit the vein and apply pressure

11. Discard the needle (in appropriate biohazard container)

12. Label the specimen before leaving the patient

13. Check the patient and apply a bandage if necessary

14. Allow the specimen for 30 minute to facilitate clotting

15. Centrifuge with medium speed for 5 minute

16. Separate serum from the blood by Micro-pipette

17. Perform lab tests and store the remaining serum at 20^0C.

Procedures for Analysis

1. 100εl positive control and negative control duplicate into individual wells.
2. Dispense 30εl of specimen dilutes into individual test wells Dispense.
3. Add 70εl of each test sample into duplicate test wells.
4. Incubate for 60 minutes at 37°C+/-1
5. Wash each well 4 times each (400εl) by filling each well with diluted wash buffer.
6. Add 100εl of Enzyme Conjugate to each well. Mix it gently by swirling the micro titer plate on flat bench for 1 minute. Do not add Enzyme Conjugate to the blank well.
7. Incubate for 60 minutes at 37°C
8. Wash the plate 4 times like step 5.
9. Add 100εl of Substrate Solution A (HRP-substrate) to each then add 100εl of Substrate Solution B (TMB) to each well gently and incubate at 18-24^{o}c in the dark place for 20 minutes.
10. Add 150ε of Stopping reagent Solution to each well to stop the color reaction. Read O.D. at 450 nm with an EIA reader

Result Interpretation

EIA Reader at 450 nm/630 (using the OD value of the blank well to correct all the reading from all wells,For the assay to be valid, average OD values of the wells with negative control must be not greater than 0.2 and with positive control not less than 1.5. the sample was considered as positive if th OD value is equal or greater than the cut-off value.

Calculate cut-off value as:

Cut-off = average OD value of Negative Control + 0.180

Where 0.180 is a coefficient defined by during statistical processing for each lot.

It is recommended to retest reactive specimens in duplicate using the óEIA-ANTI-HCV assay before final confirmation of the result.

Interpretation:

Average of NC + 0.180 = Cut-off.

Positive OD reading: ≥ Cut-off value

Negative OD reading: < Cut-off value

11.8. Annex VIII: **Laboratory Result Collection Format**

Code No _____

Laboratory result for Anti-HCV Antibodies

Code No	Anti-HCV Antibodies		remark
	positive	Negative	
001.			
002.			
003.			
004.			
005.			
006.			
007.			
008.			
009.			
010.			
011.			
012			

Declaration

I the undersigned, declare that this is my original work and has not been presented for a degree in this or any other university and all sources of materials used for this thesis have been acknowledged.

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Nigus Fikre (BSc, MSc, Ass. Professor)

Signature_____ Date _____

Wondwesson Amogne (MD, infectious disease specialist)

Signature_____ Date_____