

**HEPATITIS C VIRUS AND HUMAN IMMUNODEFICIENCY VIRUS
(HIV) COINFECTION AMONG ATTENDANTS OF VOLUNTARY
COUNSELING AND TESTING CENTER AND HIV FOLLOW UP
CLINICS OF MEKELLE HOSPITAL, MEKELLE, NORTH ETHIOPIA**

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APRIL, 2011

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**A THESIS PRESENTED TO GRADUATE STUDIES PROGRAMME, ADDIS ABABA
UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE
DEGREE OF MASTERS OF SCIENCE IN MEDICAL MICROBIOLOGY**

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APRIL, 2011

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ACKNOWLEDGEMENTS

I would like to express my best appreciative thanks and sincere pleasure to my advisor Dr. Solomon Gebre-Selassie and Dr. Adane Mihret for their constructive comments, intellectual guidance and devotion of time in correcting this paper.

I would like to acknowledge a great extent for Addis Ababa University School of Graduate studies and to the Department of Microbiology, Immunology and Parasitology (DMIP) for financial assistance. Words are not enough to express my genuine sincere gratitude to Tigray regional health bureau, Mekelle Hospital and blood bank staff members for their help and advice. On top of that, I have a great desire to forward my countless gratefulness to Wollega University for covering my living expense.

Last but not the least grateful thanks goes to all the study participants who involved in this study voluntarily.

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LIST OF ABBREVIATIONS

Ab	Antibody
Ag	Antigen
AIDS	Acquired immunodeficiency syndrome
ALT	Alanine aminotransferase
CD	Cluster designation / differentiation
CDC	Center for Disease Control
CI	Confidence interval
CSA	Central Statistical Agency
DNA	Deoxyribonucleic acid
E	Envelope
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
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
HVR	Hyper variable region
IDU	Intravenous/Injection drug users
IFN	Interferon
Ig	Immunoglobulin
IL	Interleukin
NIH	National Institute of Health
NS	None Structural
OR	Odds ratio
PCR	Polymerase Chain Reaction
PEG	Polyethylene glycol

RIBA	Recombinant Immunoblot Assay
RNA	Ribonucleic acid
SPSS	Statistical Package for the Social Sciences
STD/STI	Sexually Transmitted Disease/Infection
Th	T helper
TMA	Transcription-mediated amplification
UNAIDS	United Nation program on HIV/AIDS
VCT	Voluntary counseling and testing
WHO	World Health Organization

ABSTRACT

Background: *Because of shared routes of transmission, hepatitis C virus (HCV) infection is common in Human Immunodeficiency virus (HIV)-infected persons. HIV and HCV coinfection is major global health concern. However, limited data of this coinfection are available in Ethiopia.*

Objective: *The objective of this study was to determine the magnitude of HIV/HCV coinfection rate and to assess if sociodemographic characteristics and potential risk factors are associated with HCV seropositivity in consecutive attendants of voluntary counseling and testing (VCT) center and HIV follow up clinics of Mekelle hospital.*

Methods: *A hospital based cross-sectional survey was carried out on VCT center and HIV follow up attendees from December 2010 to January 2011. An interviewer-administered questionnaire was used to collect data on demographic information and risk factors associated with HCV infection. The rapid immuno-chromatographic test was applied for detection of HCV antibodies.*

Results: *Out of a total of 300 consecutive attendants, 135 were VCT center clients and 165 were HIV follow up cases. There were more females 181 (60.3%) than males, 119 (39.7%). The overall anti-HCV prevalence was 6.0% (18/300, 95% CI= 3.6%-9.3%). There were no significant differences in HCV seroprevalence among the different categories of age and sex ($p > 0.05$). 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 seropositive ($p = 0.006$, OR= 6.28, 95% CI= 1.42-27.82). 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 ($p > 0.05$).*

Conclusion: *This study showed a significant percentage of HCV infection in HIV positive cases. Hence, with emphasis given to HIV positive cases screening for HCV infection has importance. Based on the result obtained, recommendations were forwarded to build up nationwide hospital and community-based surveys of HIV/HCV coinfection so that to decipher the prevalence with the possible risk factors and to increase public awareness about this dual disease.*

Keywords: *HCV; HIV; coinfection; prevalence; VCT center; HIV follow up clinic; Mekelle hospital*

1. INTRODUCTION

Hepatitis C virus (HCV) is a single-stranded RNA virus that belongs to the Flaviviridae family. Since first being cloned in 1989, HCV has been demonstrated to be the causative agent of most cases of non-A, non-B hepatitis worldwide (Alter *et al.*, 1989). Currently, there are about eleven (1-11) HCV genotypes, many subtypes, and about 100 different strains (Houghton, 1996; Simmonds *et al.*, 2005). Genotypes 1-3 have a worldwide distribution. Types 1a and 1b are the most common, accounting for about 60% of global infections (Sultan *et al.*, 2009).

The reservoir of HCV is man, but the virus has been transmitted experimentally to chimpanzees (Purcell, 1994). Risk factors associated with HCV infection include injection drug use, receipt of blood products, long term haemodialysis, organ transplantation, receipt of tattoo from an unsanitary facility, vertical transmission during pregnancy and sexual or nosocomial exposure (Schreiber *et al.*, 1996). HCV is one of the main causes of cirrhosis and hepatocellular carcinoma (HCC). Acute infection leads to chronic infection and most chronic infections will lead to hepatitis and to some degree of fibrosis, which may be accompanied by relatively nonspecific symptoms such as fatigue (EASL, 1999). It is a significant healthcare problem, affecting more than 170 million people worldwide and as many as four million new infections occur annually. Of those exposed to HCV, 80% become chronically infected, and at least 30% of carriers develop chronic liver disease, including cirrhosis and HCC (Sultan *et al.*, 2009; WHO, 1997).

Coinfection with HIV and HCV is common because of their similar routes of transmission and HCV infection increases the number of complications in persons who are coinfecting with HIV (Ellen, 2001). Both can be transmitted through exposure to contaminated blood, sexual intercourse and from mother to child. Hepatitis C virus is more transmissible through percutaneous blood exposure compared to HIV. In contrast HIV is more transmissible through sexual intercourse and from mother to child compared to HCV (Sulkowski and Thomas, 2003). The WHO (1997) estimates that 3% of the world's population is chronically infected with HCV. It is also found that a consistent evidence of high HCV prevalence in many countries of Africa. The overall prevalence in Sub-Saharan Africa is estimated 3% (Madhava *et al.*, 2002). Limited data are available in Ethiopia. The overall seroprevalence of HCV was reported 2.0% by Frommel *et al.* (1993); 5.8% by Diro *et al.* (2008) and 7.5% by Gebre (2005). HIV/HCV

coinfection rates of 4.5% and 11.6% was reported locally in inhabitants of Addis Ababa; and Tikur Anbessa VCT and HIV follow up clinics by Ayele *et al.* (2002) and Gebre (2005), respectively. In some of these studies HCV infection was significantly associated with presence of HIV (Gebre, 2005; Diro *et al.*, 2008).

Diagnostic tests for HCV infection are divided into serologic assays for antibodies and molecular tests for viral particles (Houghton, 1996). The treatment of choice for HCV infection is combination therapy because it gives a better treatment response than monotherapy. The highest response rates have been achieved with pegylated interferon (Peg-IFN) and ribavirin (Makris *et al.*, 2001). There is no vaccine against HCV (Purcell, 1994). In absence of a vaccine, all precautions to prevent infection of HCV should target reduction of transmission of the virus. Screening and treatment of blood products is the only way to prevent transfusion associated cases (van der Poel, 1999).

Generally, the HIV/HCV coinfection 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 anti-retroviral (HAART) related hepatotoxicity (Michael, 2000; Sulkowski, 2002). Despite these effects, HIV infected individuals in Ethiopia are taking anti-retroviral drugs. To ensure the optimal clinical managements of HIV patients it is important to know the HCV status of the cases. Therefore, the present study estimates the magnitude of HCV infection among HIV infected individuals in Mekelle hospital. In addition, it provides baseline data for further studies aimed to study more on the magnitude of coinfection and related risk factors.

2. LITERATURE REVIEW

2.1. Hepatitis C virus

2.1.1. Virion structure and properties

2.1.1.1. Morphology and physicochemical properties

Hepatitis C virus (HCV) morphology and physicochemical properties remain unclear because HCV usually circulates in a complexed form in association with immunoglobulins. Virions consist of an envelope and a nucleocapsid. Virus capsid is enveloped by a detergent sensitive lipoprotein. Virions are spherical and measure estimated to be 30-60 nm in diameter. Capsid/nucleocapsid is round and exhibits polyhedral symmetry (ICTVdB, 2006; Murray *et al.*, 2005). The core is isometric and has a diameter of about 30 nm. HCV particles have a buoyant density of 1.24 g/cm³ in Cesium chloride (CsCl), and a sedimentation coefficient of 200 S in sucrose gradients. The density of HCV in sucrose gradients has been measured between 1.08 and 1.11 g/ml. A lighter fraction of 1.04 to 1.06 g/ml appears to be due to the association of HCV with serum beta-lipoprotein. A denser fraction of about 1.17 g/ml in sucrose appears to correspond with noninfectious immune complexes of virus and antibody. The nucleocapsid of the virus was found to have a density in sucrose of 1.25 g/cm³ (Houghton, 1996; ICTVdB, 2006).

2.1.1.2. Genome and proteins

HCV contains a non segmented, single-stranded, positive-sense RNA molecule of 9.6 kb with one long open reading frame coding for a large polyprotein of about 3000 amino acids which undergoes co- and post translational cleavage by host and viral proteases to yield individual viral proteins (Houghton, 1996). The HCV genome has been cloned in 1989 (Choo *et al.*, 1989). The complete genome is 9600 nucleotides long, is fully sequenced. The 5'-end of the genome does not have cap. The 3'-terminus has a long non-coding region. The genome has an intergenic poly(A) region (ICTVdB, 2006; Purcell, 1994). By itself, genomic nucleic acid is infectious. The genome replicates in the cytoplasm. Very little is known about the replication (life) cycle of HCV, because there is no in vitro cell culture system that is permissive for virus replication (ICTVdB, 2006; Murray *et al.*, 2005; Sultan *et al.*, 2009). However, progress has been made. HCV probably follows the replication strategy of other positive-strand RNA viruses. The virus

enters the cell and is uncoated in the cytoplasm. The viral genome is transcribed to form a complementary negative-sense RNA molecule, which, in turn, serves as a template for the synthesis of progeny positive-strand RNA molecules. The newly translated polyprotein is cleaved by a host-cell signalase as well as virus-specific non-structural proteins, NS-2 and NS-3. The enzyme capable of performing both steps of RNA synthesis is the virally encoded RNA-dependent RNA polymerase NS5b. The NS-3 of HCV also has helicase (unwindase) activity. HCV replicates by a negative-strand RNA intermediate and has no reverse transcriptase activity (Houghton, 1996; Purcell, 1994).

The genome encodes structural proteins (3 structural protein/s) and non-structural proteins (putative proteins are encoded at the 5' end). The N-terminal quarter of the genome encodes the core and structural proteins (Purcell, 1994). The glycosylated E1 and E2 molecules are anchored inside the lumen of the endoplasmic reticulum (ER). The C protein remains on the cytosol side. The rest of the genome encodes the nonstructural proteins NS2-NS5. The NS2 (250 amino acids), NS3 (500 amino acids), and NS4a proteins interact to mediate the processing of the presumed NS region of the polyprotein. NS3 (500 amino acids) is both a proteolytic cleavage enzyme and a helicase, to facilitate unwinding of the viral genome for replication. NS5b is the RNA-dependent RNA polymerase needed for viral replication. NS proteins have been localized to the membrane of the ER, suggesting that it is the site of polyprotein maturation and viral particle assembly. Little is known about the three dimensional structure of the HCV proteins. Lipids are present (but not demonstrated directly) and are located in the envelope (on the basis of solvent sensitivity) (Houghton, 1996; Murray *et al.*, 2005).

Currently, there are one through eleven (1-11) HCV genotypes, many subtypes (designated a, b, c, etc.), and about 100 different strains (numbered 1, 2, 3, etc.) based on the genomic sequence heterogeneity (Houghton, 1996; Simmonds *et al.*, 2005). Within a Hepatitis C subtype, individual viruses differ from each other ever so slightly. Such viral differences are not significant enough to form another subtype but instead form what's known as quasi-species. It is believed that within an HCV subtype, several million quasispecies may exist. The variability is distributed throughout the genome. However, the non-coding regions at either end of the genome (5'-UTR and 3'-UTR; UTR-untranslated region) are more conserved and suitable for virus detection by PCR. 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 (Houghton, 1996; Sultan *et al.*, 2009; Purcell, 1994).

Genotypes 1-3 have a worldwide distribution. Types 1a and 1b are the most common, accounting for about 60% of global infections. They predominate in Northern Europe and North America, and in Southern and Eastern Europe and Japan, respectively. Type 2 is less frequently represented than type 1. Type 3 is endemic in south-east Asia and is variably distributed in different countries. Genotype 4 is principally found in the Middle East, Egypt, and central Africa. Type 5 is almost exclusively found in South Africa and genotypes 6-11 are distributed in Asia (Sultan *et al.*, 2009). In Ethiopia the prevalence of different genotypes of hepatitis C virus (HCV) is not known. However, one local study aimed to determine the genotypes and viral load of HCV among attendees of voluntary counseling and testing center revealed a diverse HCV genotypes including genotypes 1, 2, 4, and 5 (Abreha *et al.*, 2011).

2.1.2. Immunity and Immunopathogenesis

The first line of defense against any viral agent is antigen nonspecific, and the earliest response is probably elicited by virus infected cells (Murray *et al.*, 2005). HCV enters the body either directly, through transfusion of contaminated blood products or injection with contaminated needles, or, less efficiently, by crossing over an epithelial barrier, as exemplified by perinatal or sexual transmission (Murray *et al.*, 2005; Pawlotsky, 2004). HCV specific antigens and both negative and positive strand HCV RNA have been identified in hepatocytes indicating that this cell types are the preferred site of replication after HCV reaches the liver via hepatic artery or the portal vein (Asabe, 1997; Pawlotsky, 2004). However, additional data suggested that the virus may also replicate within peripheral mononuclear cells of lymphoid or perhaps bone marrow origin (possibly B lymphocytes) (Durand *et al.*, 2010; Houghton, 1996). The outcome of HCV infection is determined by the interaction between the virus and the host immune system. The immune response to HCV is polyclonal and multispecific, both in terms of antibody and cellular immune responses. Genetic heterogeneity of HCV due to mutations as a result of absence of proofreading by NS5B RNA polymerase and immunogenetic features of the host determine heterogeneity of immune response to the virus and differences in the course of the disease and outcomes (Pawlotsky, 2004).

As for the adaptive immune response, humoral immunity may be largely ineffective despite evidence for neutralizing antibody response directed to the E2 HVR region, perhaps due to rapid selection of antibody escape variants. Viral RNAs containing spontaneous mutation within the hyper variable region-1 (HVR-1) segment of the E2 protein may be favored for survival in the host because they reduce the binding of preexisting neutralizing antibodies to the viral envelope. The persistence of infection in most HCV-infected individuals, despite the presence of HCV-directed antibodies, suggests that such antibodies fail to induce viral clearance (Houghton, 1996).

Cellular immune response does seem to play a role in the virologic outcome during acute infection based on strong association of a sustained vigorous and multispecific antiviral CD4 and CD8 T cell response with HCV clearance during acute infection. Following clearance, vigorous CD4 T cell response to HCV is maintained for many years, whereas the memory CD8 T cell response may be maintained with variable efficiency. Spontaneous elimination of HCV-infection in acute phase occurs due to these vigorous and sustained multispecific Th1-response to viral antigens. During such response proliferation of virus-specific CD4⁺ T-cells and secretion of IFN-gamma by them are observed, otherwise chronic hepatitis develops (Koziel, 1997; Pawlotsky, 2004). Cytokines are produced both locally within the liver and systemically and may play an important role in controlling viral replication and contributing to hepatocellular damage through amplification of a nonspecific immune response. In most patients, the humoral, cellular immune, and cytokine response seem insufficient to eradicate infection. In its attempt to clear the virus from the liver, the immune system contributes to the hepatocellular injury seen in most chronically infected patients (Mizukoshi and Rehermann, 2001).

2.1.3. Clinical outcome

The reservoir of HCV is man, but the virus has been transmitted experimentally to chimpanzees (Purcell, 1994). Acute HCV infection is uncommonly recognized, because it is usually accompanied by mild flulike symptoms. Clinical manifestations can occur, usually within 2-26 weeks after exposure to HCV, but the majority of persons have either no symptoms or only mild symptoms (Conry-Cantilena *et al.*, 1996). Hence, infection is infrequently diagnosed during the acute phase of infection. Acute infection leads to chronic infection in the majority of persons and spontaneous clearance of viremia once chronic infection has been established is rare. Most

chronic infections will lead to hepatitis and to some degree of fibrosis, which may be accompanied by relatively nonspecific symptoms such as fatigue. Severe complications and death usually occur only in persons with cirrhosis, which is estimated to develop in 15-20 percent of those infected (EASL, 1999). The time frame in which the various stages of liver disease develop is highly variable. Factors that accelerate clinical progression include alcohol intake, which has a pronounced effect on the course of the disease; coinfection with HIV-1 or HBV; and an older age at infection (Poynard *et al.*, 1997). Once cirrhosis is established, the risk of hepatocellular carcinoma is approximately 1-4 percent per year. Hepatocellular carcinoma can occur without cirrhosis but is rare (Colombo *et al.*, 1991).

In addition to hepatic disease, there are important extrahepatic manifestations of HCV infection. Most of these syndromes are associated with autoimmune or lymphoproliferative states and may be related to the possibility that HCV is able to replicate in lymphoid cells (Okuda *et al.*, 1999). Common extrahepatic manifestations include mixed cryoglobulinemia and porphyria cutanea tarda (Agnello *et al.*, 1992). Membranoproliferative glomerulonephritis, leukocytoclastic vasculitis, focal lymphocytic sialadenitis, lichen planus, sicca syndrome and idiopathic pulmonary fibrosis have also been linked to HCV infection and may occur in rare cases. They are believed to be secondary to immune complex deposition in association with intact virus or viral proteins. However, a clear pathophysiological role of HCV has been difficult to establish (Fargion *et al.*, 1992; Horcajada *et al.*, 1999).

Other clinically important syndromes include coinfections with other viruses, especially HIV-1 and other hepatitis viruses (HAV and HBV).

2.1.4. Epidemiology of HCV

Hepatitis C virus is a major cause of chronic liver disease in the world. HCV infection occurs among persons of all ages with the highest incidence of acute hepatitis C occurring highest among groups with specific risk factors (Ellen, 2001). The WHO (1997) estimates that approximately 3% of the world's population is to be infected with HCV and viremia persists (chronic infection) in over 80%. In developing countries, the prevalence of antibodies to HCV is much higher, with reported rates reaching 4% to 6% in some parts of Africa and the Middle East

(Darwish *et al.*, 1993). It is found that a consistent evidence of high HCV prevalence in many countries of Africa. It is estimated the overall prevalence of HCV in Sub-Saharan Africa is 3·0%. The central African region has the highest estimated prevalence of 6%, West Africa with an estimated prevalence of 2·4%, and southern and east Africa with the lowest estimated prevalence of 1·6% (Madhava *et al.*, 2002).

Risk factors associated with HCV infection include injection drug use, receipt of blood products, long term haemodialysis, organ transplantation, receipt of tattoo from an unsanitary facility, vertical transmission during pregnancy and sexual or nosocomial exposure (Schreiber *et al.*, 1996). Some studies revealed the traditional practices that involve the puncture of the skin, which have been associated with HCV transmission elsewhere (Honda *et al.*, 1993), are also likely to contribute to HCV transmission in Africa. Since the introduction of viral inactivation methods and donor screening, transmission of HCV through blood or blood product transfusion has become rare in the developed world; however, incidence rates still remain high in some developing countries. Cross-sectional studies of drug user populations have reported prevalence rates of 42% to 92% (Alter *et al.*, 1990; Thomas *et al.*, 1995); these cases account for 40% to 50% of all cases of chronic HCV (Sharara *et al.*, 1996).

Seroprevalence rates of HCV antibody have been reported to be higher in individuals with more than two sexual partners and in sex workers compared with other members of the general population, suggesting that sexual transmission may be possible (Sulkowski and Thomas, 2003). The detection of HCV in saliva, menstrual blood (Silverman *et al.*, 1994), semen (Alter *et al.*, 1990), urine (Numata *et al.*, 1993), and tears (Feucht *et al.*, 1995) provides further evidence that transmission by these means may be possible. Nosocomial transmission has been documented, such as from patient to patient by a colonoscope, during dialysis, and during surgery. Needle stick injuries in the health care setting continue to result in nosocomial transmission of the virus (Esteban *et al.*, 1996). In Ethiopia, especially in small clinics, there is a shortage of syringes and needles and they have to be reused many times often with inadequate sterilization. Therefore, these syringes and needles may be contaminated, thus being a risk factor for HCV and HIV infection (Frommel *et al.*, 1993). Abdulebar *et al.* (2007) reported a gross lack of knowledge about the transmission of HBV and HCV by considerable proportion of health care workers.

Moreover, other local studies indicate 87.8% unsafe injection practices (Damete, 2006) and a 29.1% prevalence of needle stick injury (Reda *et al.*, 2009).

Limited data are available in Ethiopia. The overall seroprevalence of HCV in 1,580 Ethiopian subjects representing urban and rural populations was reported to be 2.0% (Frommel *et al.*, 1993). Most of the studies revealed a low overall prevalence of HCV infection. These include: 0.3% in Health professionals (Yimer, 2005); 0.7% in 6361 consecutive blood donors (Tessema *et al.*, 2000); 0.9% in inhabitants of Addis Abeba (Ayele *et al.*, 2002); 1.3% in pregnant women attending antenatal clinic (Tiruneh, 2008); 1.7% in Tigray and Amahra regions (Gelaw and Mengistu, 2008). Study among blood donors from neighboring Kenya also revealed a smaller HCV rate of 0.1% (Okoth, 1996). Other local studies showed a relatively higher overall prevalence of HCV infection. These include: 5.8% in blood donors (Diro *et al.*, 2008); 7.5% in VCT and HIV follow up clinic (Gebre, 2005); 13.3% in blood donors (Dessie *et al.*, 2007).

2.1.5. Diagnostic tests

Clinical manifestations HCV infection can occur, usually within 2 to 26 weeks after exposure to HCV, but the majority of persons have either no symptoms or only mild symptoms (Conry-Cantilena *et al.*, 1996). No cell culture system has yet been developed that permits substantial HCV replication. The chimpanzee, *Pan troglodytes*, is the only non-human animal species that has been demonstrated conclusively to be permissive for HCV replication. Percutaneous inoculation of HCV-RNA-positive plasma, and in one instance saliva, has resulted in HCV infection (Basset, 1998). Diagnostic tests for HCV infection are generally divided into serologic assays for antibodies and molecular tests for viral particles (Murray *et al.*, 2005).

The primary serologic screening assay for HCV infection is the enzyme immunoassay (EIA) which detects anti-HCV. Anti-HCV is generally not detectable in patients with initial signs or symptoms of hepatitis C. Tests are not yet available to distinguish acute from chronic HCV infection; therefore, anti-HCV IgM cannot be used as a reliable marker of acute HCV infection (Pawlotsky, 2004). It can be falsely positive, especially in persons without risk factors and without signs of liver disease and therefore other tests must be used to confirm infection in these persons. Furthermore, false negative tests can occur in persons with immunocompromise, such as

HIV-1 infection (Cribier *et al.*, 1995); patients with renal failure; and those with HCV-associated essential mixed cryoglobulinemia (Agnello *et al.*, 1992). Some findings in Africa showed that high false positive rates of rapid test results by taking into account ELISA as a standard test (Duru *et al.*, 2009; Walusansa and Kagimu, 2009). But these findings disagreed with a report revealed 0.0% of sensitivity by considering ELISA as a standard test. In this case this screening assay would, therefore, have missed any infected subjects among the samples tested (Torane and Shastri, 2008). Although immunochromatographic (rapid test kits) tests are easy to perform and their manufacturers strongly recommend their use, they may give false reactive results due to their general biological and biochemical characteristics (Duru *et al.*, 2009; Torane and Shastri, 2008). In another study the HCV Ag/Ab assay did not detect HCV infection as early as the HCV RNA assay or HCV Ag assay (Laperche *et al.*, 2005). An important nonspecific laboratory test in HCV infected persons is measurement of the alanine aminotransferase (ALT) level, readily available means of identifying hepatic disease. Children should not be tested for anti-HCV before 12 months of age as anti-HCV from the mother may last until this age. Diagnosis relies on determination of ALT levels and presence of HCV RNA in baby blood after the second month of life (Ruiz-Moreno *et al.*, 1999).

The recombinant immunoblot assay (RIBA) has been used to confirm positive enzyme immunoassays. It uses antigens similar to those for the enzyme immunoassay but in an immunoblot format, so that responses to the individual proteins can be identified. A positive assay is defined by the detection of antibodies against two or more antigens, and an indeterminate assay by the detection of antibodies against a single antigen. The use of a recombinant immunoblot assay to confirm results is recommended only in low-risk settings such as blood banks (EASL, 1999). However, with the availability of improved enzyme immunoassays and better RNA-detection assays, confirmation by recombinant immunoblot assay may become less necessary (Pawlotsky, 2004). An HCV core-antigen EIA test detect for HCV core-antigen has also been established and appears to be suitable for large scale screening of blood donations, whilst its use in clinical monitoring remains to be determined (Muerhoff *et al.*, 2002).

The molecular assays are used to detect HCV RNA. Transcription-mediated amplification (TMA) technique has been developed as qualitative tests for detecting HCV RNA. Both target amplification (PCR) and branched DNA signal amplification techniques may also be used to measure HCV RNA levels (NIH, 2002). Since viral RNA is unstable, the appropriate processing of samples is critical to minimize the risk of false negative results (Walker, 1999). Viral genotyping helps predict the outcome of therapy and influences the choice of the therapeutic regimen. Different methods are available for the genotyping of HCV, most of which are based on amplification with the PCR assay. Only patients with detectable HCV RNA should be considered for pegylated interferon alfa and ribavirin therapy and the HCV genotype should be systematically determined before treatment, as it determines the indication, the duration of treatment, the dose of ribavirin and the virological monitoring procedure (Chevaliez and Pawlotsky, 2006). Histologic evaluation of a liver biopsy specimen remains the gold standard for determining the activity of HCV-related liver disease, and histologic staging remains the only reliable predictor of prognosis and the likelihood of disease progression (Yano *et al.*, 1996). A biopsy may also help to rule out other, concurrent causes of liver disease. Therefore, biopsy is generally recommended for the initial assessment of persons with chronic HCV infection. However, a liver biopsy is not considered mandatory before the initiation of treatment, and some recommend a biopsy only if treatment does not result in sustained remission (EASL, 1999).

2.1.6. Treatment

The treatment of choice for HCV is combination therapy with pegylated interferon (Peg-IFN) and ribavirin (Makris *et al.*, 2001; NIH, 2002). Pegylated-IFN is a formulation of alpha-interferon complexed with polyethylene glycol (Peg). The formulation enables sustainment of therapeutic interferon levels. At present there are two preparations: peg-alpha-2b interferon (Pegasys; Roche Products Ltd., Welwyn Garden City, UK) and peg-alpha-2a interferon (Peg-Intron; Schering-Plough Ltd., Welwyn Garden City, UK). Ribavirin is an analogue of the DNA base guanosine (Soriano *et al.*, 2004). Besides eradicating HCV, combination therapy reduces the degree of hepatic fibrosis (Manns *et al.*, 2001). During initiation of treatment, only patients with detectable HCV RNA should be considered for pegylated IFN alfa and ribavirin combination therapy (NIH, 2002). The HCV genotype should be systematically determined

before treatment, as it determines the indication, the duration of treatment, the dose of ribavirin and the virological monitoring procedure (Hadziyannis *et al.*, 2004).

2.1.7. Prevention

There is no vaccine against HCV. Research is in progress but the high mutability of the HCV genome complicates vaccine development (Purcell, 1994). In absence of a vaccine, all precautions to prevent infection of HCV should target reduction of transmission of the virus. The only means of protection are the implementation universal precautions and safe injection practices. Screening and treatment of blood products is the only way to prevent transfusion associated cases (van der Poel, 1999). HCV carriers should be strongly discouraged from drinking alcohol because there is evidence that acts as a cofactor in developing more severe liver injury (Houghton, 1996). Patients who do not have serologic evidence of immunity to hepatitis A and B should be vaccinated, especially since infection with the hepatitis A virus (HAV) in patients with chronic HCV may result in a more severe infection than in patients without HCV(Backmund *et al.*, 2005).

2.2. Human Immunodeficiency Virus

Human immunodeficiency virus (HIV) is a lentivirus (a member of the retrovirus family) that causes acquired immunodeficiency syndrome (AIDS). There are two types of HIV namely, HIV-1 and HIV-2. Both types are transmitted by sexual contact, through blood and from mother to child, and they appear to cause clinically indistinguishable AIDS. However, HIV-2 is less easily transmitted, and the period between initial infection and illness is longer in the case of HIV-2. Worldwide, the predominant virus is HIV-1. The relatively uncommon HIV-2 type is concentrated in West Africa and is rarely found elsewhere. The major routes of transmission are unsafe sex, contaminated needles, breast milk and transmission from an infected mother to her baby at birth (perinatal transmission) (Cunningham *et al.*, 2010; Murray *et al.*, 2005). HIV infects primarily vital cells in the human immune system such as helper T cells (specifically CD4⁺ T cells), macrophages and dendritic cells. When CD4⁺ T cell numbers decline below a critical level, cell-mediated immunity is lost and the body becomes progressively more susceptible to opportunistic infections (Cunningham *et al.*, 2010).

Initial tests for HIV are usually conducted using the EIA (or ELISA) antibody test or a Rapid antibody test. Rapid tests, which can produce a result in less than an hour, are becoming increasingly popular. Treatment with anti-retrovirals increases the life expectancy of people infected with HIV. The aim of antiretroviral treatment is to keep the amount of HIV in the body at a low level. It reduces both the mortality and the morbidity of HIV infection. Due to the significant increase in people receiving antiretroviral therapy, the number of AIDS-related deaths has also declined. Intensified awareness and preventive measures have also played a role. An HIV and AIDS vaccine does not yet exist, but efforts to develop a vaccine against HIV have been underway for many years (Murray *et al.*, 2005; UNAIDS, 2010).

The number of people living with HIV rose from around 8 million in 1990 to 33 million by the end of 2009. Since the beginning of the epidemic, nearly 30 million people have died from AIDS-related causes. During 2009, an estimated 1.3 million Africans died from AIDS. With around 68% of all people living with HIV residing in sub-Saharan Africa, the region carries the greatest burden of the epidemic. Hence, an estimated 22.5 million people were living with HIV in sub-Saharan Africa at the end of 2009, including 2.3 million children. In Ethiopia, more than 1.1 million people are living with HIV/AIDS and at the end of 2009 there were a total of 241,236 people ever started ART and 176,644 currently on ART (UNAIDS, 2010).

2.3. Coinfection of HIV and HCV

Coinfection with HIV and HCV is common and HCV infection increases the number of complications in persons who are coinfecting with HIV (Ellen, 2001). HIV and HCV share routes of transmission. They both can be transmitted through exposure to contaminated blood, sexual intercourse and from mother to child. HCV is more transmissible through percutaneous blood exposure compared to HIV (Sulkowski and Thomas, 2003). Although both HIV and HCV are efficiently transmitted via percutaneous exposure, HCV is about 10-fold more easily transmitted via small-volume percutaneous exposure. In contrast HIV is more transmissible through sexual intercourse and from mother to child compared to HCV (Amin *et al.*, 2004; Ruan *et al.*, 2004).

A number of studies have suggested that the presence of HIV infection accelerates the course of HCV-related liver disease in HCV/HIV coinfecting patients (Roe and Hall, 2008). The decline in

cell mediated immunity associated with progressive HIV infection is believed to permit greater HCV replication and consequently, greater infection and injury to hepatocytes. HIV infection might exert a direct cytopathic effect on liver cells (Houghton, 1996). Although, pathways of viral entry differ, both viruses can be targeted to the same host cells via binding to shared surface molecules and the envelope proteins of either virus cooperatively induce hepatocyte apoptosis via an ‘‘innocent bystander ‘‘ mechanisms. Moreover, HIV infection seems to facilitate HCV infection of extra hepatic cells (Houghton, 1996; Roe and Hall, 2008). Current data show that HIV infection adversely affects the natural history of HCV infection in several ways. First, spontaneous recovery from HCV infection occurs in only 5%-10% of persons coinfecting with HIV and HCV, compared with 15%-35% of those monoinfected with HCV (Roe and Hall, 2008; Thomas *et al.*, 2000). Second, coinfection with HIV and HCV is associated with more-rapid progression of cirrhosis, liver failure, and hepatocellular carcinoma (Soto *et al.*, 1997). A case control study of patients coinfecting with HIV and HCV via injection drug use revealed an increase in liver fibrosis, compared with matched HCV-infected patients without HIV infection (Soto *et al.*, 1997).

Whether or not HCV infection accelerates the progression of HIV infection to AIDS and death remains unclear and the mechanism has not been clearly elucidated. There are conflicting reports regarding the effect of HCV infection on the natural history of HIV disease. Reasons for the adverse effect of HCV on HIV disease are visibly unknown. Possible mechanisms that have been suggested include HCV-mediated direct impairment of CD4 cell production or sensitization of CD4 cells to apoptosis (Xiang *et al.*, 2001). There are also a number of other possible mechanisms by which coinfection with HIV and HCV could lead to a more rapid progression of HIV disease. It is known that HCV induces CD4 cell proliferation in hepatic tissue. Thus, this associated increase in CD4 cell proliferation may support increased HIV replication, especially in individuals with high HCV RNA levels. It is also possible that a direct interaction exists between HCV and HIV through interference in cell cytokine production. Such effects may be expected to lead to increased levels of both viruses (Minutello *et al.*, 1993). HCV may also negatively influence HIV disease more importantly from the perspective of HIV treatment finding that indicates coinfection with HCV increases the risk of antiretroviral-associated hepatotoxicity, requiring drug discontinuation (Sulkowski, 2002). Conversely, Rancinan *et al.*

(2002) did not detect evidence that HCV infection substantially alters the risk of dying, developing AIDS, or responding immunologically to HAART.

The exact way in which HIV accelerates chronic HCV liver disease has not been elucidated. As HIV itself does not appear to cause hepatitis directly the effect is presumably mediated via suppression of the immune response against HCV (Puoti *et al.*, 2001; Soto *et al.*, 1997). Coinfection with HIV also probably alters the response of immune cells to HCV; when CD3+/CD30+ cells are infected with both HIV and HCV, their cytokine production is skewed toward an anti-inflammatory Th2 response rather than the protective Th1 response seen when cells are infected with HCV alone. HCV liver disease has become a major cause of death in HIV infected patients stabilized on HAART (Soriano *et al.*, 2004). Hepatocellular carcinoma is a well-recognized complication of chronic HCV infection and appears to develop after a shorter duration of infection in HIV-infected patients. Because of the blood-borne nature of HCV, many individuals at risk for HIV are also at high risk for HCV infection (Garcia-Samaniego *et al.*, 2001).

Limited data on HIV/HCV coinfection are available in Ethiopia. A coinfection rate of 11.6% was reported in Tikur Anbessa VCT center and HIV follow up clinics (Gebre, 2005). Tessema *et al.* (2000) and Ayele *et al.* (2002) reported a higher HCV prevalence among HIV positives compared to HIV-negative individuals and statistically significant association was observed between HCV and HIV infections. In addition, higher prevalence of HCV antibodies among HIV positive (4.5%) compared to HIV-negative (0.8%) individuals was reported by Ayele *et al.* (2002). Likewise, lower HIV/HCV coinfection rates of 0.6%, 1.86% and 3.3% in previous studies in Africa region in different study populations were reported respectively for Gambia (Mbotto *et al.*, 2009), Nigeria (Onakewhor and Okonofua, 2009) and Uganda (Walusansa and Kagimu, 2009). Diro *et al.* (2008) indicated HCV infection was significantly associated with presence of HIV. Similarly, the HCV infection was reported as significantly higher in HIV infected individuals (11.6%) when it was compared to HIV-negative ones in VCT and HIV follow up clinics (2.6%) (Gebre, 2005). In contrast, no statistically significant difference in HCV seroprevalence rates between HIV positive and HIV-negative subjects was reported by Dessie *et al.* (2007), Duru *et al.* (2009), Lassef *et al.* (2004), and Olatunji and Iseniyi (2008).

Coinfection rate studies in VCT and HIV follow up clinics in Greece (Dimitrakopoulos, 2000), Nigeria (Agwala *et al.*, 2004) and Cameroon (kim *et al.*, 2007) revealed 7.5%, 8.2%, 8.6%, respectively. HCV study in Kenya with a larger sample size of 458 HIV positive patients showed a prevalence of 3.7% (Karuru *et al.*, 2005). However, a much higher hospital based coinfection rate has been reported in industrialized, where 30-50% in US and Europe (Veruchi *et al.*, 2004) and 30-50 % in Italy (Canta, 2004). The presence of high-risk groups such as IDU, haemodialysis and repeated transfusion in these countries could be a speculation for the observed high HIV/HCV coinfection rates. The overall prevalence of HCV infection among VCT center attendants, without HIV follow up cases, finding in various populations of Africa was reported to be 0.2-40% (Cheesbrough, 2000). This is consistent with the population based studies reported in neighboring countries like 0.7% in Kenya (Hyams *et al.*, 1993), 0.6% in Somali (Nur, 2000), and 1.4% in Eritrea (Gebrekidan, 1998).

There is no specific treatment for HIV/HCV co-infection. There are only separate treatments for HIV infection and HCV infection. In HIV-negative patients Peg-IFN/ribavirin combination therapy can achieve HCV eradication rates of just over 40% for HCV genotype 1 and up to 80% for genotypes 2 and 3 (Manns *et al.*, 2001). The standard HCV therapy in HIV-HCV coinfecting individuals is with pegylated interferon (PEG-IFN) (alpha-2a or alpha-2b) plus ribavirin (Soriano *et al.*, 2004). Coinfecting individuals with progressive HIV disease and low CD4 counts should have HAART commenced as a priority. Patients with advanced HIV infection and hepatic decomposition should not be considered for HCV treatment. Individuals with stable HIV infection and well preserved CD4 counts not on HAART should be strongly advised to consider HCV treatment (Makris *et al.*, 2001). Most clinicians would advise treating HIV disease first if the CD4 count is below 250 cells/mm³. However for most patients with good CD4 counts it is preferable to treat HCV first to avoid issues of drug interactions and HAART-hepatotoxicity (Matthews and Gregory, 2008). HIV-HCV coinfecting individuals require careful monitoring during antiviral therapy because drug interactions are a particular concern in HIV-HCV coinfecting individuals on antiretroviral therapy (Fleischer *et al.*, 2004).

3. SIGNIFICANCE OF THE STUDY

The HIV/HCV coinfection status of HIV infected Ethiopians has not been well Documented. More than 1.1 million people are living with HIV/AIDS in Ethiopia. At the end of 2009 there were a total of 241,236 people ever started ART and 176,644 currently on ART (UNAIDS, 2010). By now many studies indicate hepatitis C virus (HCV) as an opportunistic infection in HIV infected persons and known to be a risk factor for anti-retroviral (HAART) related hepatotoxicity. Despite these effects, HIV infected individuals in Ethiopia are taking anti-retroviral drugs. To ensure the optimal clinical managements of HIV patients it is important to know the HCV status of the cases. However, only a very few studies are conducted on HIV/HCV coinfection. Therefore, the findings of this study will provide baseline information on magnitude of HIV/HCV coinfection in Mekelle hospital VCT center and ART clinics.

4. OBJECTIVES OF THE STUDY

4.1. General objective

- To determine the magnitude of HIV/HCV coinfection rate and to assess sociodemographic characteristics and potential risk factors are associated with HCV seropositivity among consecutive attendants of voluntary counseling and testing (VCT) center and HIV follow up clinics of Mekelle hospital, Mekelle

4.2. Specific objectives

- To determine the magnitude of HIV/HCV coinfection among attendants of VCT center and HIV follow up clinics at Mekelle Hospital
- To identify whether there is an association between HIV and HCV among the study population
- To assess various risk factors for HCV infection

5. MATERIALS AND METHODS

5.1. Study area

The study was carried out at Mekelle Hospital, Mekelle. It is the largest hospital in the region serving as a referral hospital for more than 4.1 million people of the region and thousands of patients coming from Afar and Amhara regions. It is visited by 15-50 individuals daily for HIV screening and HIV follow-up. Mekelle is located in North Ethiopia at a distance of 783 kilometers from Addis Ababa at latitude and longitude 13°29'N and 39°28'E respectively, with an elevation of 2084 meters above sea level. Based on the figures from the Central Statistical Agency (CSA), Mekelle has an estimated total population of 215,914, of whom 104,925 are men and 110,989 are women. The woreda has an estimated area of 24.44 square kilometers, which gives Mekelle a density of 8,834.45 people per square kilometer. Mekelle is the largest city in Northern Ethiopia and sixth largest in Ethiopia (CSA, 2007).

5.2. Study period

The study was conducted from December 2010 to January 2011.

5.3. Study design

A hospital based cross-sectional study was carried out among consecutive attendants of VCT center and HIV follow up clinics, at Mekelle Hospital.

5.4. Study population

The study population was all individuals voluntarily coming to VCT center for HIV testing and those to ART clinic for immunological and chemistry tests during the study period.

5.5. Sample size and sampling technique

Taking into account the current high costs of screening test kits, the sample size was calculated using a double proportion formula by considering the prevalence of hepatitis C virus in HIV infected (p1) and HIV non-infected individuals (p2), 11.6% Vs 2.6%, respectively from previous study by Gebre (2005). Thus, it was calculated to be 124 HIV infected persons and 124 HIV negative individuals. However, a total of 300 persons could be included in the study using a

convenient sampling method. The number of samples obtained was beyond the minimum representative sample size (i.e., 248) proposed for the study.

$$n1 = n2 = \frac{\left(z_{\alpha/2} \sqrt{\left(1 + \frac{1}{r}\right) \bar{p}\bar{q}} + Z_{\beta} \sqrt{p1q1 + \frac{p2q2}{r}} \right)^2}{(p1 - p2)^2}$$

Where:

The difference in this prevalence is 9%, level of significance $\alpha=0.05$, power $1-\beta = 80\%$, $\beta = 0.2$

$\bar{p} = \frac{p1+p2}{2}$ = average proportion, $\bar{q} = 1 - \bar{p}$

r= ratio of HIV infected to controls= $n1/n2=1$ for equal sample size

p1= prevalence of HCV among HIV infected patients

p2= prevalence of HCV among the control population (HIV non-infected individuals)

p1-p2= effect size

$Z_{\alpha/2}$ = the z-score corresponding to the probability with which it is desirable to be able to conclude that an observed difference of size (p1-p2) of variables between HIV infected and non infected population will not occur by chance =1.96

Z_{β} = the score corresponding to the degree of confidence with which it is desired to certain of detecting a difference size (p1-p2) between variables of that actively present = 0.84

5.6. Data collection

Sera or plasma or whole blood of the consecutive attendants of VCT center and HIV follow up clinics at Mekelle Hospital were screened for anti-HCV. At the time of blood collection, a study format (Annex I) which focused on demographic information and risk factors associated with hepatitis C virus infection was collected using an interviewer-administered structured questionnaire after informed consent was obtained. The exclusion criterion was individuals with hemolyzed specimens. Applying these criteria, 300 attendants were included in the study.

5.7. Laboratory Diagnosis

5.7.1. Collection, handling, and transport of specimen

The blood sample from finger prick of clients visiting VCT center voluntarily and HIV positive samples (5ml) collected for other clinical investigation from HIV follow up patients were screened for HCV by anti-HCV Rapid Test kits. Blood sample (5ml) was taken from HCV reactive VCT center attendants. Clear non-hemolyzed specimens were used. The reactive samples (sera or plasma) were kept frozen at -20°C in Mekelle Hospital laboratory and were transported/shifted in dry ice to Addis Ababa for the accessibility of ELISA assay. Due to cost limitations, seven of them were retested using fourth generation Enzyme-Linked Immunosorbent assay (ELISA) to assess ELISA test results. All the necessary precautions were taken in handling the samples, as these are capable of transmitting etiological agents. Specimen containers were coded to insure confidentiality.

5.7.2. Anti-HCV detection test

Different researchers have employed different immunoassay procedures for detection of HCV antibodies. The diagnosis and identification of HCV infection can be made by evaluated Rapid Test Kits and ELISA to detect anti-HCV. Therefore, the definition of HCV antibody positive in this study was Rapid Test positive on testing. The HCV Rapid Test kits (Flavichk-HCV WB serum/plasma/whole blood test device, Qualpro diagnostics, India; and One step HCV serum/plasma test strip, Biocare TM diagnostics, China), immuno-chromatographic assays for HCV antibodies qualitative detection were used according to the manufacturers' instructions. Briefly, the supplied pipette was allowed to fill with serum/plasma/whole blood and dropped from it onto the testing device specimen port (about $50\mu\text{l}$ or two drops), and then three drops of sample running buffer was added into the other port. But, the Rapid Strip Assay (RSA) kit was immersed into the specimen (serum/plasma) and it was taken out after 10 seconds and laid on a flat surface. After fifteen minutes, if negative one colored band or if positive two colored bands could be viewed. Confirmatory test (RIBA) could not be conducted for anti-HCV positive samples in the present study due to financial constraints and unavailability of those tests. Seven HIV and anti-HCV Rapid Test positive samples were retested by fourth generation ELISA assay (HCV antibody ELISA, Human diagnostics worldwide, German) in Betezatha laboratory

diagnostics, aimed to appreciate the ELISA assay results. The assays were performed and interpreted according to the recommended protocols.

5.7.3. Detection of Antibodies against HIV

Whole blood was tested to diagnose HIV positivity at the VCT center from clients visiting voluntarily. The presence of antibodies to HIV was proven using three different immunochromatography rapid test kits, such as HIV (1+2) Rapid Test Strip (Shanghai Kehua Bio-engineering co., Ltd.; KHB), HIV 1/2 STAT-PAK Assay (Chembio Diagnostic Systems, inc.) and Uni-Gold HIV (Trinity biotech plc, Ireland) according to the manufacturer's instruction. Briefly, drops of blood produced by finger-stick from each subject were taken up from the finger-tip by the pipette supplied and dropped from the pipette onto the device. About 40µl of the sample was added to the HIV (1+2) Rapid Test cassette and then one drop of the sample diluents was added to the same area. After 2-3 minutes, one band if negative or two bands if positive could be viewed. When the anti-HIV was positive by the HIV Rapid Test from KHB, the sample was retested by the second HIV ½ STAT-PAK assay. Those subjects, whose results were positive in both tests, were considered as HIV-seropositive and counseled for positive result. However, if the result was negative by the second Rapid Test, it was further confirmed by Uni-Gold HIV Rapid Test. Lastly, if the test result was positive, it was considered as HIV seropositive and counseled for positive result while if negative, it was considered as HIV negative (Figure 2.7.4).

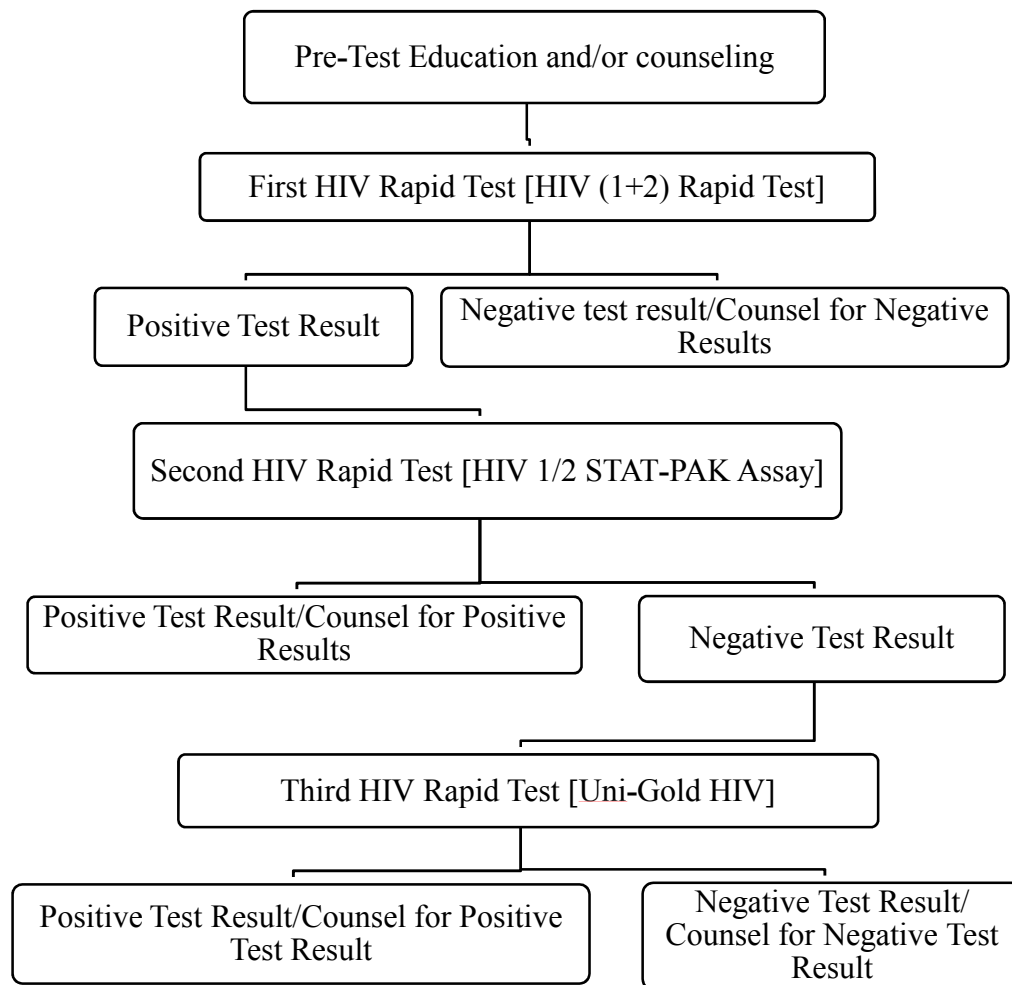


Figure 1. Algorithm for use of three HIV Rapid Tests in Mekelle Hospital VCT center

5.8. Variables

A questionnaire was administered to all consecutive attendants who consented to participate in the study to assess exposure (independent) variables such as socio-demographic characteristics (i.e., age and sex) and selected risk factors for HCV infection. HCV antibody was the outcome variable of interest (dependent variable). The HIV status of each client had serologically proven in the VCT center and HIV follow up clinics. The assessed risk factor variables in the present study included were: behavioral risks (i.e., history of STI, abortion and multiple sex partners); parenteral risks (i.e., history of frequent drug injection, dental procedure, catheterization, surgery,

blood transfusion and hospitalization); and community acquired risks (i.e., history of tattooing, scarification and bloodletting).

5.9. Data analysis and interpretation

The collected data were entered and analyzed using SPSS 17.0 statistical software. They were organized and summarized in terms of frequencies and the results of the study were presented in a descriptive measure such as a table and graph. The chi-square (χ^2) test was utilized in assessing statistical significance of association that could exist between measured variables and a P-value of less than 0.05 was considered as significant. Odds ratio (OR) and 95% confidence interval (CI) were used as a measure of the strength of association.

5.10. Ethical considerations

Ethical clearance was obtained from Department of Microbiology, Immunology and Parasitology ethical committee, Addis Ababa University (AAU). This cross sectional study was conducted in HIV screening and HIV follow up clinics at Mekelle Hospital on blood samples from finger prick of clients visiting VCT center voluntarily for HIV testing and HIV positive samples collected for other clinical investigations from follow up patients. Informed consents were obtained after explaining the aims and benefits of the study for each study individual on voluntary basis and refusal to participate involved no penalty. All these samples obtained during the study period were further investigated for HCV antibodies. Both administration of study format on HCV-related risk factors and HCV testing were commenced after obtaining permission from Tigray Regional Health Bureau and the Management Committee of Mekelle Hospital. HCV-testing were done free of charge and those whose sample became reactive for HCV were notified to their respective personnel.

6. RESULTS

6.1. Anti-HCV detection test

The gender profile of the study participants showed that 119 (39.7%) were males and 181 (60.3%) were females making the male to female ratio 0.6:1. The mean age of these participants was 28.95 years (28.95 ± 9.4) ranged from 5 months to 63 years. In addition, a relatively higher proportion of female participants in the age range of 20-39 years among all age groups (Figure 2).

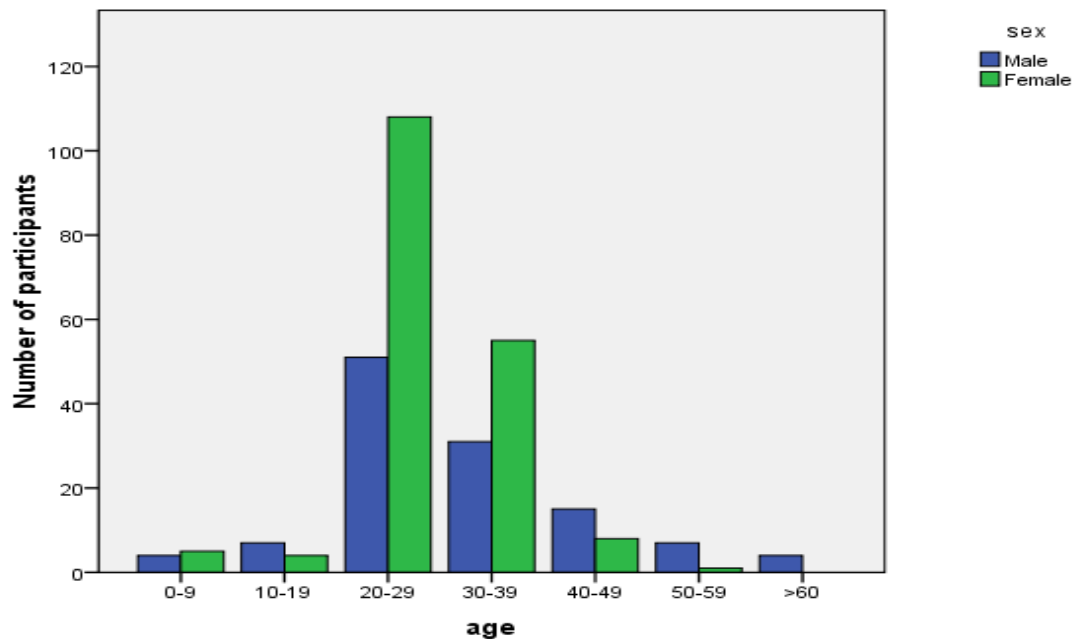


Figure 2. Age and sex distribution of the study participants

Moreover, Figure 2 shows that among all age groups, adults in the 20-29 year age group took the highest proportion (53.0%) followed by the 30-39 year age group (28.7%). Children below the age of 9 years comprised 3.0% of the study participants. The people in the 50-59 year age group accounts the lower proportion (2.7%) followed by >60 years age (1.3%).

Of the 300 study participants tested for the presence of anti-HCV, 135 (74 females and 61 males) were from VCT center and the rest 165 (107 females and 58 males) were HIV positive cases from ART clinic at Mekelle Hospital through December 2010 to January 2010. Among the 135 VCT attendants, nine clients aged above 20 years were positive and the rest 126 were negative for HIV serology. Eighteen (6.0%, 95% CI= 3.6-9.3) of the 300 attendants were positive for

HCV antibody with the Rapid Screening Test kits. Out of them, two were HIV negative participants from voluntary counseling and testing (VCT) center and the remaining 16 were HIV infected patients from HIV follow up clinics. The prevalence of HCV infection among VCT center attendants, without HIV follow up cases, was 1.5% (2/135); and 9.7% (16/165) in HIV follow up clinic cases. Seven Rapid Test reactive specimens (all were HIV positive specimens) were retested using fourth generation ELISA assay, aimed to assess ELISA antibody test results. Consequently, only one of them was found positive (Table 1).

Table 1. HCV prevalence by age and sex in attendants of VCT center and HIV follow up clinics at Mekelle Hospital (Dec. 2010-Jan. 2011)

Age group (years)	HCV-Antibody positive (%)		Total
	Males	Females	
0-9	0/4(0.0)	0/5(0.0)	0/9(0.0)
10-19	0/7(0.0)	1/4(25.0)	1/11(9.1)
20-29	2/51(3.9)	7/108(6.5)	9/159(5.7)
30-39	2/31(6.5)	3/55(5.5)	5/86(5.8)
40-49	0/15(0.0)	2/8(25.0)	2/23(8.7)
50-59	1/7(14.3)	0/1(0.0)	1/8(12.5)
>60	0/4(0.0)	-	0/4(0.0)
Total	5/119(4.2)	13/181(7.2)	18/300(6.0)

Anti-HCV antibodies were found in 18 samples using the Rapid Test kits. Hence, overall prevalence of anti-HCV antibody was 6.0% (18/300, 95% CI= 3.6-9.3) for the study population. The age specific prevalence rates were 9.1% (1/11), 5.7% (9/159), 5.8% (5/86), 8.7% (2/23), 12.5% (1/8) for the 10-19, 20-29, 30-39, 40-49 and 50-59 year age groups, respectively ($p=0.92$). However, the 0-9 and >60 year age groups had no anti-HCV positive. The males who were positive for anti-HCV were 5 (4.2%) while the females were 13 (7.2%). HCV infection was found higher proportion in females than males ($p=0.33$) and the odds ratio (OR) associated with being male was 0.57 (95% CI= 0.20-1.63) (Table 1).

As shown in the figure below, the number of HIV and HCV according to sex and age were compared. Accordingly, there were no significant differences in HCV seroprevalence among the different categories of age and sex ($p > 0.05$).

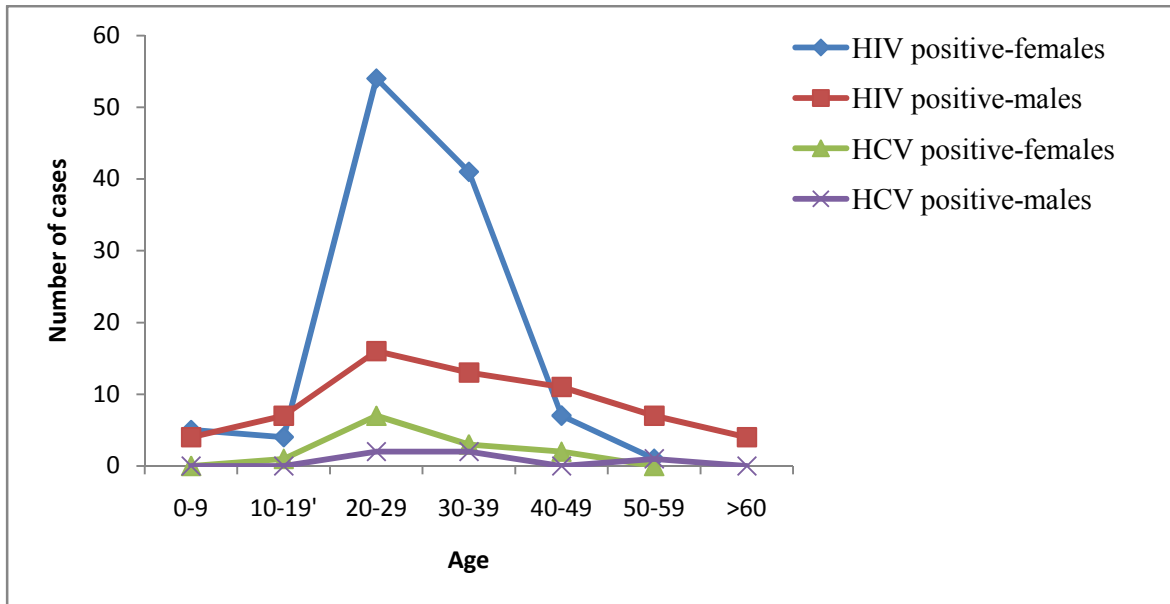


Figure 3. Seroprevalence rates of HIV and HCV infection by sex and age in attendants of VCT center and HIV follow up clinics at Mekelle Hospital (Dec. 2010-Jan. 2011)

6.2. HIV/HCV Coinfection

Of the 174 persons with HIV, 16 (5/62 males and 11/112 females) had antibodies to HCV, whereas among 126 HIV negative subjects two females (1.58%) were HCV-antibody positive. This represents 9.2% prevalence of HIV/HCV coinfection. Among the females, HIV/HCV coinfection was seen in 11 (9.8%) out of the 112 cases while among the males, HIV/HCV coinfection was found in 5 (8.1%) out of the 62 cases. The proportion of HCV infection in HIV cases was increased nearly six fold when it was compared to HIV negative individuals (9.2% Vs 1.58%). As a measure of the strength association of HIV and HCV infection, the odds ratio was 6.28 (95% CI= 1.42-27.82) and there was a significant association between HCV positivity and HIV at p value 0.05 ($p= 0.006$) (Table 2).

Table 2. Coinfection of HIV and HCV in attendants of VCT center and HIV follow up clinics at Mekelle Hospital (Dec. 2010-Jan. 2011)

HIV-Status	HCV-Antibody		Total
	Positive	Negative	
Positive	16	158	174
Negative	2	124	126
Total	18	282	300

(OR= 6.28, 95% CI= 1.42-27.82, $p= 0.006$)

The age specific pattern of HIV and HCV coinfection shows that the frequency of HCV infection was more or less in similar trend to the frequency of HIV infection in each age group of the study subjects (Figure 4).

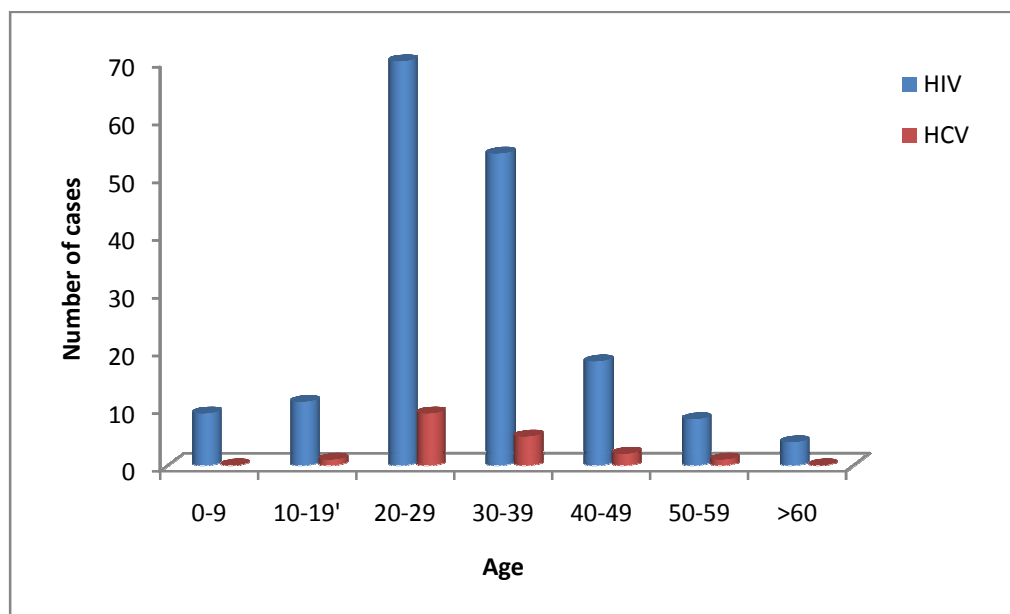


Figure 4. Specific age group in relation to HIV and HCV coinfection in attendants of VCT center and HIV follow up clinics at Mekelle Hospital (Dec. 2010-Jan. 2011)

6.3. HCV infection by risk exposure

Hepatitis C virus infection risk factors from consecutive attendants voluntarily agreed to participate in the study were collected using structured questionnaire. Some data with incomplete information were rejected and only those with complete information were included in the risk

measurements. A relatively higher proportion of HCV infection was observed in respondents admitted having had occasion on history of multiple sex partner and tattooing, 6/59 (10.2%) and 3/35 (8.6%), respectively. A total of two (8.3%) respondents admitted having been experienced to hospital admission, previously. One out of 17 (5.9%) attendants was with a history of dental procedure and one (3.1%) attendant has had history of scarification (Table 3).

Table 3. HCV prevalence by risk factors in attendants of VCT center and HIV follow up clinics at Mekelle Hospital (Dec. 2010-Jan. 2011)

Risk factors	HCV-antibody (%)			OR (95%, CI)	<i>p value</i>
	Positive	Negative	Total		
<i>Community acquired</i>					
Tattooing	3(8.6)	32(91.4)	35(14.0)	1.56(0.43-5.69)	0.45
Blood letting	0(0.0)	8(100.0)	8(3.2)	-	1.00
Scarification	1(3.1)	31(96.9)	32(12.8)	0.48(0.06-3.70)	0.70
<i>Hospital acquired</i>					
Hospitalization	2(8.3)	22(91.7)	24(9.6)	1.48(0.32-6.84)	0.65
Blood transfusion	0(0.0)	6(100.0)	6(2.4)	-	1.00
Dental procedure	1(5.9)	16(94.1)	17(6.8)	0.98(1.22-7.82)	1.00
Minor surgery	0(0.0)	16(100.0)	16(6.4)	-	0.61
<i>Behavioral acquired</i>					
Multiple sex partners	6(10.2)	53(89.8)	59(23.6)	2.16(0.78-6.02)	0.14
STI/STD	0(0.0)	30(100.0)	30(12.0)	-	0.23
Abortion	0(0.0)	23(100.0)	23(9.2)	-	0.38
Total	13(5.2)	237(94.8)	250(100.0)		

The 6.0% overall prevalence of HCV infection in the VCT center and HIV follow up clinics sampled did not show any association at *p* value 0.05 for previous history of all the abovementioned risk factors (*p* > 0.05). Therefore, no apparent risk factor that caused HCV infection was inferred from this study. As none of the study subjects admitted had history of catheterization and drug injection, it could not be examined this association.

6.4. HCV infection by occupation

Regarding the various occupational groups among the clients of VCT center and HIV follow up clinics at Mekelle hospital, two (11.1%) out of 18 farmers had positive HCV-antibody and 10.0% (5/50) of office workers had HCV prevalence, while day laborers had 9.3% (4/43) prevalence. Commercial sex workers, unemployed and housewives had 6.7% (1/15), 4.1% (2/49) and 3.6% (3/83), respectively. None significant relationship was found between occupation and HCV infection ($p= 0.786$) (Table 4).

Table 4. HCV prevalence by occupation in attendants of VCT center and HIV follow up clinics at Mekelle Hospital (Dec. 2010-Jan. 2011)

Occupational Category	HCV-antibody (%)		Total
	Positive	Negative	
Health workers	0(0.0)	2(100.0)	2(0.7)
Sex workers	1(6.7)	14(93.3)	15(5.3)
Day laborers	4(9.3)	39(90.7)	43(15.4)
Office workers	5(10.0)	45(90.0)	50(17.9)
Handicrafts	0(0.0)	12(100.0)	12(4.3)
Farmers	2(11.1)	16(88.9)	18(6.4)
Traders	0(0.0)	1(100.0)	1(0.4)
Housewives	3(3.6)	80(96.4)	83(29.6)
Drivers	0(0.0)	7(100.0)	7(2.5)
No job	2(4.1)	47(95.9)	49(17.5)
Total	17(6.1)	263(93.9)	280(100.0)

7. DISCUSSION

In the present study, the prevalence of HCV infection in HIV positive patients was 9.2% and it should be noted with astuteness that the kits used were not confirmatory. Coinfection of HIV and HCV is now a major public health concern worldwide, owing both to its high prevalence (4–5 million persons of 40 million infected by HIV) and to interactions between the two diseases in terms of their diagnosis, natural course, and treatment (Alter, 2006). Although Africa is the continent by far the most badly affected by both HIV and HCV infections, data on coinfection in the general population are lacking (Alter, 2006), particularly in Ethiopia. This finding (9.2%) is higher than earlier local research works, although direct comparison could be difficult due to methodological difference, of 4.5% in HIV infected inhabitants of Addis Ababa (Ayele *et al.*, 2002). Besides, it is higher than previous local studies in different study populations, like 0.3% in health professionals (Yimer, 2005), 0.7% in blood donors (Tessema *et al.*, 2010), 1.3% in pregnant women attending antenatal clinic (Tiruneh, 2008), 1.7% in blood donors (Gelaw and Mengistu, 2008), 2.0% in urban and rural populations of Ethiopia (Frommel *et al.*, 1993). This increase may be driven by the high-risk populations (i.e., HIV positive persons) in the present study while low-risk populations in most of the other studies revealed (for example, blood donors).

Likewise, lower HIV/HCV coinfecting rates of 0.6%, 1.86%, 1.9% and 3.3% in previous studies in Africa region in different study populations namely Gambia (Mbotto *et al.*, 2009), Nigeria (Onakewhor and Okonofua, 2009), South Africa (Amin *et al.*, 2004) and Uganda (Walusansa and Kagimu, 2009) were reported, respectively. Study among blood donors from Kenya has also revealed a smaller rate of 0.1% (Okoth, 1996). In India, a study showed that the prevalence rate of HCV-Ab in HIV-positive patients was reported to be 0% (Mahajan *et al.*, 2008) and a similar study in northern India on 620 HIV-positive patients, the rate for HCV was 1.6% (Tripathi *et al.*, 2007). In Brazil the results of a study showed the rates of 5% for HCV-Ab coinfection in HIV-positive patients (Silva *et al.*, 2006) while in Greece 7.5% (Dimitrakopoulos, 2000). The reasons for these relatively low results might be due to geographical and methodological differences with the present. Almost comparable with the present rate, reports of 8.2% in Nigeria (Agwala *et al.*, 2004) and 8.6% in Cameroon (kim *et al.*, 2007) were disclosed.

In contrast, the coinfection rate obtained in the present study (9.2%) is lower than 11.6% reported by an earlier local study in VCT and HIV follow up clinic (Gebre, 2005). Taking into consideration their similar routes of transmission, this declined rate of HCV coinfection among HIV positive people in this study may be driven by a change in HIV transmission patterns as a result of the awareness created in the community considerably through the past years. It is much lower than hospital based coinfection rate of 30-50% that has been reported in industrialized countries, such as in North America and Europe (Alter, 2006; Veruchi *et al.*, 2004), where intravenous drug use (IDU) is a major risk factor for both infections. In addition, in a similar study carried out in Iran, the co-infection rate of HCV in HIV-positive patients was found to be 74% (Alavi and Etemadi, 2007).

There was statistically significant difference in HCV seroprevalence rates between HIV positive and HIV-negative in the present study ($p= 0.006$). The rate of HCV-antibody among HIV positive and HIV negative individuals was 9.2% Vs 1.58%, respectively (OR=6.28, 95% CI= 1.42-27.82). The elevated coinfection rate in HIV-positive persons could demonstrate that they might be exposed to HCV infection prevalently by sexual contact and it suggests co-transmission of both viruses (Ayele *et al.*, 2002). Because of the blood-borne nature of HCV, many individuals at risk for HIV are also at high risk for HCV infection (Garcia-Samaniego *et al.*, 2001). The frequency of HCV transmission to sexual partners is significantly higher when HIV virus is also transmitted. This would suggest that HIV could be a co-factor for the sexual transmission of HCV infection (Pembrey *et al.*, 2001). Therefore, diagnosing HCV in HIV-positive patients is vital in order to take care of them and allot resources in health plans so that all HIV positive patients have to be tested for HCV (Bruno *et al.*, 2006). Correspondingly, this finding agreed with some studies conducted earlier in which HCV infection was significantly higher in HIV infected cases than in HIV negative individuals, 4.5% Vs 0.8% (Ayele *et al.*, 2002) and 11.6% Vs 2.6% (Gebre, 2005), respectively. In addition, Tessema *et al.* (2000) and Diro *et al.* (2008) reported the presence of a significant association between HCV and HIV infections. However, the presence of association between HIV and HCV infection contradicts the results from other local studies (Abreha *et al.*, 2011; Dessie *et al.*, 2007; Duru *et al.*, 2009; Lassey *et al.*, 2004; Olatunji and Iseniya, 2008). The possible elucidation for this variation might be due to the rapid test positive samples in the present study was not confirmed by other tests.

In the present study HCV prevalence was 4.2% for males and 7.2% for females (OR=0.57, 95% CI= 0.20-1.63, $p= 0.33$), indicating a reasonably higher proportion in females than males. This was consistent with a rate of 4.53% in females and 3.85 in males using rapid screening test (Ivantes *et al.*, 2010). In contrast, a higher prevalence rate in males than in females (10.5% Vs 5.6%) was reported locally in a similar study population by Gebre (2005). The age specific prevalence rates were concentrated among the age groups 20-49 years and was not increased with age ($p= 0.92$). Consistent with this finding, local research work from Addis Ababa showed no increase with age (Gebre, 2005). A study in neighboring Kenya has also been showing a similar epidemiological pattern of HCV infection among the 21-40 year age groups (Muasya *et al.*, 2008). In contrast, another local study by Ayele *et al.* (2002) forwarded prevalence of HCV to increase with age. There were no significant differences in HCV seroprevalence among sex and the different categories of age ($p>0.05$). This finding can suggest that HCV infection may not enhance susceptibility to gender due to risk-behavior differences by gender as well as the raising in age with time. It complies with the study done in Addis Ababa (Ayele *et al.*, 2002) where no difference by gender was reported. But significantly differ from reports made by Gebre (2005), Kim *et al.* (2007) and Mbotto *et al.* (2009) where the HCV seroprevalence was associated with age and sex.

The overall prevalence of HCV infection among VCT center attendants, without HIV follow up cases, was 1.5% (2/135). This result is nearly comparable with previous population study in Ethiopia where 2% was reported (Formel *et al.*, 1993) and agreed with 1.4% in Eritrea (Gebrekidan, 1998) and 0.2-40% finding in various populations of Africa (Cheesbrough, 2000). It is higher than other population based studies reported in neighboring countries like 0.7% in Kenya (Hyams *et al.*, 1993) and 0.6% in Somali (Nur, 2000). However, it is lower than the 5% rate observed among VCT attendants locally (Gebre, 2005). The result (1.5%) may justify, consideration of HCV testing and prevention measures in VCT centers could also be unavoidable.

Of note, out of seven rapid test reactive samples despite the high sensitivity of the assays used, only one of these sera were tested ELISA positive. The performance of rapid test and ELISA assays has not been fully validated in our country. But, similar lower ELISA test results were

already found in other studies in Africa (Duru *et al.*, 2009; Walusansa and Kagimu, 2009). The possible elucidation of this finding maybe, in immunosuppressive conditions and early stages of the disease, in which serological response is low (low antibody titers) and this could lead to negative results in serological assays. Positive ELISA test may found in those subjects with high antibody titers (Schroter *et al.*, 1999). Another explanation for variable results might be shipping and storage of serum samples in which multiple freeze-thaw cycles may result sample deterioration (Rowan *et al.*, 1994). A study in Uganda observed that those negative by EIA were positive by Rapid Strip Assay (RSA). Besides, HCV RNA positive patients had negative tests for HCV antibody by EIA; however, all of these had been rapid test positive (Seremba *et al.*, 2010). On the contrary, these findings disagreed with the result by Torane and Shastri (2008) which revealed 0.0% of rapid test sensitivity. In this case this screening assay would have missed any infected subjects among the samples tested.

The prevalence of HCV infection in the VCT center and HIV follow up clinics sampled did not show any association at p value 0.05 for previous history of all the risk factors studied ($p > 0.05$) although a number of anti-HCV positive attendants had history of these factors. Injection drug use is illegal in Ethiopia and probably rare as well, thus no data revealed on this topic in the present study. Similarly, as none of the study subjects admitted history of catheterization, it could not be examined this association. Hence, no apparent risk factor that caused HCV infection was inferred from this study. On the other hand, some studies revealed the traditional practices that involve the puncture of the skin, which have been associated with HCV transmission elsewhere, are also likely to contribute to HCV transmission in Africa (Honda *et al.*, 1993; Garcia-Samaniego *et al.*, 2001; Sulkowski and Thomas, 2003). Likewise, this finding contradicts with a similar study by Gebre (2005) who reported a significant association of catheterization and STI with HCV infection and from Cameroon by Kim *et al.* (2007) where some of the risk factors listed showed increased risk of HCV infection.

Regarding the various occupational groups, two (11.1%) out of 18 farmers had positive HCV-antibody and office workers had HCV prevalence of 10.0% (5/50), while day laborers had 9.3% (4/43) prevalence. None significant relationship was found between occupation and HCV infection ($p = 0.786$). In the present study a high prevalence of HCV was observed in farmers;

however, because of their small numbers statistically conclusive inferences may be difficult to be made. Yet, this higher rate of HCV infection in farmers might raise concern regarding need for implementation of more effective public education. In another local study HCV-antibody distribution with respect to occupation was reported uniform (Gebre, 2005). Seroprevalence rates of HCV antibody have been reported to be higher in individuals with more than two sexual partners and in sex workers compared with other members of the general population, suggesting that sexual transmission may be possible (Sulkowski and Thomas, 2003).

This study has several limitations and future studies should therefore address these limitations.

- ✓ The present study may be limited by the small samples size due to high costs of the screening tests which may restrict the generalizability of the results although the sample size in this study exceeds the minimum representative sample size determined using a local coinfection rate of 11.6% (i.e., 158). Besides, other similar studies both in small and larger sample size showed similar results. For instance, in Uganda with a smaller sample size (122 HIV positive patients) and in Kenya with a larger sample size (458 HIV positive patients) showed almost consistent coinfection rate.
- ✓ When serum samples are tested for anti-HCV antibodies using different commercial screening assays, inconsistent results often occur. Since no confirmatory test in this study was carried out, some false positive results may be expected. Supplemental anti-HCV testing (i.e., RIBA) for all anti-HCV positive confirms the presence of anti-HCV.
- ✓ The presence of screening test negative result is common in immunosuppressed individuals (HIV infection) and during window period of the disease. Hence, these assays do not exclude the possibility of exposure to hepatitis C virus for the individuals with negative results. Therefore, the anti-HCV negative results can have possibility of exposure to hepatitis C virus by verifying using other suitable tests such as HCV-core antigen test which detects viraemia.
- ✓ Freeze-thaw cycles and shipping samples long distance could possibly compromise sample quality although the samples arrived at their destination well frozen and there were no indication that they had thawed while traveling.
- ✓ Coinfection of HIV/HCV is major global health concerns. However, limited data are available in Ethiopia; as a result, the present study was deficient in local information.

8. CONCLUSION AND RECOMMENDATION

8.1. Conclusion

Coinfection rate of HIV/HCV in this study is reported to be 9.2%, which is higher than the level revealed in the general population (2.0%) but direct comparison could be difficult due to methodological difference. It demonstrates a statistically significant difference in HCV seroprevalence rates between HIV positive (9.2%) and HIV negative (1.58%) attendants. Therefore, diagnosing HCV in HIV-positive patients is vital in order to take care of them and allot resources in health plans so that all HIV positive patients have to be tested for HCV. On the contrary, there was no significant association between HCV positivity and sex and the different categories of age. This finding can suggest that HCV infection may not enhance susceptibility to gender due to risk-behavior differences by gender as well as the raising in age with time. Similarly, the prevalence of HCV in the attendants sampled did not show any association for previous history of all the risk factors studied. Hence, no apparent risk factor that caused HCV infection was inferred from this study.

Coinfection of HIV/HCV is a major global health concerns. However, limited data are available in Ethiopia and most of these available few studies conducted so far revealed lower rate of HCV infection. Accordingly, it has given less consideration to HCV infection. However, the relatively higher HCV seroprevalence in HIV positive cases than HIV negative clients in the present study and the previously documented local studies of coinfection support as HCV should not be underestimated. At last, this study which was done in Mekelle hospital VCT center and ART clinics could provide additional data on coinfection rate of HIV and HCV infection.

8.2. Recommendations

The present study has revealed statistically significant difference in HCV seroprevalence rates between HIV positive and HIV negative attendants. Therefore, based on these findings the following recommendations are forwarded:

1. HCV prevention measures should target primarily HIV infected people. Therefore, providing opportunity of screening for HCV infection in all HIV-infected patients is essential. However, under circumstances of resource constrain it may be recommended that HCV screening be limited to investigate HCV infection especially in HIV positive patients with features suggestive of liver disease in order to identify HCV as a possible cause has importance.
2. Further sero-epidemiological studies are needed to decipher the prevalence of HIV/HCV coinfection and a larger community-based study will also be needed to clarify the prevalence, HCV genotypes and the impact of repeatedly done social practices with reuse of equipments on HCV transmission in Ethiopia. This in turn help policymakers decide on the cost-effectiveness of available intervention measures.
3. The quality and sensibility of rapid immuno-chromatographic assays should be evaluated for the detection of anti-HCV antibodies because their use is essential under circumstances of resource constrain.
4. HIV infected patients who do not have serologic evidence of immunity to hepatitis A and hepatitis B should be vaccinated.
5. Implementation of more effective public health education and counseling is highly needed to highlight the dangers of HIV/HCV coinfection as early diagnosis of the coinfection may lead to prompt intervention management.

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10. ANNEXES

10.1. Annex 1

QUESTIONNAIRE

This format is prepared to obtain relevant information about risk factors for hepatitis C virus infection. Please answer correctly and honestly. Your response will be kept confidential.

Code No _____

1. Age _____ Sex _____
2. Occupation _____

Use "X" mark for the blank space accordingly

3. History of hospitalization Yes No
4. History of blood transfusion Yes No

If your answer for question 4 is yes when? _____

5. History of repeated drug injection Yes No
6. History of multiple sexual partners Yes No
7. History of STD/STI Yes No

8. History of invasive procedure

- | | | | |
|------------------|--------------------------|---------------|--------------------------|
| Tooth extraction | <input type="checkbox"/> | Minor surgery | <input type="checkbox"/> |
| Catheterization | <input type="checkbox"/> | Bloodletting | <input type="checkbox"/> |
| Scarification | <input type="checkbox"/> | Tattooing | <input type="checkbox"/> |
| Abortion | <input type="checkbox"/> | | |

Other specify _____

Only for HIV cases

9. Your HIV status? Positive Negative

THANK YOU!

11.2. Annex 2

LABORATORY DATA

Code No _____

1. Identification: Age _____ Sex _____

2. Date of specimen collection _____

3 Specimens: Serum Plasma Whole blood

4. Consistency of the specimen: Clear Hemolyzed

Icteric Lipemic

5. Test results

5.1. Rapid test: Positive Negative

5.2. Anti-HIV Abs: Positive Negative

5.3 ELISA: Positive Negative

11.3. Annex 3

CONSENT FORM

Code No _____

I have been informed about the study, which is targeted to identify hepatitis C virus (HCV) responsible for liver disease. The aim of the project and the need for taking part of the blood samples/taking blood for the analysis of hepatitis C virus infection have been informed to me in the language that I comprehend well. I have also been informed about the confidentiality of the questionnaire. Additionally I have been told that the cooperation in filling study format and participation in the study is on the voluntary bases and refusal to participate involves no penalty. Apart from this, I have been informed that the specimen is used only for research purpose and I benefit from the free laboratory investigation. Therefore, with full understanding of the importance of the study, I agreed voluntarily to participate in this activity.

Participant's name _____ Signature _____

Personnel who asked the consent _____ Signature _____

Witness _____ Signature _____

DECLARATION

I, the undersigned, declare that this M.Sc. thesis is my own original work, has not been presented as thesis work for a degree in this or other University and that all sources of material used for the thesis have been duly acknowledged.

Investigator Haftom Hadush (DVM.)

Signature _____

Date of Submission _____

Addis Ababa, Ethiopia

Advisors

Name Solomon Gebre-Selassie (MD, M.Sc.)

Signature _____

Date _____

Addis Ababa, Ethiopia

Name Adane Mihret (DVM, M.Sc.)

Signature _____

Date _____

Addis Ababa, Ethiopia