ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

Prevalence of Hepatitis B, Hepatitis C and HIV among Chronic liver disease patients in selected hospitals, Addis Ababa, Ethiopia

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List of Abbreviations

ART;-antiretroviral therapy

Ab: - Antibody

Ag: - Antigen

CDC: - Center for Disease Control

CHB;-chronic hepatitis B infection

CLD: - Chronic liver disease

DNA;-Deoxy Ribose nucleic acid

EIA: - Enzyme immunoassay

ELISA: - Enzyme linked immune sorbent assay

HAV:-hepatitis A virus

HBIG:-hepatitis B immune globulin

HBsAg: - Hepatitis B surface antigen

HBV: - Hepatitis B Virus

HCC: - Hepatocellular carcinoma

HCV: - Hepatitis C Virus

IDU: - Injection drug users

OPD: - Out patient department

RNA;-Ribose nucleic acid

SOPS: -Standard operating procedures

US -ultrasound

WHO: - World Health Organization

Abstract

Introduction; Hepatitis B virus (HBV) and hepatitis C (HCV) virus is hepatotropic virus spread mainly through contaminated blood and blood products, sexual contact and contaminated needles. Chronic infection by these viruses leads to slow progressive liver disease that over a period of up to 30 years may result in cirrhosis, chronic liver failure and hepatocellular carcinoma (HCC). HIV, HBV, and HCV infection share similar transmission routes and therefore co-infection is common. These viruses are prevalent in different parts of the world including Ethiopia there for this study shows the burden of these viruses in Ethiopia.

Objective: To determine the prevalence of HBV, HCV and HIV infection among clinically diagnosed chronic liver disease patients visited at Black Lion, St. Paul and Armed force hospital Addis Ababa, Ethiopia.

Method: Hospital based cross-sectional study was conducted in three selected hospitals of Addis Ababa over a period of 3 months (March2014- May 2014) on clinically diagnosed chronic liver disease patients. By using questionnaire brief history and risk factors was taken from each volunteering patient. Serum samples from each volunteering patients was screened for the presence of HBsAg and anti-HCV by ELISIA test kit and HIV by national test algorism.

Result: A total of 117 participants who have chronic liver disease participated in the study, where 82 of them were males and the remaining 35 were females. The age distribution range form 18-78 years and the median age was 39 years. The overall prevalence of HBsAg, HCV and HIV was 34.2%, 18.8% and 9.4 respectively. The study participants had combined HBV/HIV, HCV/HIV and HBV/HCV infection which is possible because of their common modes of transmission. History of multiple sexual partner and blood transfusion also found statically significance with HBV and HCV virus infection respectively.

Conclusion: The prevalence of HBV and HCV is high where as HIV is moderate among chronic liver disease patients and history of blood transfusion and multiple sexual partners was statistically associated with HCV and HBV infection respectively.

1. Introduction

1.1 Background

Hepatitis is an infection of the liver caused by several viruses, the most common of which are hepatitis A, B and C. Both hepatitis B virus (HBV) and C virus (HCV) are spread mainly through contaminated blood and blood products, sexual contact and contaminated needles(1).

Hepatitis B virus (HBV) and hepatitis C virus are hepatotropic virus whose primary replication occurs in the liver (2).The natural history and clinical course of hepatitis B virus (HBV) differs from that of hepatitis C virus (HCV) infection. However, similarities may be drawn in the context of chronic disease and public health burden. Chronic infection by these viruses leads to slow progressive liver disease that over a period of up to 30 years may result in cirrhosis, chronic liver failure and hepatocellular carcinoma (HCC) (3).

Hepatitis B and C virus are members of two different viral families, but both display a strong hepatotropism, whose underlying molecular mechanisms are not yet entirely understood. HBV belongs to the family of hepadnaviridae, a group of hepatotropic mammalian and avian DNA viruses, which replicate via reverse transcription of a genomic RNA intermediate. It contains a partially double stranded genomic DNA. HCV is member of the flaviviridae, where it forms its own genus, Hepacivirus. HCV is a small, enveloped positive-sense, single stranded RNA virus, and its life cycle is thought to be predominantly cytoplasmic (4).

Liver disease is the single greatest cause of non AIDS related death in patients with HIV disease, accounting for a greater proportion of deaths than non AIDS related cancers. HIV, HBV, and HCV infection share similar transmission routes and therefore co-infection is common. In patients co-infected with HIV plus HBV or HCV, fibrosis rates are accelerated compared with those infected with HBV or HCV alone, leading to faster progression to end-stage liver disease. Overall, the signs of end-stage liver disease in patients with HIV infection seem to be similar to the signs in patients without HIV infection, but survival after the first episode of decompensation is reduced(5).

WHO estimates that there are 350 million people with chronic HBV infection and 170 million people with chronic HCV infection worldwide HBV is estimated to result in 563,000 deaths and HCV result in 366,000 deaths annually (4). In Ethiopia large number of research done and it indicated that prevalence of liver disease is high It accounts for 12% of the hospital admissions and

31% of the mortality in medical wards of Ethiopian hospitals (6). This study reports the frequency of HBV, HCV, and HIV infections among clinically diagnose chronic liver diseases and the risk factors for hepatitis B and hepatitis C.

1.2 Statement of the problem

Viral hepatitis is a global public health problem affecting millions of people every year, causing disability and death around 500 million people are chronically infected with hepatitis B virus (HBV) or hepatitis C virus (HCV) in the world and approximately 1 million people die each year (~2.7% of all deaths) from causes related to viral hepatitis, most commonly liver disease, including liver cancer (7). An estimated 57% of cases of liver cirrhosis and 78% of cases of primary liver cancer result from HBV or HCV infection (8).

Viral hepatitis places a heavy burden on the health care system because of the costs of treatment of liver failure and chronic liver disease. In many countries, viral hepatitis is the leading cause of liver transplants. Such end-stage treatments are expensive, easily reaching up to hundreds of thousands of dollars per person (9). Chronic viral hepatitis also results in loss of productivity (10). Recent data indicate that from 1990 to 2005 the prevalence of HBV infection was reduced on average below 2% in Central and Tropical Latin American regions. While it remained between 2% and 4% in the Caribbean, Indian and Southern Latin American regions (11).

All countries in the African Region consider viral hepatitis an urgent public health issue. The burden of viral hepatitis, though not accurately known, is believed to be one of the highest in the world. Hepatitis A, B, C and E are the types mostly found in the Region. The prevalence of HBV is estimated at 8% in West Africa and 5-7% in Central, Eastern and Southern Africa. The prevalence of HCV is even higher in some areas, reaching levels of up to 10 % (10).

In Ethiopia 12% of medical admission and 31% of mortality in medical wards is due to liver disease. (6) Many researchers have investigated prevalence rates of HBV and HCV infections in various groups (health care workers, blood donors, medical waste handlers, and others), however the studies conducted in Ethiopia on single and co-infection of HBV, HCV and HIV among chronic liver disease, are limited to a few case series. Therefore this study was designed to determine the prevalence of HBV, HCV, and HIV among chronic liver disease patients.

2. Literature review

2.1 Chronic liver disease

Chronic liver disease (CLD) is a disease of the liver resulting from an inflammatory, infiltrative, immunologic, mechanical or metabolic injury to the liver, which has persisted for six or more months without complete resolution.(4) it has different characteristics in terms of risk factors, incubation, latency, induction and the final state of the disease process. The most common CLDs are associated with chronic viral hepatitis, alcohol use and obesity; the least common are liver cancer and those due to certain genetic, autoimmune and vascular conditions or to drug toxicity(12).

Cirrhosis is the end-stage of every chronic liver disease. It is characterized by an asymptomatic stage, known as compensated cirrhosis, followed by a rapidly progressive phase where liver dysfunction occurs, called decompensate cirrhosis. The most severe evolution condition of the cirrhosis is the hepatocelllular carcinoma (HCC), also called, primary liver cancer. Liver biopsy has been the preferred tool in the evaluation and staging of the CLD. However, its invasive nature and the development of other more accurate noninvasive alternatives have lead to a decrease on its usage for assessing the CLD. Among these alternatives, CLD staging based on ultrasound (US) data has proven to be a promising and safer alternative to biopsy (13).

Chronic liver disease is responsible for over 1.4 million deaths annually (14) and is characterized by permanent inflammatory processes that predispose to liver cancer and in particular hepatocellular carcinoma (HCC). In healthy liver, inflammatory processes stimulate growth and repair and restore normal liver architecture. However, if liver inflammation becomes chronic, the balance of damage versus regeneration in the liver is disrupted and can lead to the formation of excessive scar tissue, termed fibrosis. In the long-term, an exacerbation of fibrosis will lead to cirrhosis, which is characterized by abnormal liver architecture and function and is associated with a significant reduction in overall health and well being. At cirrhotic stages, the liver damage is often irreversible or difficult to treat. Cirrhosis leads frequently to death from liver failure or due to hepatocellular carcinoma (HCC) (11).

2.2 Viral hepatitis

Hepatitis is an inflammation of the liver, most commonly caused by a viral infection (15).Viral liver diseases are among the most important communicable disease worldwide different species of viruses, including Cytomegalovirus, Epstein-Barr, Herpes simplex, Adenovirus, Coxsackie virus, Mumps, Yellow fever, and other cause parenchymal hepatic inflammation, but the term viral hepatitis generally implies the five hepatotropic viruses: Hepatitis A, B, C, D and E virus(13).

Infections of HBV and HCV are by far the most prevalent, and their consequences can be serious. Long term chronic infection with one or both of these viruses is the most common cause of liver fibrosis and cirrhosis, leading to liver failure and hepatocellular carcinoma (16). According to the World Health Organization (WHO), 2 billion people have been infected with the hepatitis B virus (HBV), and more than 350 million have a chronic HBV infection (17). In addition, it has been estimated that up to 3% of the world's population has been infected with hepatitis C virus (HCV), of which 170 million people are chronically infected, and an additional 3 to 4 million people are infected each year (18).

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 hepatocellular carcinoma are responsible for at least 12% of medical admissions and over 20% of hospital mortality in many parts of Africa(6).

2.2.1 Hepatitis B Virus

Hepatitis B virus (HBV) is a small DNA virus and belongs to a group of hepatotropic DNA viruses (hepadnaviruses). The virus consists of a nucleocapsid and an outer envelope composed mainly of three hepatitis B surface antigens (HBsAgs) that play a central role in the diagnosis of HBV infection. The nucleocapsid contains hepatitis B core antigen (HBcAg), a DNA polymerase reverse transcriptase, the viral genome as well as cellular proteins (19).

The viral genome of HBV is a partially double stranded circular DNA of approximately 3, 200 base pairs that encodes four overlapping open reading frames. The S gene, which codes for HBsAg; two pre-S region genes (pre-S1 and pre-S2) that code for the hepatocyte receptor binding site; the C gene, which codes for HBcAg and HBeAg; the P gene, which codes for a DNA polymerase; and the X gene that activates viral and cellular promoters. Although HBV is a DNA virus, it replicates in a way similar to retroviruses, making an intermediate RNA transcript (20).

Natural history and clinical manifestation

Acute Hepatitis

The first encounter with HBV results in an acute hepatitis B infection and in the majority of it is asymptomatic. However, 20-30% of patients may exhibit symptoms of acute infection (21). Such as fever, malaise, anorexia, nausea followed by jaundice. The chronological profile of serologic markers that appear during acute hepatitis infection is shown in Fig-1. In more than 95% of patients with acute HBV infection, the disease is self-limiting leading to complete recovery and life-long immunity (22). However, in 1-5% of immune competent adults the disease may progress to chronic hepatitis B infection (CHB). The age of acquisition of the infection plays an important role in determining the course of the infection (22). Up to 90% of those who acquire the infection during the perinatal period progress to CHB. Less than 1% of individuals with acute hepatitis B infection may develop fulminant hepatic failure that has high mortality rate reaching approximately 80% (23).

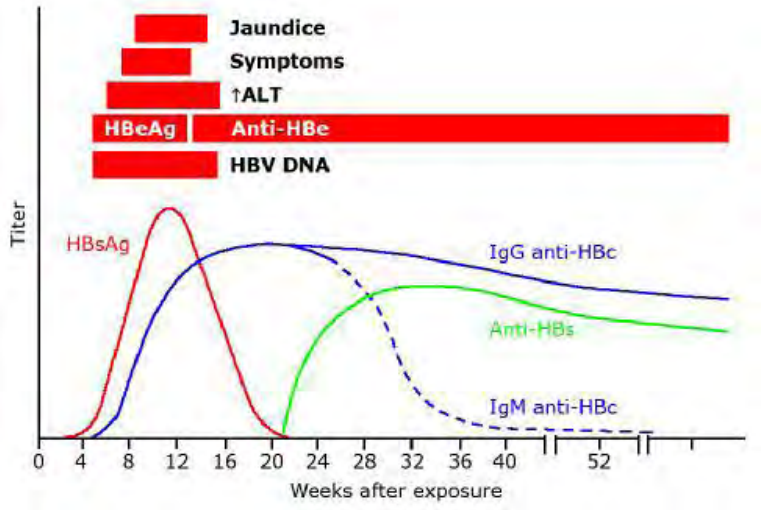

Fig.1. the chronological profile of serologic markers appearing during the course of acute hepatitis B infection (24).

Chronic Hepatitis

Chronic hepatitis B infection is defined as persistence of high levels of HBsAg for 6 months or more following acute infection (21). The serological markers during CHB infection are shown in

Fig-2. The infection runs a long and variable clinical course, with different outcomes in terms of severity of the underlying liver disease and the extent of its progression (24).

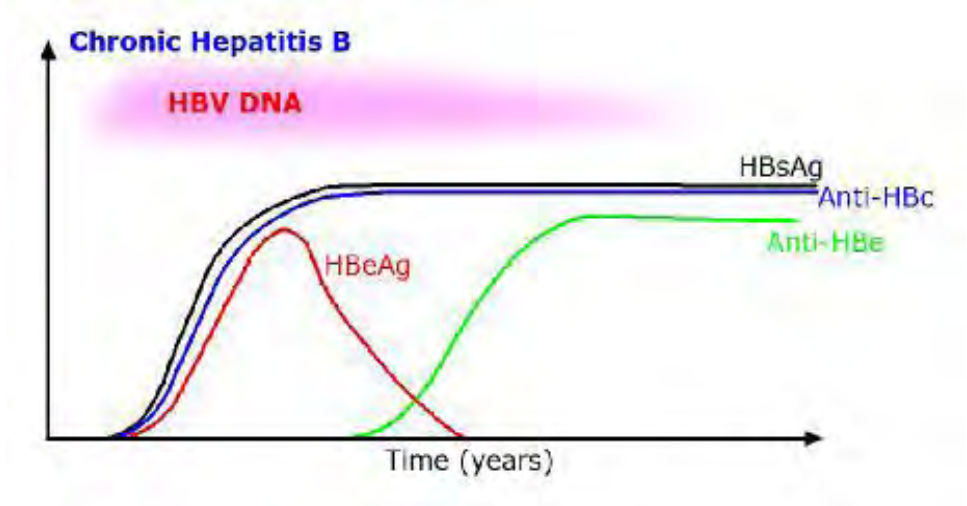

Fig.2. Serological markers during chronic HBV infection (24).

Chronic active infection requires active HBV viral replication with presence of HBV DNA. Presence of HBsAg without detectable HBV DNA or HBeAg defines chronic carrier state. These patients usually have anti-HBeAg and normal liver chemistries. Small amounts of HBV DNA might be detected as long as HBsAg antigens are present. This indicates that presence of HBsAg could diagnose chronic infection without determining the presence or absence of HBV DNA, HBeAg or anti-HBeAg. Most patients with chronic hepatitis B are clinically asymptomatic. Some may have nonspecific symptoms such as fatigue (24).

In most instances, significant clinical symptoms will develop only if liver disease progresses to decompensated cirrhosis with jaundice, ascites, peripheral oedema, and encephalopathy accompany it accordingly, physical examination will be normal in most instances. In advanced liver disease there may be stigmata of chronic liver disease such as splenomegaly, spider angiomata, Caput medusae, palmar erythema, testicular atrophy, gynecomastia, etc (25).

Transmission

Transmission of hepatitis B virus results from exposure to infectious blood or body fluids containing blood such as saliva, nasopharyngeal washings, semen, menstrual fluid and vaginal secretions. Possible forms of transmission include sexual contact, blood transfusions, re-use of contaminated needles and syringes, and vertical transmission from mother to child during childbirth. Without intervention, a mother who is positive for HBV confers a 20% risk of passing the infection to her offspring at the time of birth. This risk is as high as 90% if the mother is also positive for HBeAg (26).

Global Epidemiology

Hepatitis B is one of the major diseases of mankind and is a serious global public health problem. Of the 2 billion people who have been infected with the HBV, more than 350 million have chronic (lifelong) infections. HBV infections result in 500,000 to 1.2 million deaths per year caused by chronic hepatitis, cirrhosis, and hepatocellular carcinoma (27). Hepatitis B surface anti-gen (HBsAg) positivity of more than 8% in a community is considered to be high. In countries which include the far east, parts of the middle east, sub-saharan africa and the amazon basin. In these regions, serologic evidence of prior HBV infection (anti-hepatitis B core antigen (anti-HBcAg) or anti-hepatitis B surface antigen (anti-HBsAg) positivity is present in the vast majority of individuals (28).

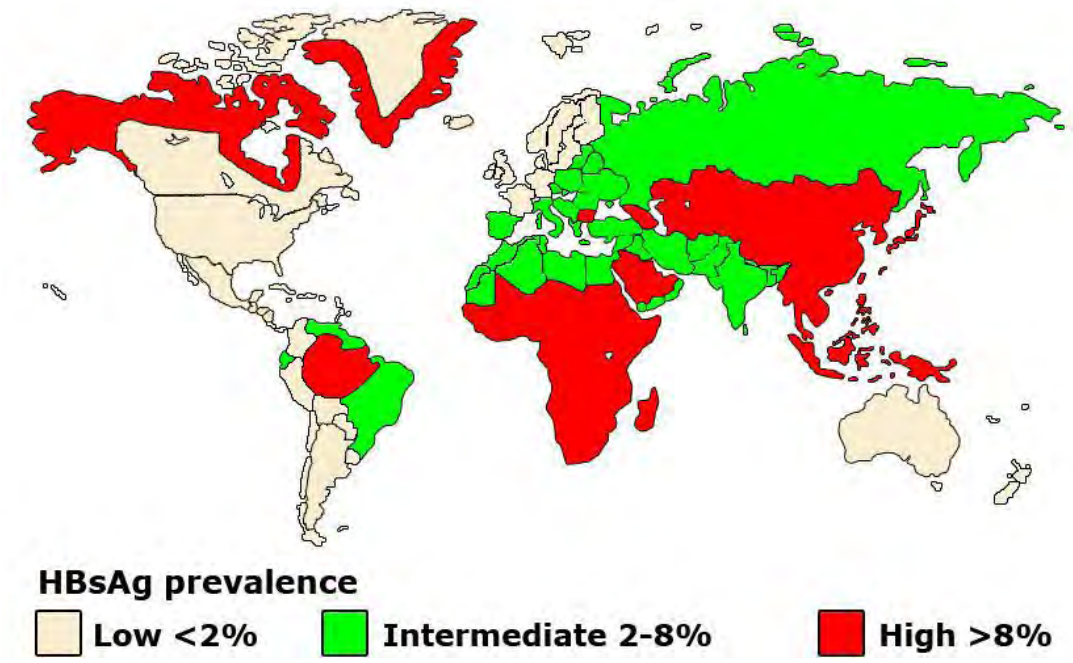

Fig.3. Estimated global prevalence of hepatitis B virus infection (11).

Countries like Japan, India, central Asia and the Middle East including Eastern and Southern Europe, as well as parts of South America, are all areas with intermediate (2% to 7% HBsAg positive) prevalence of chronic HBV infection. Low prevalence (<2% HBsAg positive) of chronic HBV is found in regions including the United States, Northern Europe, Australia, and the southern part of South America (27).

Approximately 45% of the world population lives in areas where chronic HBV infection is highly endemic (≥8% of the population are HBsAg-positive); 43% live in areas of intermediate endemicity (2-7% HBsAg-positive); and 12% live in areas of low endemicity (<2% HBsAg-positive) (25).

In Africa, infections with HBV play a major role in the etiology of most liver diseases. By country, estimated HBsAg seroprevalence ranges between 5% and 19%, and the total number of carriers may approach 58 million with as many as 12.5 million likely to die prematurely due to hepatitis B-induced liver disease (28).

In Ethiopia as in other Sub-Saharan Africa, the prevalence of liver disease is high. They account for 12% of the hospital admissions and 31% of the mortality in medical wards of Ethiopian

hospitals (6). A nationwide sero-epidemiological study of hepatitis B markers prevalence was conducted in Ethiopia on 5,270 young males from all regions of the country. Overall prevalence rates were 10.8% for HBsAg and 73.3% for "at least one marker positive"; a remarkable geographical and ethnic variability of marker prevalence was observed, reflecting the wide differences existing in Ethiopia in socio-cultural environment and activities such as tribal practices and traditional surgery. Sexual practices and medical exposure also play some role as determinants of hepatitis B marker prevalence in Ethiopia (29).

Another community based seroprevalence study done in the capital city of Ethiopia; Addis Ababa has shown a 7% seroprevalence of HBsAg, higher in males than females. Overall HBV seroprevalence rose steadily to over 70% in 49 year olds. The age at which 50% had evidence of infection was around 20 years (29). Similar study subjects of other countries, 44.6% HBsAg in india among chronic liver disease (26) 42.9% HBsAg in Ghana among HCC patients (25) and 36% HBsAg % and 24% amog HCC patient in Zimbabwe (28).

Prevention of hepatitis B

Several vaccines have been developed for the prevention of HBV infection. These rely on the use of one of the viral envelope proteins (hepatitis B surface antigen or HBV). The vaccine was originally prepared from plasma obtained from patients who had long-standing hepatitis B virus infection. However, currently, it is made using a synthetic recombinant DNA technology that does not contain blood products. One cannot be infected with hepatitis B from this vaccine (26).

The risk of vertical transmission to the newborn can be drastically reduced from 20%- 90% to 5%-10% by administering to the newborn hepatitis B vaccine (HBV 1) and hepatitis B immune globulin (HBIG) within 12 hours of birth, followed by a second dose of hepatitis B vaccine (HBV 2) at 1-2 months and a third dose at and no earlier than 6 months (24 weeks). Since 2% of infants vaccinated will not develop immunity after the first three dose series, infants born to hepatitis B positive mothers are tested at 9 months for hepatitis B surface antigen (HBV) and the antibody to the hepatitis B surface antigen (anti-HBs); if post-vaccination test results indicate that the child is still susceptible, a second three dose series at (0, 1 and 6 months) is administered. If the child is still susceptible after the second series, a third series is not recommended (30).

Treatment of hepatitis B

Acute hepatitis B infection does not usually require treatment because most adults clear the infection spontaneously (31). Early antiviral treatment may only be required in less than 1% of patients, whose infection takes a very aggressive course (fulminant hepatitis) or who are immune compromised. On the other hand, treatment of chronic infection may be necessary to reduce the risk of cirrhosis and liver cancer. Chronically infected individuals with persistently elevated serum alanine aminotransferase, a marker of liver damage, and HBV DNA levels are candidates for therapy (32).

2.2.2 Hepatitis C Virus

HCV is classified as the type member of the genus Hepacivirus within the virus family Flaviviridae (33). 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) that undergo variation during infection due to hyper variable regions within their genes (34).

HCV has been suggested to have six genotypes, which differ from each other by 31–33% at the nucleotide level and are further classified into several subtypes (33) the genotype 1, 2, and 3 appears to have a worldwide distribution and their relative prevalence varies from one geographic area to another. HCV genotype 4 appears to be prevalent in North Africa and the Middle East, and genotype 5 and 6 seems to be confined to 6 South Africa and Hong Kong respectively (34).

Clinical outcome of hepatitis C

HCV infects only humans and chimpanzees. Patients infected with HCV have an 80 to 85 percent chance that the HCV infection will persist and that they will go on to have a chronic HCV infection. Chronic HCV infection has multiple manifestations, the most common of which is chronic progressive liver disease associated with inflammation and fibrosis that is some patients may progress to cirrhosis. Cirrhosis or end–stage liver disease is associated with multiple complications including variceal bleeding, ascites, encephalopathy, and hepatocellular carcinoma. These conditions are common causes of morbidity and mortality in HCV infected patients (35).

HCV infection causes an acute or chronic necroinflamatory disease of the liver. Only relatively small fractions of HCV infections are symptomatic. Most infected individuals remain asymptomatic and presumably, undiagnosed (36). Major clinical manifestation is progressive hepatic fibrosis, which leads to cirrhosis and an increased risk of hepatocellular carcinoma (37).

Primary infection with HCV leads to persistent viremia in approximately 85% of patients with development of chronic liver disease in >60% of cases. Approximately 20% of individuals, as shown in figure1.3 below, with chronic hepatitis C eventually develop medically significant sequel including cirrhosis end stage liver disease or hepatic cellular carcinoma (38).

Transmission

There are multiple routes of transmission of HCV. Since it is a blood-borne infection which is transmitted sexually and vertically and by iatrogenic, occupational, cultural and recreational activities. Unsafe transfusions and therapeutic injections and acupuncture are examples of iatrogenic transmission. Intravenous drug use, tattooing, scarification and ear-piercing are examples of recreational and cultural activities that may spread HCV. It may also be transmitted by needle-stick injuries (39).

Epidemiology

Epidemiological studies of HCV are challenging since most cases of HCV infection are asymptomatic and indistinguishable clinically from other causes of hepatitis. A laboratory diagnosis is therefore essential, but not always available, particularly in resource- limited settings. There are consequently few population-based epidemiological studies of HCV. Most studies are based on select high risk (e.g. intravenous drug users) or low risk (e.g. blood donor) populations which either overestimate or underestimate the true prevalence respectively (40).

HCV is endemic worldwide, with an estimated global prevalence of 3%, which represents 170 million people. HCV is four to five times more prevalent than HIV globally and is the commonest chronic blood-borne infection in the United States Three to four million people is infected by HCV every year (41).

There is great variation in the geographical distribution of HCV, with the highest prevalence in Africa and Asia, and lower prevalence in industrialized countries (Figure 4). Even within developing nations, there is great variation in prevalence. 0.9% in India and 3.2% in China, to the highest reported prevalence in Egypt of 22%. Some of this variation may be explained by differences in study and reporting methods. Actual differences in HCV prevalence may be due to variation in risk factors in different parts of the world. Injection drug use is the single most important risk factor in developed regions of the world. Unsafe therapeutic injections and blood

transfusions are important risk factors in developing nations where these practices occur. Occupational, peri-natal and sexual exposure, and tattooing, body-piercing, acupuncture and scarification are other modes of HCV transmission. The relative contribution of these risk factors to the prevalence of HCV is poorly defined, particularly in developing regions of the world (40).

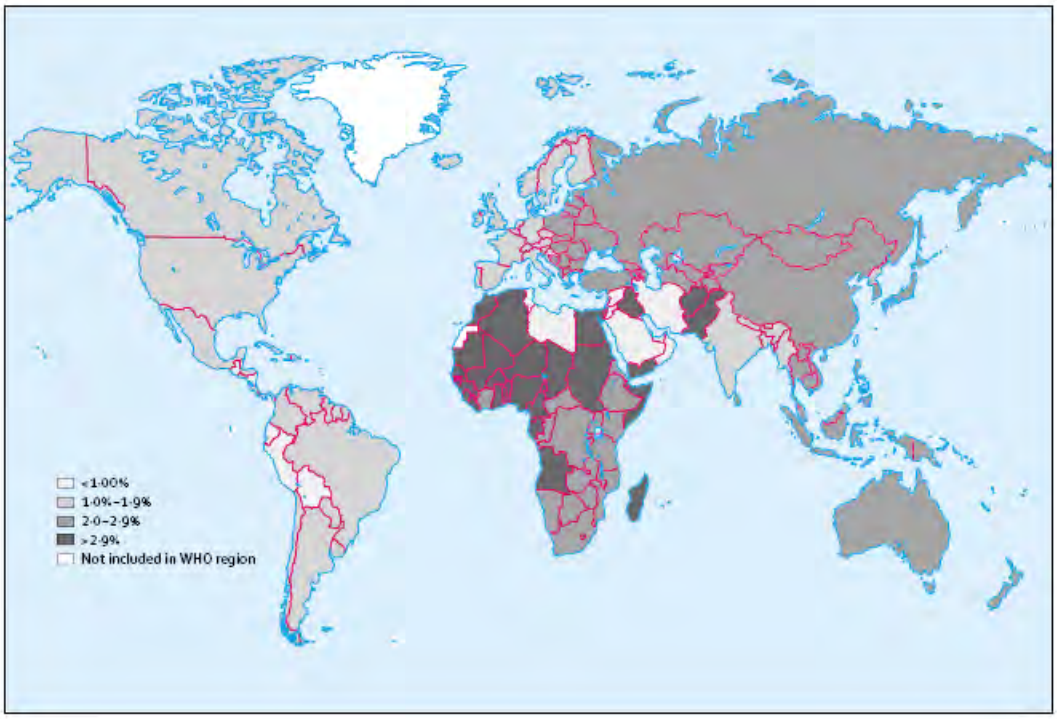

Figure 4. Estimated global prevalence of hepatitis C virus infection (41).

Sub-Saharan Africa has the highest prevalence of HCV (5.3%) among the WHO world regions. Within this region there are remarkable differences in prevalence: the central African region has the highest prevalence (6%), and Southern and East Africa, the lowest (1.6%). Cameroon has the highest national prevalence of 12% and South Africa the lowest (0.1%) among blood donors (42).

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% (43). Most of the studies revealed a low overall prevalence of HCV infection. These include: 0.3% in Health professionals (44). 0.7% in 6361 consecutive blood donors (45). 0.9% in inhabitants of Addis Ababa (46). 1.3% in pregnant women attending antenatal clinic (47). 1.7% in Tigray and Amahra regions (48).

Prevention

There is no vaccine against HCV. Research is in progress but the high mutability of the HCV genome complicates vaccine development (49). 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 (50). HCV carriers should be strongly discouraged from drinking alcohol because there is evidence that acts as a cofactor in developing more severe liver injury (51).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 (52).

Treatment of Hepatitis C

Treatment of acute hepatitis is mainly supportive, consisting of bed rest and balanced diet with small frequent nitrous meals and hospitalization reserved only for cases of sever disease (53).The goal of treatment is to achieve a sustained viral response (SVR), as defined by the absence of viremia 6 month after stopping the medications; SVR is associated with improved histology and decreased risk of morbidities (54).

2.3 Human Immunodeficiency Virus

Human immunodeficiency virus (HIV) is a lenti virus (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 (prenatal transmission) (55).

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 than1.1 million people are living with HIV/AIDS and at the end of 2009 (56).

Most liver disease among HIV-infected individuals is secondary to confection with HCV and/or HBV. These diseases spread in similar ways, especially, through the usage of shared syringes and sexual activity. Many HIV positives might be co-infected with HBV or HCV, or both. It might result in cancer, cirrhosis, liver failure and death (57). Prevalence HIV among chronic liver disease in Ethiopia has not been known for sure.

3. Significance of the study

Viral hepatitis is a global public health problem affecting millions of people every year, causing disability and death Around 500 million people are chronically infected with hepatitis B virus (HBV) and hepatitis C virus (HCV) in the world and Approximately 1 million people die each year (7). In Ethiopia 12% of medical admission and 31% of mortality in medical wards is due to liver disease (6). Co-infection of HIV with hepatitis B (HBV) and/ or hepatitis C (HCV) is one of the serious health problems in many societies (58).There for This study provide information on the pattern of hepatitis B hepatitis C and HIV among chronic liver disease in the study site and the related circumstances as well as the risk factor for hepatitis disease . This study also useful to health managers and planners to develop appropriate preventive services, allocate resources, decide on priorities and target certain populations.

More over the study provides information for the others who want to search more on magnitude of hepatitis B, hepatitis C and HIV among CLD patients and assessment of related risk factors.

4. Objective of the study

4.1. General Objective

- To determine the prevalence of HBV, HCV and HIV infection among clinically diagnosed chronic liver disease patients visiting at Black Lion, St. Paul and Armed force hospital in Addis Ababa, Ethiopia.

4.2. Specific Objective

- To determine HBV sero- prevalence among clinically diagnosed chronic liver disease patients
- To determine HCV sero- prevalence among clinically diagnosed chronic liver disease patients
- To determine HIV sero- prevalence among clinically diagnosed chronic liver disease patients
- To determine the risk factors for HBV and HCV infection among clinically diagnosed chronic liver disease patients.

5. Materials and Methods

5.1. Study design and study period

A hospital based cross sectional study was conducted at Black Lion, St. Paul and Armed force hospital from March to May 2014.

5.2. Study Area

The study was conducted in Addis Ababa; the capital city of Ethiopia, established in 1887. A population of 3,384,569 (2007 census) Located in the geographic center of the country which Lies at an altitude of 7,546 feet (2,300 meters) with a grassland biome. There are a total of 12 Government owned Hospitals in Addis Ababa city, Four are under the Federal Ministry of Health (one of which is a teaching Hospital), one is a university Hospital under the Addis Ababa University, Five are general hospitals and under the Addis Ababa regional Health Bureau the other two are Army and Police Hospitals.

All of these government hospitals were built before 30 years ago. Five of the hospitals are also referral hospitals serving people coming from all over Ethiopia. Based on having organized gastrointestinal unit and availability patient Black Lion, St. Paul and Armed force hospital selected for this study.

5.3. Study Variables

Dependent variables: - Hepatitis B, Hepatitis C and HIV sero- status

Independent variables: - Age, sex, marital status, occupational status, risk factors for ,HBV and HCV (multiple sexual partners, blood transfusion history, abortion, history of surgical procedure, uvuloctomy, ear/nose piercing, circumcision, contact with jaundiced patients, dental extraction, tattooing, shaving by barbers)

5.4. Sample size estimation and Sampling technique

First the required sample size was calculated by applying standardized statistical formula. Then non probability convenient sampling method was employed to get the chronic liver disease patients till the calculated sample size was achieved.

Sample size was calculated by taking over all Hepatitis B surface antigen prevalence from other studies conducted among blood donors 8.3% (58), at 95% Confidence interval and 5 % margin of error.

Sample size was derived using the following formula:-

$$N = \frac{Z^2 P (100-P)}{E^2}$$

Where: - N = Sample size required

Z: Standard normal distribution abscissa corresponding to 95% confidence interval (1.96)

d= desired level of precision/Marginal error (0.05).

P= Proportion in the target population to have HBV.

According to the formula the calculated sample size was 117.

5.5. Source population

All patients who were visiting gastrointestinal unit at Black Lion, St. Paul and Armed force hospital during the data collection period where the source population.

5.6. Study population

Clinically diagnosed CLD patients who were visiting at Black Lion, St. Paul and Armed force hospital, during the study period were the study population. The clinical diagnostic criteria for grouping patients as chronic liver disease patients were based on the presence of patient history of either:-

a) Clinical signs like ascites, hepatomegaly, splenomegaly with other clinical features including jaundice, palmar erythema, clubbing, edema, axillary and pubic hair loss, Spider nevi, flapping tremors, drowsiness, confusion and coma.

b) Ultra sound showing coarse hepatic texture, changes in liver size, increased portal vein diameter, and splenomegaly.

c) Impaired liver function tests including raised level of alanineaminotransferase (ALT).

5.7. Questionnaire Administration

Structured questionnaire was used to obtain demographic data, and risk factors associated with HBV and HCV infection which include sex, age residence occupation religion, maternal status, history of sexual transmitted disease, abortion etc. The questionnaire was administered to all participants enrolled in the each study prior to specimen collection.

5.8. Eligibility criteria

5.8.1. Inclusion criteria

- All voluntary adult patients (age 18 and above) with clinically recognizable chronic liver disease in the three hospitals during the study period were eligible.

5.8.2. Exclusion criteria

- All Patients unable to communicate and under comma were excluded.

5.9. Data collection procedure

Clinically diagnosed chronic liver disease patients were sort by the clinical history on their patient history sheet. All patients, whose clinical history report they are chronic liver disease patients was contacted. Socio-demographic data and other risk factor data was collected with a structured questionnaire from the study participants by the investigator and 5 ml of venous blood was collected aseptically by vacuntainer needle in serum separating tube, samples were left at room temperature for 30 minutes to facilitate clotting and were centrifuged at 3000rpm for 5 minutes to get clear serum for serological analysis. HIV test was performed immediately and Serum was secondly aliquated in nunc tubes and stored at -20°C awaiting transport to Armed Force General hospital laboratory for Hepatitis B and hepatitis C ELISA testing. Repeated freezing and thawing was avoided. All Regulation was strictly followed during packaging and transportation. HIV results were issued to the CLD patients immediately after post-test counseling. Other results for HBsAg, and anti HCV, were given to the patients during the next attendance.

5.10. Serological Test Principle

5.10.1. HBsAg EIA Test

Serological testes for determining HBV by ELISA third generation immunoassay kits, (DIALAB CHEMISCH-TECHNISCHEN, JOAQUIN COSTA, PLANTA, GESELLSCHAFT, AUSTRIA) it is based on a one step “Sandwich principle”. Antibody to HBsAg coupled to horseradish peroxidase (HRP) is served as conjugate with 3, 3´, 5, 5´- Tetramethylbenzidine (TMB) and peroxidase as a substrate. Upon completion of the test, a color developed which is directly proportional to the amount of the HBsAg in the sample. For detection HBsAg in human serum or plasma (60).

5.10.2. HCV Antibody EIA

Serological testes for determining HCV by ELISA third generation immunoassay kits, (HUMAN BIOCHEMICA UND DIAGNOSTICA, MBH, GERMANY) This assay based on formation of stable Ag-Ab complex when a positive sample is added into a well coated with recombinant Ag representing HCV epitopes. The conjugate is a rabbit antihuman IgG labeled with horseradish peroxidase and enzyme substrate, when it was added, a blue color developed. This color changes to yellow after blocking the reaction with sulphuric acid. The intensity of color is proportional to the anti-HCV concentration that present in the sample (62).

5.10.3. HIV TEST

HIV-test to detect HIV, the anti-HIV antibody test was used for screening according to the manufacturer's instructions (rapid test currently used in national algorithm for Ethiopia; (KHB, Shangha Kehua Bioengineering Co., Ltd. China) for screening and positive samples were re-tested with STAT-PACK (Chembio HIV 1/2 STAT-PAK™ Assay, CHEMBIO DIAGNOSTIC SYSTEMS, INC., MEDFORD, NY, USA). Samples giving discordant results in the two tests were reexamined using tie-breaker, (Uni-Gold HIV, Trinity Biotech PLC, Co.Wicklow, Ireland).

5.10.3.1. KHB HIV

KHB rapid test determine by kit (KHB SHANFAHI KEHUA BIO-ENGINEERING CO, LTD) with principle of the gold-gp160 conjugate and gold-gp36 conjugate are coated to the conjugate pad in advance. The test line (HIV type I+II antigens) and the control line (monoclonal antibody against gp160) and pre-coated on the surface of NC membrane. When the sample that added to the sample pad migrate through the conjugate pad, it reconstitutes and mixes with colloidal gold-antigen conjugates. The mixture continues to migrate through the NC membrane to the pre-coated antigens or antibody present on the membrane. A purple red test line will be visible in the strip if there are enough antibodies to HIV-1/HIV-2 in the sample. If antibodies to HIV-1/ HIV-2 are absent, or are present at every level, then no color will appear in the test line. The control line purple red is used as quality control only and does not affect the result of the test (64).

5.10.3.2. STAT PACK HIV

STAT PACK rapid test determine by kit (CHEMGIO DIGNOSTIC SYSTEMS, INC) with principle of the specimen/buffer mixture migrates along the test strip by capillary action, reconstituting the conjugate. If present, the antibodies bind to the colloidal gold conjugated antibody binding protein. In a reactive sample, the dye conjugated immune complex migrates on the nitrocellulose membrane and is captured by the antigens immobilized in the TEST (T) area producing a pink/purple line. In the absence of HIV-1 and HIV-2 antibodies, the sample continues to migrate along the membrane and produces a pink/purple line in the CONTROL (C) area containing immunoglobulin G antigens (66).

5.10.3.3. UNIGOLD

UNIGOLD rapid test determine by kit (TRINTI BIO TEC BRAY, CO. WICKLOW, IRELAND) with principle of Recombinant proteins representing the immune dominant regions of the envelope proteins of HIV-1 and HIV-2, glycoprotein gp41, gp120 (HIV-1) and glycoprotein gp36 (HIV-2) respectively are immobilized at the test region of the nitrocellulose strip. These proteins are also linked to colloidal gold and impregnated below the test region of the device. A narrow band of the nitrocellulose membrane is also sensitized as a control region during testing two drops of serum, plasma or whole blood is applied to the sample port, followed by two drops of wash buffer and allowed to react. Antibodies of any immunoglobulin class, specific to recombinant HIV-1 or HIV-2 proteins will react with colloidal gold linked antigen. The antibody protein-colloidal gold complex moves chromatographically along the membrane to the test and control region (68). (For detailed all laboratory method see annex I)

5.11. Quality control

Prior to the beginning of any data collection, all data collectors were trained by the principal investigator on an overview of the assessment and its objectives. During the entry of data it was cross checked to assure the right data was entered correctly. All specimens were collected according to the standard operating procedure of specimen collection. The quality of test results were maintained using the internal quality control of the test kits for ELISA method and by using a known negative and positive samples an external quality control.

5.12. Data analysis and interpretation

Data was entered and analyzed using the statistical soft ware SPSS version 15.0.The seroprevalence for HBsAg, HCV, and HIV was expressed in percentages for the entire study group and results obtained were presented in tables, figures and graphs. The chi-square test was used to determine the association between variable. Odds ratios (OR) and 95% confidence intervals (CI) were used a measure of the strength of association.

5.13. Ethical Considerations

Ethical clearance was obtained from departmental research and ethics committee of School of Addis Ababa University Medical laboratory technology and official permission to collect data has gotten from the Management Committee of selected hospitals. The study participants were informed about the purpose of the study and written informed consent was obtained from each participant during testing no names were used in the data collection process, only unique identity numbers was used. For those whose HIV status did not know pre and post test counseling was given by physician or trained nurse. Sample taken from each patient was coded and results obtained were kept confidential. The results were notified to study participants. Individuals found to be positive for HBV, HCV and/or HIV were linked to physicians for monitoring and further management.

5.13.1. Dissemination of results

This study on completion could serve as a reference material to researchers, experts or policy makers for intervention. To reach these bodies the finalized paper will be submitted to School of laboratory technology, Addis Ababa University. So it can serve as a reference in the library. In addition, a copy of this material will be given to Black Lion, St. Paul and Armed force hospital. The result will also be disseminated through publication in peer reviewed local and international journals and through presenting it in relevant workshops and seminars.

6. Result

All the respondents who provided informed written consent were above 18 years of age, and clinically diagnosed with CLD. A total of 117patients were included in the final analysis, Out of these, 54.7% (n=64) were from Black Lion, 21.4% (n=25) were from St. Paul's general Specialized Hospital, and the remaining 23.9% (n=28) were from Armed force teaching Hospital.

6.1 Socio demographic characteristics

Among the 117 analyzed clients, 70.1% (n=82) represents male participants while 29.9% (n= 35) represents female participants with a male to female ratio of 2.3:1. The set age range was 18 to 78 years of age with a median age of 39 and range 60. Most of the analyzed patients were in the age group of 28 to 37 years of age. This represents a percentage mark of 31.6% (n= 37), compared to the older aged group of ≥ 68years which was represented by only 5.1% (n= 6).

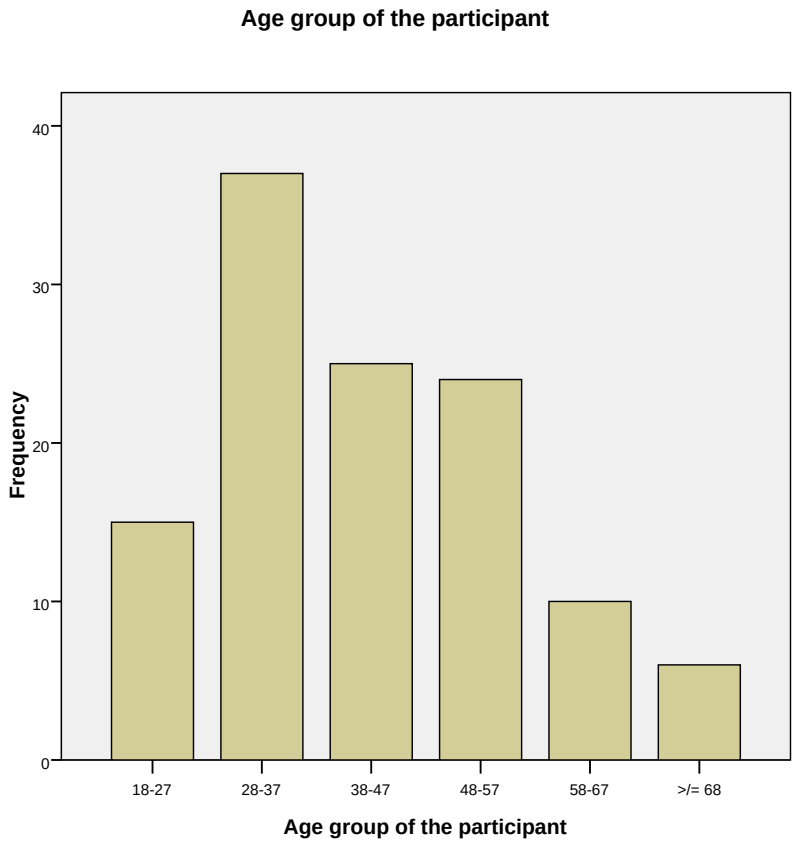

Figure 5; Age distribution of chronic liver disease patients at Black Lion, St. Paul and Armed force hospital from March 2014 - May 2014**.**

Most of the clients were found to be married. Among the 117 analyzed clients, 73.5% (n= 86) were married. , 19.7% (n=23) were single and 1.7% (N=2) divorced, whereas the remaining 5.1% (N=6) were widow with no statistical difference.

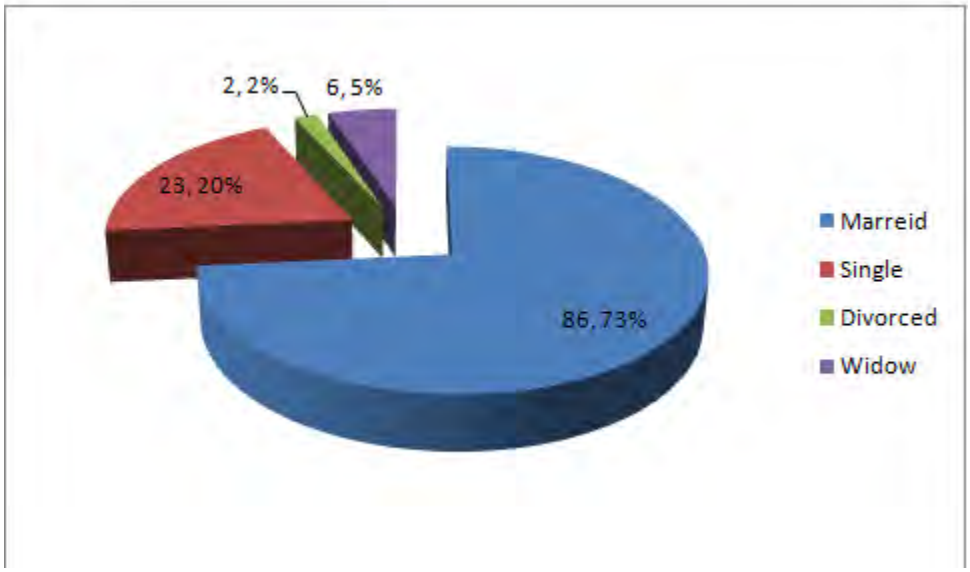

Figure 6. Marital status of chronic liver disease patients at Black Lion, St. Paul and Armed force hospital from March 2014 - May 2014.

Concerning ethnicity majority 44.4% (n=52) of chronic liver disease patients were Oromo Followed by 31.6% (n=37) Amhara, 12% (n=14) Tigare, 8.5% (n=10) Gurage and 3.4(n=4) Wolita. There was no significant difference between being Oromo, Amhara or other ethnic group in relation to HBV, HCV and HIV infection. On the religious background, most of the clients were Christians. Represented by 85.5% (n=100) while14.5% (17) number of participants self identified as Muslim. In relation to living area, 59.8% (n=70) of the study participants were urban dwellers and the remaining40.2 % (n=47) were rural dwellers.

In relation to educational status the study revealed two extremes; in which 37.6% (n=44) of the study participants were not educated and/ had not received formal education and.23.1% (n=27) of the study participants had above grade 12 education But there was no statistically significant relation between illiterates and the educated in acquisition of HBV, HCV and HIV infection (P> 0.05).

6.2. Prevalence of HBV, HCV and HIV

The results of the study showed that from 117 samples of clinically diagnosed CLD patients, 62.3% (n=73) samples were marker positive. From overall positive samples 34.2% (40) were HBsAg positive, 18.8% (n=22) were Anti- HCV positive, while 9.4% (n=11) samples were positive for HIV. Concerning to confection 3.4% (n=4) were HBV / HIV positive, 2.5% (n=3) were HCV/HIV and 2.5% (n=3) were HCV/HBV.

6.2.1. Magnitude of Hepatitis Bvirus infection by socio demographic characteristics

The prevalence of Hepatitis B virus was determined by the presence of Hepatitis B surface Antigen in individual's serum using an Enzyme immunoassay. The prevalence of HBsAg in chronic liver disease was 34.2%. The sex specific prevalence rate was higher in males 35.3% (29/82) than females 31.8% (11/34) but the difference did not reach statistical significance (P=0.68). The prevalence of HBV by specific age group was highest 30% (12/40) in 28-37 and lowest 2.5 %(1/40) in the age groups of ≥68 (Figure 7).

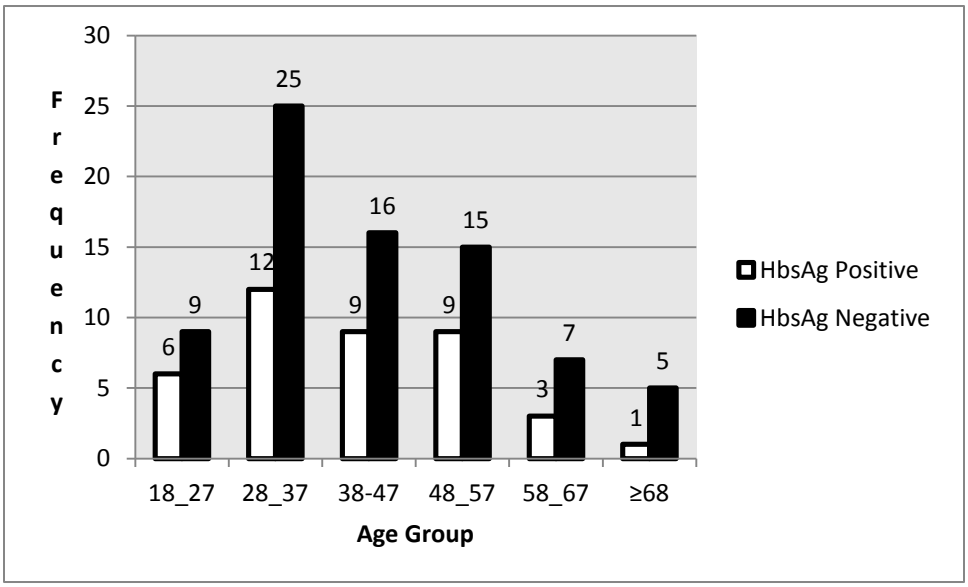

Figure 7. The distribution of HBsAg by age among CLD patients at Black Lion, St. Paul and Armed force hospital form March 2014 - May 2014.

Majority 14.5% (17/117) of HBsAg positive CLD patients were in the ethnic group Oromo(male 14, female 3) followed by Amhara and Tigrae 6.8% (8/117) where 50% of them were males and 50% females for Amhara and 87.5 male and 12.5 female for Tigrae . In relation to Religion

distributions of HBsAg positivity was higher among Christians accounting 37.7% (37/98) and it was 5.7 (3/19) among Muslim showing no statistical difference (P=0.08). Concerning to educational status highest percent was found in study participants with an education level elementary 12(30%) followed by Illiterate 10(25%) and least Read and write level which were not statically significance (P=0.58).

With regard to living area 23% (27/117) HBsAg tested positive study participants were urban Dwellers and 11.1% (13/117) were rural dwellers. In relation to marital status the highest percent of positivity was taken by married with a prevalence of 24.7% (29/117) followed by singles 5.9% (7/117) and a least prevalence was observed in Divorced 0.85% (1/117).

Table1. Seroprevalence of Hepatitis B, Hepatitis C and HIV in relation to socio demographic characteristics among clinically diagnosed CLD patients attending at Black Lion, St. Paulo's and Armed Force hospital form March 2014 - May 2014.

Variable	HbsAg Positive No. (%)	χ2	P-value	HCV Positive No. (%)	χ2	Pvalue	HIV Positive No. (%)	χ2	P-value
Age group									
18-27	6(15)			2(9.1)			0		
28-37	12(30)	0.38	0.54	6(27.3)	2.94	0.09	5(45.2)		
38-47	9(22.5)			4(18.2)			2(18.2)	0.01	0.92
48-57	9(22.5)			4(18.2)			3(27.3)		
58-67	3(7.5)			3(13.6)			1(9.1)		
≥68	1(2.5)			3(13.6)			0(0)		
Sex									
Male	29(72.5)	0.17	0.68	17(77.3)	0.70	0.42	8(72.7)		
Female	11(27.5)			5(22.7)			3(27.7)	0.41	0.84
Residence									
Urban	27(67.5)			15(68.2)			11(100)		
Ruler	13(32.5)	0.22	1.51	7(31.8)	0.80	0.37	0	12	0.99

Etinicity									
Oromo	17(42.5)	0.47	0.22	8(36.4)	0.7	0.25	7(63.6)	0.8	0.28
Amhara	8(20)			6(27.3)			3(27.3)		
Tigrea	8(20)			4(18.2)			1(9.1)		
Gurage	6(15)			3(13.6)			0		
Wolita	1(2.5)			1(4.5)			0		
Marital status									
Married	29(74)	0.44	0.51	14(63.6)	1.77	0.16	9(81.8)	0.01	0.92
Singe	7(20.8)			5(22.7)			1(9.1)		
Divorced	1(1.3)			1(4.5)			0(0)		
Widow	3(3.9)			2(9.1)			1(9.1)		
Occupation									
Self employee	15(37.5)	0.35	0.89	4(18.2)	3.71	0.061	3(27.3)	0.55	0.46
Student	3(7.5)			2(9.1)			1(9.1)		
House wife	4(10)			1 (4.5)			1(9.1)		
Farmer	1(2.5)			4(8.2)			1(9.1)		
Military	9(22.5)			2(9.1)			3(27.3)		
Government	7(17.5)			5(22.7)			2(18.2)		
Pension	1(2.5)			(18.2)			1(9.1)		
Education									
Illiterate	25(25.5)	035	0.89	6(27.3)	0.68	0.79	2(18.2)	0.58	0.45
Read and write	8(2.5)			4(18.2)			0		
Elementary	16(30)			5(22.7)			4(36.4)		
Secondary	10(20)			1(4.5)			3(27.3)		
Collage	18(22.5)			6(27.3)			2(18.2)		

6.2.2. Magnitude of Hepatitis C infection by socio demographic characteristics

The prevalence of Hepatitis C virus was determined by the presence of Hepatitis C antibody in individual's serum using an Enzyme immunoassay assay. Twenty two 18.8 % (22/117) of the study participants were positive for anti-HCV Ab. The sex specific prevalence rate was higher among male 20.7% (17/82) than female (5/35) 18.4% the difference was not statistical significance (P= 0.42). The highest prevalence of HCV by specific age group was 27% (6/22) in the age group of 28-37 and was lowest (5%) in the age group of 18-27. (Figure 8).

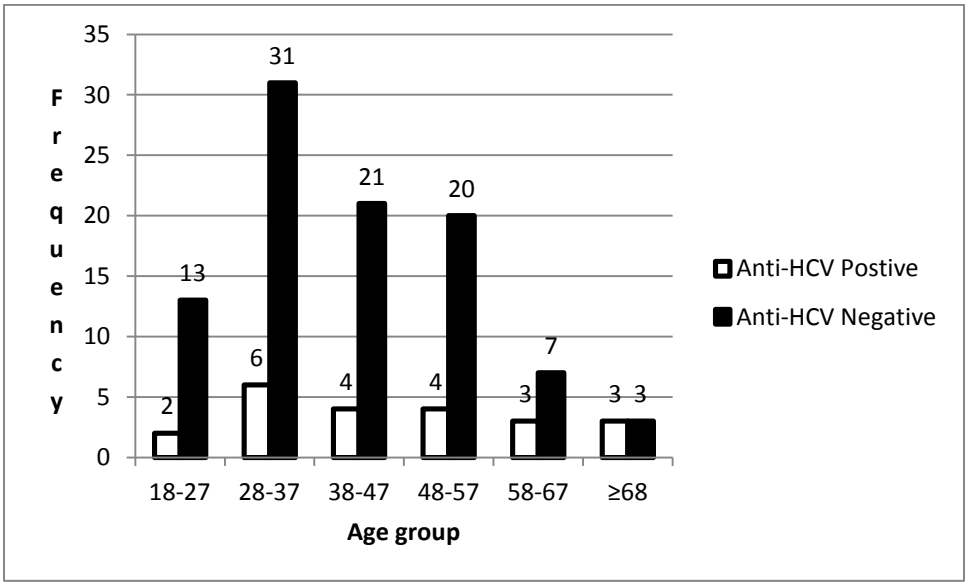

Figure 8. The distribution of anti-HCV Ab by age among CLD patients at Black Lion, Armed Force and St. Paul hospitals from March 2014 -May 2014.

With respect to marital status sero-positivity for anti-HCV Ab was highest among married 11.9% (14/117) followed by single 4.2% (5/117). Concerning religion and ethnicity, similar to HBV positivity, the ethnic group Oromo and Christians took the highest portion for anti-HCV Ab positivity with figure of 6.8% (8/117) and 13.6% (16/117) respectively and it were not statically significant(P=0.16).

6.2.3 Magnitude of HIV infection by socio demographic characteristics

The prevalence of HIV virus was determined by using national test algorism. Out 117 specimen tested, HIV was detected in 11 CLD patients giving a prevalence of 9.4%. The distribution of the HIV infection among CLD patients according to age group was analyzed and the result shown in Figure 5. There was no significant association (P =0.92) between the age of participants and the infections. For HIV infection CLD patients, in age group 28-37 years had the highest prevalence of 4.2% (5/117) while CLD patients both age groups 18-27 and ≥68 years had the lowest prevalence.

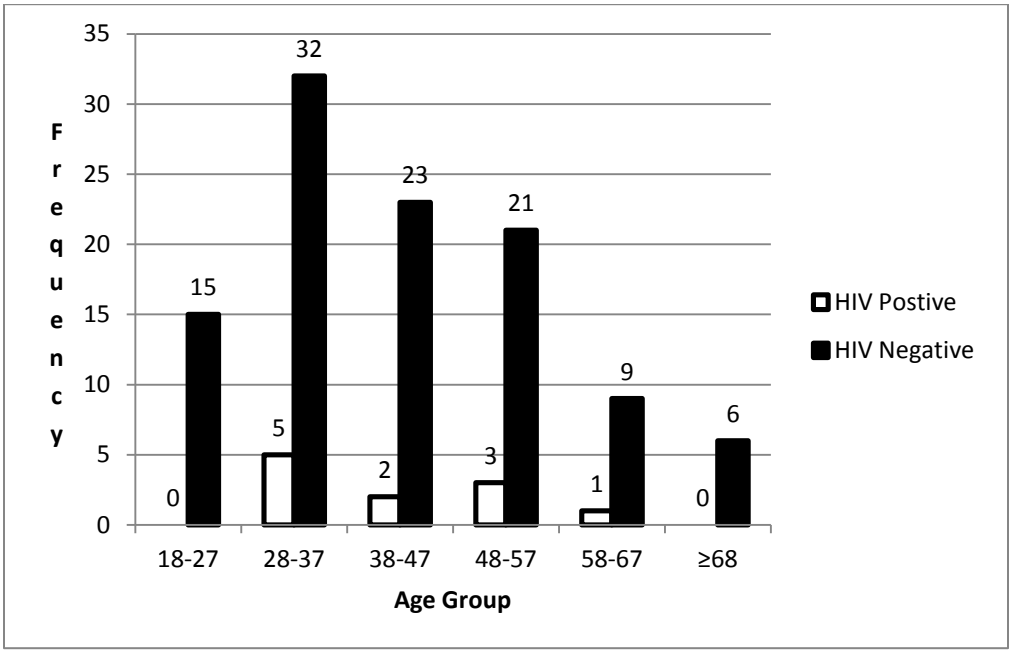

Figure 9. The distribution of HIV by age among CLD patients at Black Lion St. Paul and Armed force hospital form March 2014 - May 2014.

In relation to sex prevalence rate was higher among male 6.8% (8/117) than female 2.5% (3/117) the difference was not statistical significance (P= 0.84). The distribution of HIV according to marital status was analyzed and the result shown in Table1. The highest prevalence of HIV was found among married 7.6 %(9/117) as against Divorced 0% and There was no significant association (P=0.92) between marital status and HIV infection.

With respect to living area 9.4% (11/117) HIV tested positive study participants were urban Dwellers and 0% were rural dwellers there was no significant association (P = 0.99) between residence and HIV infection.

The distribution of HIV according to educational status was analyzed and the result showed that there was no significant association (P=0.45) between the educational status and presence HIV-Abs .CLD patients who had only elementary education had the highest prevalence 3.4 %(4/117) while those with both illiterate and collage had the lowest prevalence 1.7 %(2/117) (Table1).

6.3. Risk factors of HBV and HCV virus infection

Among 117 CLD patients risk factors studied multiple sexual partners and blood transfusions were to be associated with hepatitis B and hepatitis C infection respectively. Other risk factors like, history of STD/STI, dental extraction at health facility, circumcision, surgical procedure, hospital admission, ear piercing, uvuloctomy, tattooing on body, tattooing on gum, contact with jaundiced patient, alcohol consumption, abortion shaving by barber were not associated with both infection.

Concerning to multiple sexual partner 22.2% (26/117) of CLD patients reported that they had history of multiple sexual partner. From those 11.9% (14/117) were positive for HBsAg (AOR= 6.8; 95% CI: 1.6-27, P= 0.008). Those who had multiple sexual partner were 6.8 time more likely to have infection with HBV than their counter parts and difference was statistically significant (P<0.05).

In relation to blood transfusion 35.8% (42/117) of CLD patients reported that they had history of blood transfusion from those 11.1 % (13/117) were positive for anti-HCVAb (AOR= 4.7; 95% CI: 1.6-13.8, P= 0.05). Those who had blood transfusion history were 4.7 time more likely to have HCV infection than those who had not and difference was statistically significant (P<0.05).

Table 2. Bivarate Assessment of risk factor for hepatitis B infection at Black Lion, St. Paul and Armed force hospital from March 2014 - May 2014.

Variable	Frequency	No of HbsAg positive	$\chi 2$	p-value
Community acquired				
Tattooing on gum	12	3	0.53	0.48
Tattooing on body	15	5	0.06	0.94
Shaving by barber* **	68	27	2.59	0.14
Body piercing	11	5	0.66	0.41
Uvuloctomy	75	27	0.30	0.58
Ear piercing	34	11	0.72	0.79
Contact with jaundice patient	15	6	0.25	0.61
Circumcision	91	3	0.80	0.38
Hospital acquired				
Hospitalization	70	26	0.68	0.41
Blood transfusion	42	12	0.93	0.33
Dental extraction	46	12	2.25	0.13
Surgical procedure	22	10	1.48	0.22
Behavioral acquired				
Alcohol consumption	59	20	0.04	0.95
History of STI/STD	24	10	0.73	0.39
Multiple sexual partner	26	7	5.50*	0.019*
Delivery by TBA**	8	2	0.33	0.57
Abortion**	10	3	0.00	0.93

= concerning female *= concerning male *statically significant ($P \leq 0.05$).

The identified risk factors behavioral acquired found to be associated with hepatitis B ($p<0.05$).

Table 3. Bivariate Assessment of risk factor for Hepatitis C infection at Black Lion, St. Paul and Armed force hospital from March 2014 - May 2014.

Variable	Frequency	No of anti-HCV positive	$\chi 2$	p-value
Community acquired				
Tattooing on gum	12	3	0.32	0.56
Tattooing on body	15	3	0.16	0.90
Shaving by barber* **	68	12	1.41	0.22
Body piercing	11	2	0.03	0.96
Uvuloctomy	75	5	0.33	0.96
Ear piercing	34	14	0.55	0.47
Contact with jaundice patients	15	2	0.36	0.56
Circumcision	91	17	0.004	0.95
Hospital acquired				
Hospitalization	70	12	0.31	0.58
Blood transfusion	42	13	6.1	0.015*
Dental extraction	46	10	0.63	0.24
Surgical procedure	22	3	0.50	0.49
Behavioral acquired				
Alcohol consumption	59	11	0.002	0.96
History of STI/STD	24	4	0.58	0.31
Multiple sexual partner	26	7	1.35	0.23
Delivery by TBA**	8	4	0.24	0.64
Abortion**	10	3	1.74	0.18

= concerning female *= concerning male *statically significant ($P \leq 0.05$).

The identified risk factors hospital acquired found to be associated with hepatitis C ($p<0.05$).

6.3.1. Multivariate Analysis of risk factor for hepatitis B infection

In bivariate analysis, it was found that significant risk factors for HBV infection among studied hospital personnel were: multiple sexual partners (COR= 2.9; 95% CI: 1.2-7.1, P= 0.019) Multiple logistic regression analysis was applied for controlling confounders and for evaluating the effects of risk variables on HBV infection among studied group after analysis statically significance was found (AOR= 6.8; 95% CI: 1.6-27.8, P= 0.008)

Table 4. Multivariate analysis of risk factor for hepatitis B infection at Black Lion, St. Paul and Armed force hospital from March 2014 - May 2014.

Characteristics	OR (95%, CI)	p-value	aOR (95%, CI)	p-value
Multiple sexual partner				
No	1		1	
Yes	2.9 (1.2-7.1)	0.019*	6.8(1.6-27.8)	0.008*

*statically significant (P≤0.05)

6.3.2. Multivariate Analysis of risk factor for hepatitis C infection

In bivariate analysis, it was found that significant risk factors for HCV infection among studied hospital personnel were: blood transfusion (COR= 3.3; 95% CI: 1.3-8.5, P= 0.015) Multiple logistic regression analysis was applied for controlling confounders and for evaluating the effects of risk variables on HCV infection among studied group. After analysis statically significance was found (AOR= 4.7; 95% CI: 1.6-13.8, P= 0.005)

Table 5.Multivariate Analysis of risk factor for hepatitis C infection at Black Lion, St. Paul and Armed force hospital from March 2014 - May 2014.

Characteristics	OR (95%, CI)	p-value	AOR (95%, CI)	p-value
Blood transfusion				
No	1			
Yes	3.3(1.30-8.54)	0.015*	4.7 (1.6-13.8)	0.005*

*statically significant (P≤0.05)

7. Discussion

In the present study conducted in 117 clinically diagnosed cases of chronic liver disease 34.2% were positive for HBsAg, 18.8% were positive for HCV antibodies and 9.4% were positive for HIV antibodies. Comparable 35.8% HBsAg and 22.5% HCV Ab seropositivity was reported previously from Ethiopia may 2011 (4). This implies that there has been no appreciable change in the seroprevalence of HBV in the area over the last 3 year period.

Concerning HCV Ab it was higher than our study (22.5%) and it could be due to using non confirmatory test kits for analysis of HCV Ab. When the finding of the current study were compared with results reported from similar study subjects of other countries, 44.6% HBsAg and 26% HCVAbs in India among chronic liver disease patients (26) 42.9% HBsAg and 7.1 HCV Antibody in Gana among HCC patients (25) and 36% HBsAg % and 24% HCV Antibody among HCC patients in Zimbabwe (28). In relation to HBsAg the reasons for the relatively lower rate of seroprevalence in our study compared with other studies cannot be completely discerned. But, the difference in demographic characteristics of the study population, the difference in hepatitis epidemiology in these countries, awareness of the routes of HBV transmission, efforts made to implement universal precautions by health professionals and preliminary benefits due to the initiation of national programs of immunization in other countries might explain these discrepancies.

Liver disease has become an important cause of morbidity and mortality in patients with HIV/AIDS due largely to complications derived from chronic active hepatitis involving co-infection with hepatitis B or C virus. HIV alone can also cause hepatic involvement with other Systemic disease in advanced HIV stage (57). It has been estimated that approximately one-third of the deaths of patients with HIV infection are in some way related to liver disease. Prevalence of HIV found 9.4% in the present study. In another study it has been reported to be 3% in India (55) the difference may be due to difference in demographic characteristics of the two populations.

Age distribution of CLD in our study population showed that 73.5% of CLD cases were before fifty years of age. 3.4% of patients were up to 20 years of age, about 15.3 in second and 34.1% in third decade and 17% in fourth decade of life. A comparable high age distribution (75.8%) of CLD cases age <50 years was observed by Abel et al., 2010(4). A high prevalence of HBV was recorded in the age group 28-37 with a prevalence of 30% (12/40) and HCV it was 27.3% (6/22) in the age group 28-37. The reason could be an early childhood infection with the

Hepatitis viruses. The chronic infection with hepatitis viruses leads to slow progressive liver disease. It may end up in cirrhosis, chronic liver failure and hepatocellular carcinoma over a period of up to 30 years (3).

In relation to sex the present study HBV, HCV and HIV prevalence was 35.3% for males and 31.4% for females ($\chi2$=0.17, P=0.68), 20.7 %males and 14.2% for females ($\chi2$=0.7, P=0.42) and 9.7% for male and 8.5% for females ($\chi2$=0.4, P=0.48) respectively indicating a reasonably higher proportion in males than females in three diseases could be a reflection of more males coming for treatment in our setting. Besides it could be due to more social mobility in males than females and thus greater vulnerability to be infected. However, there were no significant differences (p>0.5) on other hand Comparable results were reported in a number of studies concerning HBV infection, in Ethiopia 5.4 in males and 2.7% in females (27), other study in India 72% in males and 28% in females (25).

In our study, cases of HCV associated CLD was much less common than the HBV associated cases and this higher HBV to HCV pattern is well recognized in Ethiopia for the last few years as many studies have shown on different study populations, in Gondar HBV to HCV was 4.1%/1.3% (47), in Amhara and Tigray regional state 6.2%/1.7% (40) . Contrary to the above reports different studies reported higher prevalence of HCV to HBV in different study populations the higher prevalence of HCV to HBV infection in the other studies could be explained by geographical variation and with the fact that Hepatitis C virus has a higher propensity for causing liver disease than Hepatitis B virus.

This study found among the risk factors studied to be associated with Hepatitis B included multiple sexual partners (aOR: 6.8, 95%CI: 1.6-27.8) Other risk factors like blood transfusion, tooth extraction in health facility, ear piercing, tattooing ,Hospital admission , Surgical procedure were not associated with Hepatitis B virus infection. In study conducted by Lavanchy D (28) results suggested that younger age of sexual initiation and multiple sex partners are significant risk factors for the acquisition of Hepatitis B Virus. Sexual transmission has also been suggested to play a role in East Africa as demonstrated in the ugandan study (25). The finding that scarification is a risk factor for acquiring H epatitis B is however different from a Southwestern Uganda study which found low rates of scarification (24). This might be due to variation of culture of underlying population studied between the two areas.

Transfusion of blood products has been a leading cause of transmission of HCV; however, due to

improved screening, transmission through transfusions has decreased in most developed countries. In Japan, incidence of post-transfusion non-A non-B hepatitis among those with less than 10 transfusions dropped from 4.9% (1988-Oct '89) to 1.9% (Nov'89-90) after screening with first-generation anti-HCV test was introduced. (50) In the US, incidence of post-transfusion hepatitis C dropped from 3.84% to 0.57% per patient (0.03% per unit blood) after HCV screening was introduced in 1990. (49)In England, the frequency HCV infected donations dropped from 1 in 520,000 (1993-98) to 1 in 30 million (1999-2001) when donations were tested for HCV RNA (50).

Multivariate analysis showed a strong association between blood transfusion and HCV infection. The overall prevalence of HCV among chronic liver disease patients who had history of blood transfusion was 30.9% (13/54) (aOR: 4.7, 95%CI: 1.6-13.8). Those who had history of blood transfusion were 4.7 time more likely to have infection with HCV than their counter parts and the difference was statistically significant. This might be explained by poor screening of blood donor. Correspondingly, this finding agreed with a study conducted in among Haemodialysis Patients in Gaza Strip, Palestine the risk of infection increased sharply if patient received more than 15 blood units for HBV infection, and 10 blood units for HCV infection (50). However, no other apparent risk factor that caused HCV infection was inferred from our study.

Concerning the various occupational groups the distribution of HBV infection was, self employed 37.5% (n=15) house wife 10 %(n= 4) student 7.5 %(n=3) military 22.5%(n=9) farmer 5%(n=1) government worker 17.5%(n=7) and pension 2.5%(n=1). No significant relationship was found between occupation and HBV infection ($p>0.05$). In the present study a higher prevalence of HBV was observed in self employee workers; however, because of their small numbers statistically conclusive inferences may be difficult to make. Similarly the distribution of HCV infection in the various occupational groups was house wife 4.5 %(n= 1) student 9.1 %(n=2) military 9.1%(n=2) farmer 18.2%(n=4) government worker 22.7%(n=5) and pension 18.2%(n=4). For HCV the higher sero-positivity was recorded among government worker it was not significantly associated with none of the occupational groups.

In summary this study has revealed high prevalence of HBV and HCV in chronic liver disease patients. However, neither associated risk factors nor associated socio-demographic characteristics have been significantly associated with acquisition of infection except patients

who had history blood transfusion and multiple sexual partners with respect to HCV and hepatitis B infection respectively.

8. Limitations of the study

Limitation

- Chronic liver disease patients with occult HBV infection were not identified.

- Our study was only set and able to measure the prevalence of HBV , HCV and HIV in clinically diagnosed chronic liver disease patients (in general term) and therefore this study didn't measure the prevalence of these viruses in the different sub-groups of chronic liver disease classification due financial limitation.

9 .Conclusion and recommendation

Conclusion

This study showed that HBV, HCV and HIV prevalence among clinically diagnosed liver disease patients 34.2% (n=40), 18.8 %(n=22) and 9.4% (n=11) respectively. There was no statistical significance difference in acquisition of HBV, HCV and HIV infection With respect to socio-demographic characteristics but statically difference was found CLD patients who had history of multiple sexual partner and history of blood transfusion for hepatitis B and hepatitis C respectively.

Therefore we conclude HBV and HCV infection is high where as HIV is moderate among chronic liver disease patients and those CLD patients who had history of blood transfusion have 4.7 time higher risk of acquisition of HCV infection than those who do not have history of blood transfusion and those who had history of multiple sexual partner have 6.8 time higher risk of acquisition of HBV infection than their counter parts.

Recommendation

Since HBV and HCV are major health problem in chronic liver disease patients and therefore the following recommendations must be carried out for its prevention.

- ✓ All clinically diagnosed CLD patients should be tested for HBV and HCV sero status
- ✓ Vaccination against HBV is instrumental in the prevention viral CLD, and is recommended for all newborns and individuals who are at increased risk for infection especially People with multiple sex partners.
- ✓ Awareness of people on modes of transmission of the hepatitis B and hepatitis C important to prevent viral infection.
- ✓ Adoption of highly sensitive method to screening of blood donor for HCV in blood bank.

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Annex I Laboratory method

1. HBsAg EIA Test

INTRODUCTION

The HBsAg EIA Test Kit is a third generation immunoassay for the qualitative detection of the presence of Hepatitis B Surface Antigen in serum or plasma specimen. The test utilizes monoclonal antibodies to selectively detect various subtypes of HBsAg in serum or plasma.

HBsAg is one of the earliest markers that appear in the blood following infection with Hepatitis B virus (60). This infection of the liver is transmitted through sexual contact, blood borne exposure, transmission from mother to child during delivery, sharing of objects that pierce the skin, child-to-child and household contact. The four major HBsAg subtypes include adw, adr, ayw, and ayr, all sharing the common determinant 'a'. The HBV infection causes a wide variety of liver damage such as acute self-limiting infection, fulminating hepatitis, chronic hepatitis with progression to cirrhosis and liver failure, and asymptomatic chronic carrier state. In HBV infected people, the virus persists for the rest of their lives and can be passed on to others. Therefore, Hepatitis B has become a global public health problem. Infection with HBV results in the appearance of a number of serological markers and one of the first of such markers is Hepatitis B Surface Antigen (HBsAg). HBsAg appears 1-10 weeks after exposure and before biochemical evidence of liver disease or jaundice. (61). Three weeks after the onset of acute hepatitis almost half of the patients will still be positive for HBsAg. In the chronic carrier state, HBsAg persists for 6-12 months with no seroconversion to the corresponding antibodies (59).

PRINCIPLE

The HBsAg EIA Test Kit is a solid phase qualitative enzyme immunoassay based on a sandwich principle for the detection of HBsAg in human serum or plasma. The microwell plate is coated with monoclonal antibodies specific to various subtypes of HBsAg. During testing, the specimen and the enzyme-conjugated HBsAg antibodies are added to the antibody coated microwell plate and then incubated. If the specimen contains HBsAg, it will bind to the antibodies coated on the microwell plate and simultaneously bind to the conjugate to form immobilized antibody-HBsAg-conjugate complexes. If the specimen does not contain HBsAg, the complexes will not be formed. After initial incubation, the micro well plate is washed to remove unbound materials.

Substrate A and substrate B are added and then incubated to produce a blue color, indicating the amount of HBsAg present in the specimen. Sulfuric acid solution is added to the micro well plate to stop the reaction which produces a color change from blue to yellow. The color intensity, which corresponds to the amount of HBsAg present in the specimen, is measured with a micro plate reader at 450/630-700 nm or 450 nm. (60)

STORAGE AND STABILITY

• Unopened test kits should be stored at 2-8°C upon receipt. All reagents are stable through the expiration date printed on the box. Return reagents to 2-8°C immediately after use.

• Allow the sealed pouch to reach room temperature before opening the pouch and removing the required number of strips to prevent condensation of the micro well plate. The remaining unused strips should be stored in the original resalable pouch at 2-8°C and can be used within 1 month of the opening date.

• Concentrated Wash Buffer may be stored at room temperature to avoid crystallization. If crystals are present, warm up the solution at 37°C. Working Wash Buffer is stable for 2 weeks at room temperature.

• Do not expose reagents especially the Substrate to strong light or hypochlorite fumes during storage or incubation steps.

SPECIMEN COLLECTION AND PREPARATION

• The HBsAg EIA Test Kit can be performed using only human serum or plasma collected from venipuncture whole blood.

• EDTA, sodium heparin, and ACD collection tubes may be used to collect venipuncture whole blood and plasma specimens. The preservative sodium azide inactivates horseradish peroxide and may lead to erroneous results.

• Separate serum or plasma from blood as soon as possible to avoid hemolysis. Grossly hemolytic, limpid or turbid samples should not be used. Specimen with extensive particulate should be clarified by centrifugation prior to use. Do not leave specimens at room temperature

for prolonged periods. Serum and plasma specimens may be stored at 2-8°C for up to 7 days prior to assaying. For long term storage, specimens should be kept frozen below -20°C.

• Bring specimens to room temperature prior to testing. Frozen specimens must be completely thawed and mixed well prior to testing. Specimens should not be frozen and thawed repeatedly.

PROCEDURE

1. An aliquot of 50µl of sera sample and control dispense into assigned coated wells

2. Add 50µl of conjugate to each well except for blank well

3. Mix gently and cover the plate with an adhesive seal, and incubated at 37 ºC for 60 min.

4. Wash each well 5 times with 350 µL of Working Wash Buffer

5. Add working substrate in to each well

6. Mix than cover the micro well palate with adhesive seal and incubated at the plate 37 ºC for 30

7. Add 100µl of TMB substrate to each well and incubate at 20-25 ºC for 30 min.

8. Add 100µl of 1 mol / L of H2SO4 to stop the reaction

9. read with photometric at 450 nm, with air blank

10. The cut-off value (COV) calculated as mean of negative control (PCx) +0.050. The sample is reactive if the absorbance ≥ COV.

INTERPRETATION OF RESULT

Non Reactive: specimen with absorbance less than Cut-Off Value is non-reactive for HBsAg and may be considered negative.

Reactive: Specimen to the Cut-Off Value is considered initially reactive for HbsAg. The specimen should be retested in duplicate before final interpretation. Specimens that are reactive in at least one of the re-test are presumed to be repeatedly reactive and should be confirmed using other HBV markers or confirmatory testing. Specimens that are non-reactive on both retests should be retested in duplicate before final interpretation. Specimens that are reactive in at

least one of the re-test are presumed to be repeatedly reactive and should be confirmed using other HBV markers or confirmatory testing. Specimens that are non-reactive on both retests should be considered non-reactive.

Note: Specimens with values within ±10% of the Cut-Off Value should be retested in duplicates for final interpretation.

2. HCV Antibody EIA

INTRODUCTION

The HCV Antibody EIA Test Kit is a third generation immunoassay for the qualitative detection of the presence of IgG antibodies to HCV in serum or plasma specimen. The test utilizes recombinant HCV antigens encoded by the genes for both structural (nucleocapsid) and non-structural proteins to selectively detect antibodies to HCV in serum or plasma (61).

Hepatitis C Virus is a small, enveloped, positive-sense, single-stranded RNA virus. HCV is now known to be the major cause of parenterally transmitted non-A, non-B hepatitis. HCV infection causes a wide variety of chronic liver disease, cirrhosis and liver cancer. The main route of transmission of the virus is via transfusion of blood and blood products, organ transplantation, and sharing contaminated needles and syringes. Antibodies to HCV is found in over 80% of patients with well-documented non-A, non-B hepatitis. Cloning the viral genome has made it possible to develop serologic assays that use recombinant antigens. Compared to the first generation HCV EIA tests using single recombinant antigen, new serologic tests incorporate recombinant protein and/or synthetic peptide antigens to avoid nonspecific cross-reactivity and to increase the sensitivity (62).

PRINCIPLE

The HCV Antibody EIA Test Kit is a solid phase qualitative indirect simultaneous enzyme Immune assay for the detection of IgG antibodies to HCV in human serum or plasma. The micro

Well plate is coated with HCV recombinant antigens. During testing, the specimen diluents and the specimens are added to the antigen coated micro well plate and then incubated. If the specimens contain antibodies to HCV, it will bind to the antigens coated on the micro plate to

form immobilized antigen-HCV antibody complexes. If the specimens do not contain antibodies to HCV, the complexes will not be formed. After initial incubation, the microwell plate is washed to remove unbound materials. The enzyme-conjugated anti-human IgG antibodies are added to the micro well plate and incubated. The enzyme-conjugated anti-human IgG antibodies will bind to the immobilized antigen-HCV antibody complexes present. After the second incubation, the micro well plate is washed to remove unbound materials. Substrate A and substrate B are added and then incubated to produce a blue color indicating the amount of HCV antibodies present in the specimen. Sulfuric acid solution is added to the micro well plate to stop the reaction producing a color change from blue to yellow. The color intensity, which corresponds to the amount of HCV antibodies present in the specimen, is measured with a micro plate reader at 450/630-700 nm or 450 nm. (61)

STORAGE AND STABILITY

• Unopened test kits should be stored at 2-8°C upon receipt. All reagents are stable through the expiration date printed on the box. Return reagents to 2-8°C immediately after use.

• Allow the sealed pouch to reach room temperature before opening the pouch and removing the required number of strips to prevent condensation of the micro well plate. The remaining unused strips should be stored in the original resalable pouch at 2-8°C and can be used within 1 month of the opening date.

• Concentrated Wash Buffer may be stored at room temperature to avoid crystallization. If crystals are present, warm up the solution at 37°C. Working Wash Buffer is stable for 2 weeks at room temperature.

• Do not expose reagents especially the Substrate to strong light or hypochlorite fumes during storage or incubation steps.

SPECIMEN COLLECTION AND PREPARATION

• The HCV Antibody EIA Test Kit can be performed using only human serum or plasma collected from venipuncture whole blood.

• EDTA, sodium heparin, and ACD collection tubes may be used to collect venipuncture whole blood and plasma specimens. The preservative sodium azide inactivates horseradish peroxide and may lead to erroneous results.

• Separate serum or plasma from blood as soon as possible to avoid hemolysis. Grossly hemolytic, lipoid or turbid samples should not be used. Specimen with extensive particulate should be clarified by centrifugation prior to use. Do not leave specimens at room temperature for prolonged periods. Serum and plasma specimens may be stored at 2-8°C for up to 7 days prior to assaying. For long term storage, specimens should be kept frozen below -20°C.

• Bring specimens to room temperature prior to testing. Frozen specimens must be completely thawed and mixed well prior to testing. Specimens should not be frozen and thawed repeatedly.

PROCEDURE

1. Add 100 µL Specimen in respective wells including Negative Control, Positive Control, Blank and specimen wells.
2. Mix gently and cover the plate with an adhesive seal, and incubated at 37 ºC for 60 min
4. Wash each well 8 times with 350 µL of Working Wash Buffer
5. Add 100 µL of Conjugate to each well except for the Blank well
6. Cover the micro well plate with the Plate Sealer and incubate at 37°C for 30 min
7. Repeat Step 4
8. Add 100µl of TMB substrate to each well and incubate at 37ºC for 30 min.
9. Add 100µl of 1 mol / L of H2SO4 to stop the reaction
10. Read with photometric at 450 nm, with air blank

11. The cut-off value (COV) calculated as mean of negative control (PCx) + 0.10. The sample is reactive if the absorbance ≥ COV.

INTERPRETATION OF RESULT

Non-Reactive: Specimens with absorbance less than the Cut-Off Value are non-reactive for antibodies HCV and may be considered negative.

Reactive: Specimens with absorbance greater than or equal to the Cut-Off Value are considered initially reactive for antibodies HCV. The specimen should be retested in duplicate before final

interpretation. Specimens that are reactive in at least one of the re-test are presumed to be repeatedly reactive and should be confirmed using other HBV markers or confirmatory testing. Specimens that are non-reactive on both retests should be considered non-reactive.

Note: Specimens with values within ±10% of the Cut-Off Value should be retested in duplicates for final interpretation.

3. KHB HIV TEST

Principle

The gold-gp160 conjugate and gold-gp36 conjugate are coated to the conjugate pad in advance. The test line (HIV type I+II antigens) and the control line (monoclonal antibody against gp160) and pre-coated on thesurface of NC membrane. When the sample that added to the sample pad migrate through the conjugate pad, it reconstitutes and mixes with colloidal gold-antigen conjugates. The mixture continues to migrate through the NC membrane to the pre-coated antigens or antibody present on the membrane. A purple red test line will be visible in the strip if there are enough antibodies to HIV-1/HIV-2 in the sample. If antibodies to HIV-1/ HIV-2 are absent, or are present at every level, then no color will appear in the test line. The control line purple red is used as quality control only and does not affect the result of the test (63).

QUALITY CONTROL

Built-in control feature the control line serves as a built-in internal control and gives confirmation of sample addition and proper test performance. A pink/purple line will appear in the CONTROL (C) area if the test has been performed correctly and the device is working properly (Please see section: Interpretation of Test Results)

SPECIMEN COLLECTION

KHB Assay is performed on fingerstick whole blood, venous whole blood, serum orplasma specimens. Fingerstick Whole Blood: Prepare to perform the fingerstick blood collection procedure. Clean the finger of the person being tested with an antiseptic wipe. Allow the finger to dry thoroughly or wipe dry with a sterile gauze pad. Using a sterile lancet, puncture the skin just off the center of the finger and wipe away the first drop with sterile gauze and avoid

squeezing the fingertip to accelerate bleeding as this may dilute the blood with excess tissue fluid. Collect the sample from the second drop touching the disposable Sample Loop provided to the drop of blood until the Sample Loop is full. Test immediately, following Test Procedure Instructions.

Venous Whole Blood: Draw blood following laboratory procedures for obtaining venous blood. Collect sample in a tube containing citrate, heparin, or EDTA. Be sure the tube of blood is well mixed. Serum or Plasma: Draw blood following laboratory procedures for obtaining serum or plasma specimens. Collect specimen in a tube not containing any anticoagulant (serum), and in a tube containing citrate, heparin, or EDTA (plasma). Collect specimen in a clean container following standard laboratory procedures. Venous whole blood, serum and plasma specimens may be tested immediately after collection. If specimens are not tested immediately, refrigerate them at 2 to 8°C (36 to 46°F) following collection. These specimens should be tested within 3 days of collection. If specimens are not tested within 3 days of collection, serum or plasma specimens should be frozen at -20°C (-4°F) or colder.

Procedure

1. Remove a test cassette from a foil pouch, and place it on a flat surface.

2. Use the sample of either serum/plasma or whole blood: Add 40µl of sample (precision pipette) to the sample area first and then slowly instill one drop (40µl) of sample diluents to the same area. Direct contact of the diluents bottle with the sample area should be avoided.

3. The result can be seen within 2-3 minute with strong positive samples. Please do not interpret the test result after 30 minutes. Do record the result on cassette.

RESULT INTERPRETATION

Positive result (two bands)

A reddish-purple band appears both on the control line(C-line) and the test line (T-line) of the cassette.

Negative result (one band)

A reddish purple band appears only at the control line(C-line) of the cassette.

Invalid result (no band)

No reddish purple band appears neither at the control line nor the test line of the cassette. (81)

4. STAT PACK HIV TEST

Introduction

The Chembio HIV 1/2 STAT-PAK™ Assay is a single-use immunochromatographic test for the detection of antibodies to Human Immunodeficiency Virus Type 1 (HIV-1) and Type 2 (HIV-2) in fingerstick whole blood, venous whole blood, and serum or plasma specimens. The Chembio HIV 1/2 STAT-PAK™ assay is intended for use as a point of-care test to aid in the diagnosis of infection with HIV-1 and HIV-2. This test is suitable for use in multi-test algorithms designed for the statistical validation of rapid HIV test results. When multiple rapid HIV tests are available, this test should be used in appropriate multi-test algorithms(65).

Principle

The specimen/buffer mixture migrates along the test strip by capillary action, reconstituting the conjugate. If present, the antibodies bind to the colloidal gold conjugated antibody binding protein. In a reactive sample, the dye conjugated immune complex migrates on the nitrocellulose membrane and is captured by the antigens immobilized in the TEST (T) area producing a pink/purple line. In the absence of HIV¬1 and HIV¬2 antibodies, the sample continues to migrate along the membrane and produces a pink/purple line in the CONTROL (C) area containing immunoglobulin G antigens (66).

STORAGE

The Chembio HIV 1/2 STAT-PAK™ Assay should be stored in its unopened pouch at 8 to 30°C (46 to 86°F). Do not freeze. Do not open the pouch until you are ready to perform a test. When stored as indicated, test devices are stable until the expiration date marked on the pouch. Running Buffer should also be stored at 8 to 30°C (46 to 86°F) in its original vial.

SPECIMEN COLLECTION

The Chembio HIV 1/2 STAT-PAK™ Assay is performed on finger stick whole blood, venous whole blood, and serum or plasma specimens. Finger stick Whole Blood: Prepare to perform the finger stick blood collection procedure. Clean the finger of the person being tested with an antiseptic wipe. Allow the finger to dry thoroughly or wipe dry with a sterile gauze pad. Using a sterile lancet, puncture the skin just off the center of the finger and wipe away the first drop with sterile gauze and avoid squeezing the fingertip to accelerate bleeding as this may dilute the blood with excess tissue fluid. Collect the sample from the second drop touching the disposable Sample Loop provided to the drop of blood until the Sample Loop is full. Test immediately, following Test Procedure Instructions.

Venous Whole Blood: Draw blood following laboratory procedures for obtaining venous blood. Collect sample in a tube containing citrate, heparin, or EDTA. Be sure the tube of blood is well mixed.

Serum or Plasma: Draw blood following laboratory procedures for obtaining serum or plasma specimens. Collect specimen in a tube not containing any anticoagulant (serum), and in a tube containing citrate, heparin, or EDTA (plasma). Collect specimen in a clean container following standard laboratory procedures. Venous whole blood, serum and plasma specimens may be tested immediately after collection. If specimens are not tested immediately, refrigerate them at 2 to 8°C (36 to 46°F) following collection. These specimens should be tested within 3 days of collection. If specimens are not tested within 3 days of collection, serum or plasma specimens should be frozen at -20°C (-4°F) or colder. DO NOT FREEZE WHOLE BLOOD!

QUALITY CONTROL

Built-in control feature the control line serves as a built-in internal control and gives confirmation of sample addition and proper test performance. A pink/purple line will appear in the CONTROL (C) area if the test has been performed correctly and the device is working properly (Please see section: Interpretation of Test Results).

Procedure

1. Remove the required number of HIV1/2 STAT-PAK test devices from their wrappers by tearing the wrapper and place them on a flat surface (It is not necessary to remove the desiccant).

2. Label the test unit with patient name or identification number.

3. Touch the 5 µl sample loop provided to the material to be tested allowing the opening of the loop to fill with the liquid.

4. Holding the sample loop vertically, touch it to the sample pad in the center of the SAMPLE (S) well of the device to dispense ~5 µl of sample (serum, plasma or whole blood) onto the sample pad.

5. Invert the running buffer bottle and hold it vertically (not at an angle) over the sample well. Add the buffer slowly drop wise, 3 drops (~ 105 µl) into the sample(S) well.

6. Read the test result between 15 and 20 minutes after the addition of running buffer. Reactive test results (see interpretation of test results section) may be observed and read earlier than 15 minutes. To verify a non reactive test result, wait the entire 15 minute after starting the test. Do not read the results after 20 minutes

INTERPRETATION OF RESULTS

Non Reactive

One pink/purple colored line in the control (C) area, with no colored line in the test (T) area indicates a non reactive result. A non reactive test result means that HIV1/2 antibodies were not detected in the specimen. The test result is interpreted as Negative for HIV1/2 antibodies However; this does not exclude possible infection for HIV

Reactive

Two pink/purple colored lines, one in the test (T) area and one in the control (C) area indicate a reactive result. The line in test (T) area may look different from the line in the control (C) area. The intensity of the line in the test (T) area will vary with the concentration of specific antibodies, from barely visible to very dark.

Invalid

A pink/purple colored line should always appear in the control area, no matter if the test line appears or not. If there is no distinct pink/purple line visible in the control area, the test is invalid. It is recommended that the test be repeated with a new device (81).

5. **UNIGOLD HIV TEST**

INTRODUCTION

Human Immunodeficiency Virus (HIV) has been recognized as the etiological agent of the acquired immunodeficiency syndrome (AIDS). The Trinity Biotech Uni-Gold™ HIV test is a rapid immunoassay based on the immunochromatographic sandwich principle (67).

PRINCIPLES

Recombinant proteins representing the immunodominant regions of the envelope proteins of HIV-1 and HIV-2, glycoprotein gp41, gp120 (HIV-1) and glycoprotein gp36 (HIV-2) respectively are immobilized at the test region of the nitrocellulose strip. These proteins are also linked to colloidal gold and impregnated below the test region of the device. A narrow band of the nitrocellulose membrane is also sensitized as a control region. During testing two drops of serum, plasma or whole blood is applied to the sample port, followed by two drops of wash buffer and allowed to react. Antibodies of any immunoglobulin class, specific to the recombinant HIV-1 or HIV-2 proteins will react with the colloidal gold linked antigens. The antibody proteincolloidal gold complex moves chromatographically along the membrane to the test and control regions of the test device. A positive reaction is visualized by a pink/red band in the test region of the device. A negative reaction occurs in the absence of human immunoglobulin antibodies to HIV in the analyzed specimen. Consequently no visually detectable band develops in the test region of the device. Excess conjugate forms a second pink/red band in the control region of the device. The appearance of this band indicates proper performance of the reagents in the kit. (68)

STORAGE AND STABILITY

The Trinity Biotech Uni-Gold™ HIV test device and wash solution can be stored at 2-27°C. No-

Kit components should be used after the kit expiry date.

SPECIMEN COLLECTION AND STORAGE

Whole blood, serum or plasma may be used. Whole Blood: If finger stick whole blood is used, drops of blood produced should be taken up from the finger-tip by the pipette supplied and dropped from the pipette onto the device. Blood droplets should not be dropped directly from the fingertip onto the device as their size may vary. Whole blood specimens should be used within ten minutes of collection for optimum performance. If a specimen has started to clot, do not remix before testing. In such instances, the clear serum should be pipette off the clotted specimen and used for analysis. If an anticoagulant has been used in the blood sample, whole blood can be used directly on the device using the pipette supplied. If testing is not to be carried out immediately, samples should be stored at 2-8°C for up to three days, or preferably, the sample should be centrifuged and the plasma retained for future testing. Serum or Plasma: Serum or plasma may be kept for seven days at 2-8°C. Samples should be frozen for longer storage. Avoid repeated freezing and thawing of samples.

QUALITY CONTROL

Good Laboratory Practice necessitates the use of control specimens to ensure proper device performance at least once daily. A built in procedural control on the test device indicates that the test is functioning correctly. A pink/red band should always appear at the control window. Please note certain commercial controls designed for ELISA may not perform properly with the Trinity Biotech Uni-GoldTM kit. For further information please contact Trinity Biotech**.**

PROCEDURE

1**.** Check the expiration date. Do not use expired kits.

2. Remove the test device from its protective wrapper.

3. Label with the appropriate patient/client identification.

4. Using one of the disposable pipettes supplied, fill with sample.

5. Holding the pipette over the sample port, carefully add two drops of sample (approx. 60µl).

6. Add 2 drops (approx. 60µl) of the appropriate wash reagent to sample port.

7. Allow 10 minutes from the time of wash reagent addition for reaction to occur. The result should be read immediately after the end of the 10 minute incubation time but it stable for a further 10 minute after the incubation time. Do not read results after 20 minutes following sample addition

INTERPRETATION OF RESULTS

Positive result

A reddish-purple band appears both on the control line(C-line) and the test line (T-line) of the cassette.

Negative result

A reddish purple band appears only at the control line(C-line) of the cassette.

Invalid result

No reddish purple band appears neither at the control line nor the test line of the cassette.

Annexe II Questionnaire

Addis Ababa University

Faculty of Medicine

Department of Medical laboratory technology

(To be translated to Amharic)

For data collectors: For each question please put a cross clearly inside one box don't worry If you make a mistake; simply cross out the mistake and put a cross in the correct box.

1. Identification

1.1. Code----------

1.2. Sex A. Male □ B. Female□

1.3. Age----------

2. Back ground information

2.1. Residence □ A. Urban □ B. Rural □

2.2. Current occupational status

A. Self employed □ B. Driver □ C. House w ife □

D. Student □ E. Farmer □ F. Other specify----------

2.3. Religion A. Christian □ B. Muslim □

C. Doesn't have □ D. Other (Specify) ----------

2.4. Marital status A. Married □ B. Single □

C. Divorced □ D. Widow □

2.4. Ethnicity A. Oromo □ B. Amhara □ C. Tigrae □

D. Gurage □ E. Wolita □ F. Others ….

2.5. Educational Status

A. Illiterate □ B. Read and write only □

C. 1-8 grade □ D -12 grade □ E > 12 □

Have you have or ever practiced the following?

3.1 History of STD/STI A. yes ☐ B. No ☐

3.2 Multiple sexual prater A. yes ☐ B. No ☐

3.3. Blood transfusion A. yes ☐ B. No ☐

3.4. Abortion A. yes ☐ B. No ☐

3.5. Dental extraction at health facility A. yes ☐ B. No ☐

3.6. Circumcision A. yes ☐ B. No ☐

3.7. Hospital admission A. yes ☐ B. No ☐ If yes why?

3.8. Surgical procedure A. yes ☐ B. No ☐

3.9. Venous or body piercing for treatment A. yes ☐ B. No ☐

3.10. Delivery by TBA A. yes ☐ B. No ☐

3.11. Ear piercing A. yes ☐ B. No ☐

3.12. Nose piercing A. yes ☐ B. No ☐

3.13. Uvuloctomy A. yes ☐ B. No ☐

3.14. Tattooing on body A. yes ☐ B. No ☐

3.15. Tattooing on gum A. yes ☐ B. No ☐

3.16. Shaving A. yes ☐ B. No ☐

3.17. Contact with jaundiced patient A. yes ☐ B. No ☐

3.18. Frequent alcohol consumption A. yes ☐ B. No ☐

አዲስ አበባ ዩኒቨርስቲ
የህክምና ፋኩልቲ

የላቦራቶትሪ ትምህርት ክፍል

ለመረጃ ስብሳቢዎች ፤ ጥያቄዉን ከጠየቃችሁ በኃላ መልሱን በተሰጠዉ ሳጥን ውስጥ ከተሰጡት አማራጮች አንዱን የክኤስ ምልክት ይጻፉ ።።

1. <u>ጠ ቅ ላ ላ ጥ ያ ቄ</u>

1. 1. **ኮድ----------**

1.2. ፆታ ***ሀ.*** **ወንድ** ☐ **ለ . ሴት** ☐

1.3. እድሜ----------

1.4. መኖሪያ አካባቢ ***ሀ.*** **ገጠር** ☐ **ለ . ከተማ** ☐

1.5. ሥራ ***ሀ. የግል*** ☐ **ለ . ሹፌር** ☐ **ሐ . የቤትእመቤ ት** ☐

መ. ተማሪ ☐ **ሠ. ገበሬ** ☐ **ረ. ሌላ (ይገለጽ)----------**

1.6. ሃይማኖት ***ሀ.*** **ክርስቲያን** ☐ **ለ . ሙስሊም** ☐ **ሐ . የለም** ☐ **መ. ሌላ (ይገለጽ)**

1.7. የጋብቻ ሁኔታ ***ሀ.*** **ያገባ /ች** ☐ **ለ . ያላገባ /ች** ☐ **ሐ . የፈታ /ች** ☐ **መ. የሞ ተበት /ባት** ☐

1.8. ብሔር ***ሀ.*** **ኦሮሞ** ☐ **ለ . አማራ** ☐ **ሐ . ትግሬ** ☐ **መ. ጉራጌ** ☐

ሠ . ወላይታ ☐ **ረ . ሌ ላ (ይገለጽ) ----------**

1.9. የትምህርት ደረጃ

ሀ . **ያልተማረ** ☐ **ለ . መፃፍና ማንበብ የሚች ል** ☐

ሐ . ከ *1*ኛ እስከ *8*ኛ ☐ **መ. ከ *9*ኛ እስከ *12* ኛ** ☐ ***ሠ.*** **ከ *12* በላይ** ☐

2.ሄፓታይተስ “ቢ ” እና “ሲ ”ን በተመለከተ ጥያቄዎች

ከዚህ በታች ያሉትን በህይወት ህ /ሽ አድርገህ /ሽታዉቃለ ህ /ቂያለሽ ?

2.1 ያባለዘር በሽታ ሀ. አዎ □ ለ . አይደልም □

2.2 ከአንድ በላይ የትዳር ጓደኛ ሀ. አ ዎ □ ለ . አይደልም □

2.3 ደም መቀበል ሀ. አዎ □ ለ . አይደልም □

2. 4 ማስወረድ ሀ. አዎ □ ለ . አይደልም □

2.5 ጤና ድርጅት ጥርስ ማስነቀል ሀ. አ ዎ □ ለ . አይደልም □

2.6 ግርዛት ሀ. አዎ □ ለ .አይደልም □

2.7 ሆስፒታል መተኛት ሀ. አዎ (ለ ምን) ------- □ ለ . አይደልም □

2.8 ማንኛዉም አይነት ቀዶ ጥገና ሀ. አ ዎ □ ለ . አይደልም □

2.9 ሞኝ ባገኝ መቆረጥ ሀ. አዎ □ ለ . አይደልም □

2.10 በልምድ አዋላጅ መዉለ ድ ሀ. አ ዎ □ ለ . አይደልም □

2.11 ጆሮ መበሳት ሀ. አዎ □ ለ . አይደልም □

2.12. አፍንጫ መበሳ ት ሀ. አዎ □ ለ . አይደልም □

2.13. እንጥል ማስቆረጥ ሀ . አዎ □ ለ . አይደልም □

2.14 ሰዉነት መነቀስ ሀ. አዎ □ ለ አይደልም □

2.15. ድድ መነቀስ ሀ. አዎ □ ለ. አይደልም □

2.16 ፀጉር ቤት ዊም መላጨት ሀ. አዎ □ ለ . አይደልም □

2.17ወፍ ከያዘዉ ሰዉ ጋር ንክኪ ሀ. አ ዎ □ ለ . አይደልም □

2.18. አዘዉትሮ መጠጥ መጠጣት ሀ. አ ዎ □ ለ. አይደልም □

Annexe III Patient information

Purpose

We have planned to conduct a study with objective of investigating the magnitude of HBV, HCV and HIV infection among Chronic liver. The knowledge gained from this work is believed to help the management and control chronic liver disease.

Participation

We are asking you and others to voluntarily participate in this study. What is expected from everyone is to be examined for HBV, HCV and HIV before collecting blood pre and post counseling will given by trend nurse or physician and you will asked to answer few questions in relation to risk factor. The laboratory examination involves collection of 5 ml venous blood. All samples are collected using sterile and disposable equipments: tubes, syringes and needles.

Risks and discomforts associated

Taking 5ml of blood doesn't have any harm to your health except minor needle brick injury pain which lasts only for micro second. However if you have any discomfort you will be seen by physician.

Benefits

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

Confidentiality

Any information that we collect about you during this research will be kept confidential. Information about your identity will be put away after recording you file; and kept in a secured place. Only the principal investigators will be able to link your identity with the code number, should this become necessary to assist you medically.

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 won't bear any information relevant to your personality e.g. your

name or identity. We assure you the confidentiality of such information. Thus we also need your permission to use the test results for writing a report.

Right to refuse

Since participation in this study is entirely voluntary. You can refuse to participate in this research at any time. Your refusal to participate in this study will not affect any of the benefits you are supposed to get from the center.

ለ ጥናቱ መረ ጃ ና ተሳታፊ ነት መግለጫ ቅጽ

የጥናቱ ዓላማ

ሄፓታ ይተስ “ቢ” ፣ ሄፓታይተስ “ሲ” እና “ኤች አይ ቪ” ቫይረሶች በጉበት ህመ ማን መካከል ያ ለዉን ስርጭት ለማጥናት የታቀደ ነዉ፡ ፡

በጥናቱ ስለ መሳ ተ ፍ

በዚህ ጥናት መሳተፍ በሙሉ ፈቃደኝነት ላይ የተመሰረተ ነዉ፡ ስለሆነም በጥናቱ እንዲሳተፉ ፈቃደኛነትዎን እንጠይቃለን ፡ለመሳተፍ ከፈቀዱ ፤5ሚሊሊትር የደም ናሙና ከክንድዎ ተወስዶ የላቦራቶሪ ምርመራ ይደረግሎታል ፡፡ የላቦራቶሪ ምርመራውም ሄ ፓታይተ ስ “ቢ ” “ሲ ” እና “ኤች አይቪ ” ቫይረስን በደም ዉስጥ መኖርና አለመኖር ማረጋገጥይሆናል ፡ ፡ ደም ከመወሰዱ በፊት እና ከውጤት በሁዋላ በሰለጠነ ባለ ሙያ የምክር አገልግሎት ያገኛሉ፡ የደም ናሙናዉም የሚወሰደዉ ንጽህናዉ በተጠበቀ አዲስ እና በታሸገመርፌ ና ስሪነጅ ነዉ፡ ፡

በጥናቱሊከሰቱ የሚችሉ ተያያዥ ችግሮች

5 ሚሊሊትር የደም ናሙናዉን ለመዉሰድ መርፌ ሲተባ ከሚፈጥረዉ የቅጽበትየ ህመም ስ ሜት በስተቀር የጎላ ችግር አያመጣም ነገር ግን ምቾት ካልተሰማዎት ሀኪም እንዲያይዎት ይደረጋል ፡ ፡

በጥናቱ በመሳተፍ የሚገኝ ጥቅም

የደም ናሙና የላብራቶሪ ዉጤት ምንም አይነት ችግር ካሳየ የመድሃኒት ትእዛዝና የባለ ሙያ ምክር ይሰጥዎታል ፡ ፡

የጥናቱ መረጃዎች ሚስጥራዊነት

በጥናቱ ውስጥ የተሰበሰቡ ማናቸዉም ግላዊ መረጃዎች ሚስጥራዊነታቸዉ የተጠበቀ ይሆናል ፡፡ ከማንነትዎ ጋር በቀጥታ ተያያዥነት ያላቸዉ መረጃዎች በሙሉ በዋና ተመራማሪዉ ሚስጥራ ዊ በሆነ የመረጃ ጥንቅር ዘዴ ከተቀየሩ በኃላ ብቻ ለምርምር ሂደቱ የሚዉሉ ይሆናሉ ፡፡

የጥናቱን ዉጤት ስለ ማሳወቅ

የዚህ ጥናት ዉጤት በተለያዩ የህትመት ውጤቶች የሚቀርብ ሲሆን ይህ ከማንነትዎ ጋር የተያያዘ ምንም አይነተ መረጃን አያካትትም፡ ፡ ስለዚህም የጥናቱን ዉጤት በሪፖርት እናቀርበዉ ዘንድ ፈቃዶን እነጠይቃለን ፡ ፡

ከጥናቱ ስለ መዉጣ ትና ስለ ማቋረጥ

ይህ ጥናት በፈቀደኝነት ላይ የተመሰረተ እንደ መሆኑ መጠን በማንኛዉም ወቅት በፈቃድዎ ከጥናቱ መዉጣት ይችላሉ ፡ ፡ ከጥናቱ ቢወጡም እንኳን የተለመደዉን የህክምና እርዳታ በጤና ተቋ ሙዉስጥ በማንኛዉም ጊዜ የማግኘት መብት አልዎት ፡፡

Annexed IV Consent form

Consent form

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.

I----------do herby give consent to Dr/Mr./Mrs/Miss---------- 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: ----------

Patient's signature: ----------

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

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

Date: ----------

Witness: ----------

<u>ስለ ስምምነቱ ማረጋገጫ ፊርማ</u>

እኔ ስሜ ከታች የተገለፀዉ የጥናቱ ተሳታፊ ለመሆን ስወስን የጥናቱን አላማዎች አሰራሮችና ቅድመሁኔታዎች በግልጽ በመረዳትና ከጥናቱ ተሳታፊነት ፈቃደኛነቴን በማንኛዉም ደረጃ የማንሳት መብቴን በማረጋጥ ነዉ፡፡

እኔ ---------- በጥናቱ ተሳታፊ መሆኔን በፊርማዬ እያረጋገጥሁ ይህንን ስወስን በጥናቱ ሳቢያ ሊከሰቱ የሚችሉ አደጋዎች በሚገባ የተረዳሁና ከጥናቱ በማንኛዉም ደረጃ እራሴን ለመሰረዝ ብወስን ተገቢ የሆኑ ህክምናዎችና እገዛዎች ሁሉ እነደማይነፍጉኝ በማመን ነዉ፡፡ እነዚህ መረጃዎች ሁሉ በሚገባ በምረዳዉ ቋንቋ የተገለጸልኝ መሆኑን በፊርማዬ አረጋግጣለሁ ፡፡

የበሽተኛዉ ሙሉ ስም---------- ፊርማ----------

የተመራማሪዉ ሙሉ ስም፤ዶ/ር፤ኡቶ፤ወ/ሮ፤ወ/ት ---------- ፊርማ------

የምስክር ሙሉ ስም ---------- ፊርማ----------

Annex V: 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.

Name: Yacob Mohammed

Signature _____

Place _____

Date of submission _____

This thesis has been submitted with my approval as University

Advisor

Name _____

Signature _____

Place _____

Date of submission _____

ADDIS ABABA UNIVERSITY
COLLEGE OF HEALTH SCIENCES
SCHOOL OF ALLIED HEALTH SCIENCES
DEPARTMENT OF MEDICAL LABORATORY SCIENCES

Prevalence of Hepatitis B, Hepatitis C and HIV among Chronic liver disease patients in selected hospitals, Addis Ababa, Ethiopia

By Yacob Mohammed (BSc.)

Approved by the Examining Board

_____	_____
Chairman, Dep. Graduate Committee	Signature
_____	_____
Advisor	Signature
_____	_____
Examiner	Signature