ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES

DEPARTMENT OF MEDICAL LABORATORY SCIENCES

Prevalence of Hepatitis B and Hepatitis C Virus Infections and Associated Risk Factors among Sexually Transmitted Infection Syndromic Diagnosed Patients Attending at Selected Government Health Facilities, Addis Ababa, Ethiopia

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ABBREVIATION

AAU	Addis Ababa University
Ab	Antibody
Ag	Antigen
AIDS	Acquired Immunodeficiency Syndrome
AOD	Adjusted Odds Ratio
ART	Antiretroviral Therapy
CDC	Center for Disease Control
DNA	Deoxyribonucleic Acid
DRERC	Departmental Research and Ethics Review Committee
ELISA	Enzyme Linked Immunosorbent Assay
HBcAg	Hepatitis B core Antigen
HBsAg	Hepatitis B surface Antigen
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
MSM	Male sex with male
OD	Odds Ratio
PI	Principal Investigator
PLHIV	People Live With Human Immunodeficiency Virus
RNA	Ribonucleic Acid
SOP	Standard Operating Procedure
STD	Sexually Transmitted Disease
STI	Sexually Transmitted Infections
TBA	Traditional Birth Attendant
WHO	World Health Organization

Abstract

Background: Hepatitis B and hepatitis C are among the main public health concerns in the world and are serious infectious diseases. In the first 6 months, the most infections with that viral hepatitis have no symptoms; therefore, diagnosis of the virus in the early stage is difficult. Syndromic approach for management is widely used to control and reduce the burden of sexually transmissible diseases; however the magnitude of viral hepatitis B and hepatitis C infections is still underreported in Ethiopia among sexually transmitted syndromic diagnosed patients.

Objective: The aim of our study was to assess the prevalence of hepatitis B virus and hepatitis C virus infections and associated risk factors among sexually transmitted infection syndromic diagnosed Patients attending at Selected Government Health Facilities in Addis Ababa, Ethiopia.

Methods: A health facility based cross-sectional study was conducted from January to May 2024. The socio-demographic data was taken from each study participant using pre-structured standard questioner. The 5 ml of whole blood was collected using serum separator test tube and serum sample extracted to detect the presence of HBsAg and anti-HCV antibody by using rapid test and ELISA test kits. The data was inserted into Microsoft excel and exported in to SPSS Version 23 for analysis of the result. Descriptive statistics, bivariate or multivariate analysis were used and p value lower than 0.05 were considered as statically significant.

Result: A total of 355 study subjects who have sexually transmitted syndromes were participated in the study. The overall prevalence of HBsAg and HCV anti-body was 5.6% and 3.1 % respectively. History of sexually transmitted disease and blood transfusion found to be statically significant with HBV with (AOR 7.4; 95 % CI: 1.6-32.9, P= 0.009) viral infection and history of blood transfusion with HCV with (AOR of 7.4; 95%CI: 1.9-28.8, p=0.003) viral infections.

Conclusion: This study showed that HBV and HCV are still public health problems which need awareness and health education on the risky behaviors and mode of transmission of HBV and HCV among individuals with Sexually Transmitted Infection syndromes.

Keywords: HBV, HCV, STI/STD, syndromic patients, Addis Ababa, Ethiopia

1. INTRODUCTION

1.1 Background

One of the leading causes of death and morbidity in the world today is viral hepatitis [1]. The burden of hepatitis is rising globally, in contrast to the decreasing trend of HIV prevalence. The two main kinds of viral hepatitis, hepatitis B virus (HBV) and hepatitis C virus (HCV), account for 96% of hepatitis-related deaths. The illnesses brought on by these viruses are regarded as health problems even though they can both be avoided and HCV is even curable [1].

More than 300 million people are infected with hepatitis B virus (HBV) in the world, which is a common cause of liver illness and liver cancer [2]. The small DNA virus known as HBV, which belongs to the Hepadnaviridae family, has peculiar characteristics that resemble those of retroviruses. HBV can incorporate into the host genome and replicates via an RNA intermediary. The HBV replication cycle's special characteristics provide the virus a unique ability to survive in infected cells [2].

The HCV virus is an enveloped positive sense RNA virus that involved in to the Hepaciviridae family of flaviviridae viruses. It is linked to potentially fatal diseases such as hepatocellular carcinoma and liver cirrhosis. The hepatitis C virus kills 700,000 individuals worldwide each year by infecting about 71 million people. One-fourth of the entire health burden is caused by the chronic hepatitis C virus linked with extrahepatic factors [3]. There were over 10 million cases of hepatitis C virus (HCV) infection in Pakistan. It has a significant illness and death rate and causes hepatocellular cancer or liver cirrhosis. The main ways that HCV infection is spread are through unprotected sexual contact, prenatal exposure from a mother to her child, and percutaneous contact with contaminated blood [4].

The largest frequency of hepatitis B and C virus infection is seen in sub-Saharan Africa, where these infections are endemic. Female sex workers' high-risk sexual contact and restricted access to healthcare resources put them at risk for sexually transmitted infections, like HBV and HCV [5]. Over 60 million instances of chronic hepatitis B and over 10 million cases of chronic hepatitis C infections were reported in sub-Saharan Africa (SSA) in the World Health Organization's (WHO) 2019 Progress Report on HIV, viral hepatitis, and sexually transmitted infections [5].

People who have had a sexually transmitted infection (STI) in the past or present are more likely to have an HBV infection because unprotected intercourse can spread both STIs and HBV infections. Consequently, the CDC advises anyone seeking STI evaluation or treatment to get vaccinated against hepatitis B. The American Association for the Study of Liver Disease and the American College of Physicians both recommend hepatitis B testing as a best practice for individuals with a history of STI, despite the CDC's recommendation against pre-vaccination testing for those with current or past STIs. Sexual contact is the usual way that hepatitis B is spread; however, it is unknown how common hepatitis B is among people who have had or are currently experiencing a STI [6].

Sexually transmitted infections were common among several population groups, especially female sex workers (FSWs), in Ethiopia and other places. Numerous characteristics, including having genital sores, age, marital status, educational attainment, length of time as FSW, early sexual debut, and client count, were linked to their incidence among FSWs [7, 6]. Within this type of population group, there was also a noteworthy prevalence of HBV and HCV. Data on the seroprevalence of HBV and HCV in syndromic STI patients in Ethiopia were scarce [8].
Therefore this study aimed to assess the prevalence of HBV and HCV, as well as associated risk factors, among STI patients who are syndromic at a selected government health facility in Addis Ababa, Ethiopia.

1.2 Statement of the problem

Numerous etiological factors can cause viral hepatitis, an infectious disease with unique epidemiological, clinical, and laboratory characteristics that contribute significantly to global morbidity and mortality. Over 257 million people worldwide were projected to have a chronic hepatitis B virus (HBV) infection in 2015, whereas 71 million people have a chronic hepatitis C virus (HCV) infection [9, 10]. The vast majorities of individuals, approximately 40–80%, who suffer from chronic hepatitis B or C, are not aware of their serostatus and continue to spread the infectious agent to others. In economically underdeveloped nations, chronic liver disease caused by HBV and HCV remains the most difficult issue to solve [10]. Research revealed that active replication of long-term HBV and HCV infections was responsible for 80% of cases of hepatocellular carcinoma (HCC) and liver cirrhosis [11].

Worldwide, hepatitis B virus (HBV) and hepatitis C virus (HCV) were prevalent infections [12]. One third of people on the planet are thought to have been exposed to HBV, and 350 million of those cases were chronic infections. About 350 million populations have been infected with HBV, and the disease's chronic consequences claim the lives of one million people annually. The majority of people with chronic HBV infection live in Asia, the Middle East, and Africa, despite the virus's global prevalence. An estimated 1.2 million people worldwide pass away each year from liver cancer, cirrhosis, and chronic HBV infection [13, 14].

Approximately 2 billion individuals worldwide carried the Hepatitis B virus (HBV), and an additional 360 million were at risk of problems as a result of the infection. Out of the 2 billion people who were infected, about 65 million of them lived in Africa and were consequently at risk of developing issues connected to HBV. Hepatocellular carcinoma and liver cirrhosis are two of these consequences [15, 27, 28]. Approximately 100 times more virulent than HIV, HBV is more contagious than other blood-borne viral pathogens. The increased viral load in blood, prolonged environmental survival (>7 days at room temperature), and transmissibility in the absence of visible blood are some of the factors that contribute to HBV's infectiousness [15, 31].

Among high-risk Chinese populations, such as drug users (18–30% to 66–97%) and dialysis patients (pooled prevalence rate: (41–51%), the prevalence of HCV infection is higher [16].

Worldwide, more than one million STIs (sexually transmitted infections) are contracted every day, most of which have no symptoms. An estimated 374 million new cases of one of the four treatable STIs like; chlamydia, gonorrhea, syphilis, and trichomoniasis occurred a year. Herpes simplex virus, also known as herpes, is thought to infect more than 500 million people between the ages of 15 and 49 [17].An infection with the human papillomavirus (HPV) is linked to about 311,000 cervical cancer deaths annually [18].In 2016, an estimated 1 million pregnant women were expected to have contracted syphilis, leading to about 350,000 unfavorable delivery outcomes [19].STIs can raise the risk of HIV and have a direct influence on sexual and reproductive health through stigmatization, infertility, malignancies, and pregnancy difficulties. One of the biggest obstacles to lowering the global burden of STIs is drug resistance. The incidence among young Ethiopians increased from 1.15 percent in 2005 to 4 percent in 2011[20].

According to certain reports, Ethiopia has a high prevalence of HBV and HCV infections as well as associated risk factors [21, 22, 32]. On the other hand, prevalence and trends of STI Syndrome patients have not yet been found in a number of locations, including the current study area. Thus, the purpose of this study was to assess the prevalence of HBV and HCV among patients with syndromic diagnoses of sexually transmitted infections at a selected government health facility in Addis Ababa, Ethiopia.

1.3 Significance of the study

Studying prevalence of HBV and HCV infections and the risk factors associated with them will be beneficial to patients, medical professionals, researchers, and Ministry of Health policymakers.

This research will benefit the general public as well as the community in terms of early infection prevention, diagnosis, prognosis, and treatment, particularly for those with STI-diagnosed patients.

The study may again persuade our nation to recognize the hepatitis virus as one of new community health issues that, if ignored, might have a major negative impact on society.

This study would give baseline data for future research because the prevalence of HBV and HCV among individuals with STI-diagnosed illnesses has not been studied before.

2. LITERATURE REVIEW

2.1 Hepatitis B virus and C Virus

2.1.1 Epidemiology of HBV

Between 5 and 10% of people living with HIV (PLHIV) worldwide are reported to also have hepatitis B virus (HBV) infection. HIV and HBV were common risk factors, and there was a higher chance of HBV co-infection (HIV/HBV co-infection) in populations where many global HIV epidemics occurred. One third of people worldwide are estimated to be infected with HBV is one of the main causes of mortality and morbidity. A quarter of carriers acquire serious liver disease, and about 5% are chronic carriers [23].

2.1.2 Epidemiology of Hepatitis C Virus

The primary cause of chronic liver disease is the hepatitis C virus (HCV), and cirrhosis, hepatocellular cancer, liver failure, and mortality are all linked to chronic HCV infection. There are an estimated 70.1 million people with active viremia worldwide. About 500,000 people die each year from complications related to HCV infection, primarily in lower middle-income countries. Approximately 3% of the world's population (170-200 million individuals) has a chronic HCV infection. The vast majority of HCV patients were never able to get rid of their HCV infection. An early diagnosis at this asymptomatic stage is necessary for prompt intervention, to prevent progression to advanced liver disease and death, as HCV infection stays silent until the formation of decompensated cirrhosis [24, 25]. The likelihood of progression occurring after stem cell transplantation (SVR) is probably higher in many cases where there are notable liver co-morbidities or risk factors such alcohol intake or fatty liver disease. Generally speaking, the evolution of fibrosis in HCV patients might be accelerated by the co-occurrence of multiple liver diseases, such as hemochromatosis and alcohol intake or chronic viral hepatitis and alcoholic liver injury [26, 27].

In Japan, a population-based cohort study was carried out from April 2012 to August 2018, yielding a total of 6,422 HCV patients. 31/6422, 95% CI [confidence interval]: 0.33–0.68% was the HIV prevalence rate of 0.48%. In 3.2% (1/31) of cases, HIV was diagnosed after HCV, 58.1% (18/31) before HCV, and 38.7% (12/31) concurrently. HCV or HIV co-infected patients had a lower median age (37–51 years, $p < 0.001$), were most of them to be male (30/31 [96.8%] - 3059/6391 [47.9%], $p < 0.001$), had a higher likelihood of having other STDs (38.7% [12/31].

0.9% [56/6391], p < 0.001), and resided in Tokyo, the highest populated capital city in Japan (67.7% [21/31] vs. 11.6% [742/6391], p < 0.001). In Tokyo, 18.6% (13/70; 95% CI) of males in their 20s to 30s with HCV were HIV positive. Males aged 20–30 had an HCV prevalence of 18.6% (13/70; 95% CI, 10.3–29.7%) [28].

Individuals who tested positive for HIV and who had co-infections with other STIs had a higher risk of morbidity and mortality. This has implications for clinical practice. The occurrence of sexual transmitted diseases has sharply increased, with HIV-positive people being more susceptible to syphilis, gonorrhea, Mycoplasma genitalium, and hepatitis C virus (HCV). It may be possible to prevent co-infection of STIs in HIV-positive people by reducing risk-behavior, increasing testing, and managing the condition carefully, as well as eventually getting rid of the germ. The growing corpus of data about drug-drug interactions and antibiotic resistance should guide the management of individuals who were previously co-infected [29, 30].

From October to December 2019, observational multicenter a cross-sectional study was conducted at the rest stops frequented by the miners along FG's two borders with Suriname and Brazil. Both the number of sexual partners and the frequency of condom use were higher among alcohol consumers. HIV, HCV, HBV, and syphilis were more common than in the local population, with prevalence rates of 0.5% (95% CI: 0.1–2.1), 2.1% (95% CI: 0.7–3.6), 1.6% (95% CI: 0.3–2.8), and 12.4% (95% CI: 9.0–15.7), respectively [31].

In a community-based study carried out in eastern China between 2011 and 2012, 149,175 people from 60 communities across three counties in Jiangsu province were examined to determine the burden of hepatitis C virus infection and risk factors in the general population. Of these, 1175 subjects (0.79%) tested positive for HCV antibodies. The incidence was low in children (0.09%) but gradually rose in adolescents (0.20%) and adults (0.35%) over the age of 21. In most age categories, the magnitude of HCV infection was highest in females than to males.In summary, even though the population's HCV prevalence was lower than that of the country, the overall reservoir of infection is still substantial and calls for public health initiatives like health education to reduce the scope of the issue [32].

To assess the risk factors and epidemiological characteristics of syphilis, HBV, and HCV infection among HIV-positive patients at West China Hospital, SCU, a retrospective study involving HIV-positive patients was carried out between 2014 and 2016. There were found

serum makers for syphilis, HBV, and HCV. The prevalence of co-infections with HIV/HBV, HIV/HCV, and HIV/syphilis was found in 894 HIV-positive patients to be 14.4%, 5.7%, and 18.9%, respectively. The triple co-infection of HIV/HBV/HCV, HIV/HCV/syphilis, and HIV/HBV/syphilis was 7 (0.7%), 12 (1.3%), and 29 (3.2%), respectively. [33].

In four regions of Ukraine, a multi-site random sample biobehavioral health study was carried out among inmates in 13 prisons who were scheduled for release in six months. Participants completed structured health assessment questionnaires then had rapid serology test for syphilis, HIV, and viral hepatitis after giving their consent. Twenty-one percent of the 402 participants (mean age = 31.9 years) were women. Regional variations were noted in the burden of HBV, HCV, HIV and syphilis, which were 19.4% (95% CI = 15.5%–23.3%), 60.2% (95% CI = 55.1%–65.4%), 5.2% (95% CI = 3.3%–7.2%), and 10% (95% CI = 7.4%–13.2%), respectively. The prevalence of HCV was 28.6%. Of the 78 prisoners with HIV, 50.7% were not aware of their status, and 44 (56.4%) had CD4, 350 cells/mL; of these, only five (11%) were getting antiretroviral medication [34].

Africa is regarded as having a high HBV endemicity (≥8%). Hepatitis B surface antigen (HBsAg) sero-prevalence has been estimated to be between 6 and 20% of the population, despite the fact that it is challenging to determine the precise burden of HBV in Africa. There was a higher incidence of 9.7% to 16.6% in other emerging nations. Rwanda is one of the few nations in Africa that has made a commitment to end HIV, HBV, and HCV by 2030. Treatments for HIV, HBV, and HCV should be expanded in order to potentially lower morbidity and death rates. According to research conducted across various risk groups in Kenya, the prevalence of HBV infection ranged from 5 to 30% [35].

An additional retrospective investigation was carried out at the hospital in Aïoun, Mauritania, between January 2010 and December 2015. During the course of the five-year trial, 1,123 donors were gathered. 182 of them tested positive for HIV, representing a 16.2% overall prevalence with a 5.2 sex ratio man/woman predominance in the male population. The donors' age range 17 -73 years, with an average year of 32.7 ± 10. The age group of 21–30 years old was most represented (40.5%). 1.2% for HIV, 11.8% for HBV, 0.2% for HCV, and 3% for syphilis were

the seroprevalences observed. To guarantee blood safety for the recipient, a strict selection and screening process for blood donors was strongly advised [36].

An investigation was carried out to evaluate the frequency of hepatitis C virus infection in Africa: anti-HCV antibodies in the general population and in individuals with primary liver cancer or cirrhosis. Serum samples from 410 adults residing in Tunisia, Senegal, Burundi, and Madagascar as well as 209 patients with liver illnesses from Senegal and Tunisia were examined for anti-hepatitis C virus (anti-HCV) antibodies. 4.2% of African adult population had anti-HCV antibodies, as did 51% of patients with liver cirrhosis and 37% of patients with primary liver cancer. Nonetheless, HBsAg+ patients had greater percentages of anti-HCV antibodies than HBsAg-patients did [37].

An institutional cross-sectional study was carried out from January to June 2013 on 318 pregnant patients who visited the antenatal clinic at Bahir Dar health facilities. Participants in the study provided pertinent data. Hepatitis C virus sero-prevalence was assessed by employing an ELISA kit to find HCV immunoglobulin. Among pregnant women, the hepatitis C virus was present in 0.6% of cases overall. In summary, there was a low frequency of the Hepatitis C virus among pregnant patients visiting Bahir Dar medical facilities, and the expected factors did not reach statistical significance [38].

In Gambella, Ethiopia, 453 refugees participated in a cross-sectional survey that was carried out between January and May of 2018. Among refugees, the overall incidence of HBsAg was 7.3% (33/453) and anti-HCV was 2.0% (9/453). Of these, 1.4% (5/370) and 6.8% (25/370) of the females and 9.6% (8/83) of the mens tested positive for HBsAg and anti-HCV, respectively. There was a correlation between HCV infection and the age groups of 18–29 and 30-41 ($P = 0.003$ and $P = 0.020$). In a sizable camp for refugees in Ethiopia, this study revealed an intermediate incidence of HBV and HCV virals [10].

At the Dire Dawa Blood Bank in Eastern Ethiopia, a retrospective examination of the records of consecutive blood donors from July 2010 to June 2013 was carried out. 5647 (88.57%) of the 6376 blood donors who were tested were replacement donors, while 729 (11.43%) were voluntary donors. Of them, 5430 (85.16%) were male and 4492 (70.45%) were in the 18–32 age range. 450 donors, or 7.06% of the total, had serological result of a minimum of one pathogen

infection. HBV, HIV, HCV, and syphilis positive rates overall were 4.67%, 1.24%, 0.96%, and 0.44%, respectively [39].

In 1994, a community-based seroepidemiological survey was carried out in Addis Ababa, Ethiopia, to gather information on the dynamics of hepatitis B virus (HBV) infection transmission and its management. Using commercial ELISAs, venous blood from 4736 people under 50 years old who were chosen from 1262 households using stratified cluster-sampling was tested for HBV markers. In comparison to girls (5%; 4-6), the burden of HBsAg was higher in men's (9%; 7-10) at 7% (95% CI 6-8). Of HBsAg positives (18–29), 23% had an HBeAg prevalence, while less than 1% of women of reproductive age had an HBeAg positive. The overall seroprevalence of HBV (any marker) increased gradually with age, reaching over 70% in individuals aged 40-49, suggesting a notable transmission during childhood and adulthood [40].

From October to December 2020, 387 people with presumed PTB participated in a cross-sectional study. Participants in the study were 44.2 years old on average. In all, the percentage of those who tested positive for HBV, HIV, or TB was 14, 3.6%, 7.2%, and 9.6%, respectively. HBV-HIV co-infection occurred in just one patient (0.3%). Six people (1.6%) had the TB or HIV co-infection found in them. Several sexual partners, alcohol consumption, having body pierced, and being divorced from your partner were all found to be significantly linked with HBV infection in a multivariate analysis [41].

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3. Objective

3.1 General objective

To determine the prevalence of HBV and HCV infections and associated risk factors among sexually transmitted infection syndromic diagnosed patients attending at selected government health facilities in Addis Ababa, Ethiopia.

3.2 Specific Objective

- To determine burden of HBV among sexually transmitted infection syndromic diagnosed patients attending at selected government health facility in Addis Ababa, Ethiopia January to May 2024.

- To determine prevalence of HCV among sexually transmitted infection syndromic diagnosed patients attending at selected government health facility in Addis Ababa, Ethiopia January to May 2024.

- To determine risk factors associated with occurrence of HBV and HCV among sexually transmitted infection syndromic diagnosed patients attending at selected government health facility in Addis Ababa, Ethiopia January to May 2024.

4. Hypothesis

Prevalence of HBV and HCV are equal in the general community and in patients with STI-syndrome diagnoses.

HO; there is no difference on the prevalence of HBV and HCV between general community and in patients with STI-syndrome diagnoses.

HA; there is difference on the prevalence of HBV and HCV between general community and in patients with STI-syndrome diagnoses.

5. MATERIALS AND METHODS

5.1 Study area

The study was conducted in Addis Ababa at selected government health facility, Addis Ababa, Ethiopia. Addis Ababa is the capital City of Ethiopia and administratively divided in to 11 sub cities. According to Central Statistical Agency, Ethiopian Demographic Health Survey 2022 has an estimation projection of population of 5,228,000 [35].Based on the 2012 (EFY) Health and Health related indications repoted by MoH, Addis Ababa has 13 Hospitals and 98 Health Centers. Saint peter specialized hospital, Kebena health center, Addis Ketema Kifle Ketema health center and Addisu Gebeya health center were selected by simple random sampling by considering Sub Cities as selection unit.

5.2 Study design and period

A health facility based cross-sectional study was conducted from January to May, 2024 to determine the magnitude of HBV and HCV Infections and associated risk factors among sexually transmitted infection syndromic diagnosed patients attended at selected government health facility in Addis Ababa, Ethiopia.

5.3 population

5.3.1 Source of population

All STI syndromic patients attending at selected government health facility in Addis Ababa were our source of population.

5.3.2 Study population

All STI syndromic Patients at selected government health facility in Addis Ababa that satisfy the selection criteria were taken as the study populations

5.4 Inclusion and Exclusion criteria

5.4.1 Inclusion criteria

Those participants syndrome STI diagnosed cases that would give informed consent and above the age of 20 was included in this study

5.4.2 Exclusion criteria

Those individuals syndrome STI diagnosed cases who have previously known to have HBV, and/or HCV (or hepatitis) infections using medical history or questionnaire.

5.5 Study variables

5.5.1 Dependent variables

-Prevalence of HBV

- Prevalence of HCV

5.5.2 Independent variables

Socio-demographic Characteristics

-Age

-Sex

-Marital Status

-Educational Status

-Occupation

Associated Risk factors

-Blood transfusion history, abortion, history of surgery, history of STI cases, uvuloctomy, ear piercing, circumcision, tooth extraction, body piercing for blood collection for treatment, hospital admission, alcohol consumption tattooing on the body, tattooing on gum and shaving by barber

5.6 Sample size determination and sampling technique

5.6.1 Sample size determination

The sample size is about 355 study participants by taking previous study in Dessie referral and Kemise general Hospital Northeastern, Ethiopia as a reference [42].

$$n = \frac{\frac{(Za)^2}{2} \times p(1-p)}{(d)^2}$$

Where; Z α/2 = the corresponding Z score of 95% CI (confidence interval) =1.96

P = HBV prevalence from previous study=27.4%

P=prevalence= 0.27≈0.3

d= Margin of error (5%) = 0.05

n= required sample size

n = (1.96)2 (0.3) (0.7)/ (0.05)2

= (3.84) (0.21)/ (0.0025) =323

For the calculation, 95% CI and 5% margin of error were used. To reduce errors arising from complaints non-response rate, 10% of sample size added giving a sample of 355. The study subjects were selected by systematic random sampling technique.

5.6.2 Sampling technique

Using simple random sampling method one hospital and three health center was selected from 13 Hospitals and 98 health centers of Addis Ababa government health facility and then the calculated sample size was distributed to each health facility by population proportion to size applying proportional allocation formula, based on three months report of STI in each selected government health facility. The study subject were selected from list of STI registration book by using systematic random sampling technique every "K" value=18, which was obtained through dividing the total number of STI cases in three month report from selected government health facility to the required sample size. The first study participant was selected by randomly from 1 to 18, then the rest of the study subject were included every "18" value.

$$K = \frac{\text{Total number of STI syndromes in three month report}}{\text{Required sample size}} = \frac{6500}{355} \approx 18$$

Proportional to size allocation formula was used to select study unit in each selected government health facility.

$$\frac{nf \times ni}{N}$$

nf = the final sample size

ni = the number of three months STI syndromic diagnosed patients in each government health facility

N= the total number of STI syndromic diagnosed patients in the selected government health facility.

$$\text{SPSH} = \frac{355 \times 2400}{6500} = 131.07 \approx 131$$

$$\text{KHC} = \frac{355 \times 1200}{6500} = 65.53 \approx 66$$

$$\text{AKHC} = \frac{355 \times 1500}{6500} = 81.92 \approx 82$$

$$\text{AGHC} = \frac{355 \times 1400}{6500} = 76.46 \approx 76$$

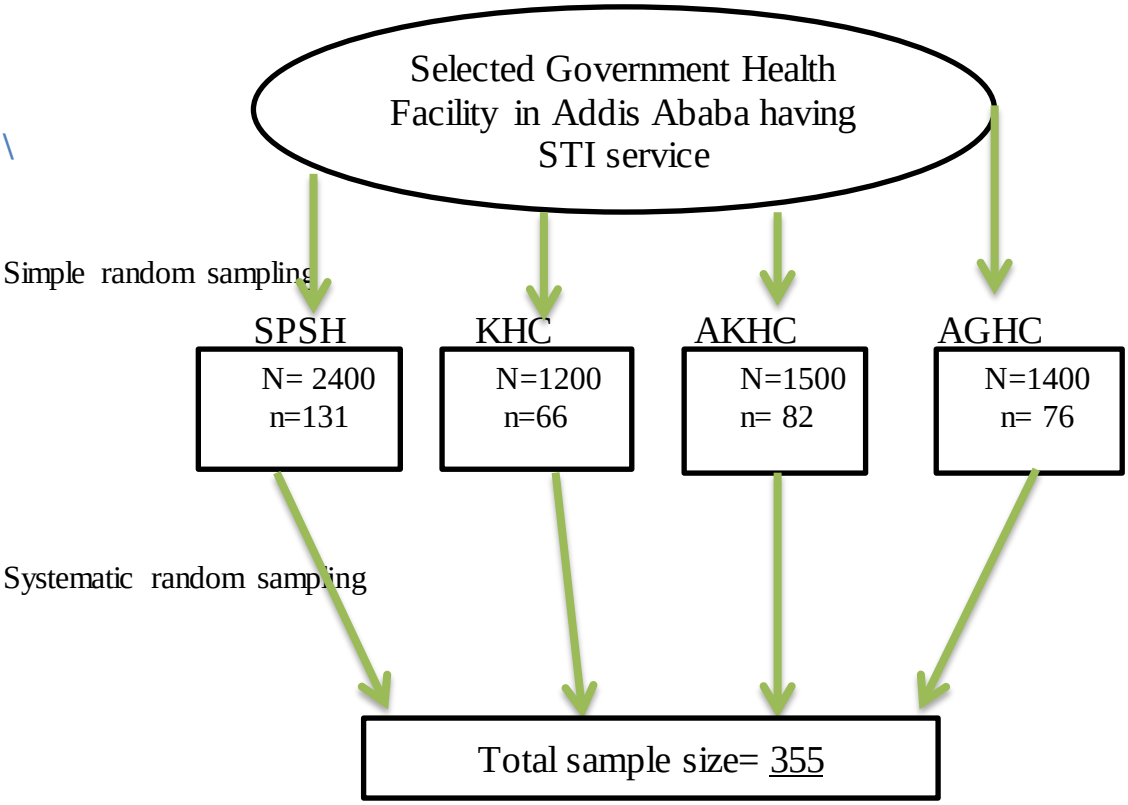

Figure 1: Diagrammatic presentation of sampling procedure for the STI syndromic diagnosed patients at selected four government health facility in Addis Ababa, Ethiopia, 2024.

5.7 Measurement and Data collection

5.7.1 Data collection procedure

The ideas, risks, advantages of study participation and the mandate to withdraw from the study at any time were explained to participants. Following complete explanation of the standards and aims of the study, written consents was got from each study participants. Then individuals would assess whether they fulfill the well-organized exclusion/ inclusion criteria. Associated risk factors and socio demographic information was collected using pre-structured questionnaire. Then 5ml of blood was collected by professional laboratory personnel and the serum samples were separated after the blood is clotted and centrifuged at3000rpm and stored at -20 °C until analysis. Then detection of HCV and HBV was undertaken by the principal investigator. All collected samples that fulfill acceptance criteria would tested for HCV and HBV using anti-HCV antibody and HBsAg using rapid test that follows an immune-chromatographic method. Positive results using rapid screening kit were further confirmed by using ELISA test. By using ELISA (Murex version 3) test kit following the manufacturer's instruction and the standard operating procedure of the Debre Markos Blood Bank laboratory. Serum sample with absorbance value of

above the cutoff value were taken as positive for HBSAg and HCV Ab, lower than the cutoff value was reported as negative.

5.7.2 Principles of each Laboratory analysis

HBsAg rapid test principle

All sera were tested for the screening of HBV by the HBsAg rapid test kit. The test kit is an immune-chromatography, which has two unique sites for immune-assays on a membrane. The test sample flows through the membrane assembly of the cassette, the color monoclonal anti-HBsAg, and colloidal gold conjugate complexes with HBsAg in the sample. This complex moves further down the membrane to the test region where immobilized by another monoclonal anti-HBsAg antiserum coated on the membrane. The pink-purple color band formation confirms a positive test result and the absence of the color band in the test region indicates a negative test result. Unreacted conjugate and unbound complex, if any, move further on the membrane and were subsequently immobilize by the anti-rabbit antibodies coated on the membrane at the control region, forming a pink/purple color band. This control band serves to validate the test result [66].

HBsAg ELISA test principle

All sera that were positive by screening HBsAg rapid test was further analyzed by sandwich HBsAg ELISA test. This Sandwich ELISA method uses polystyrene micro-well strips pre-coated with monoclonal antibodies specific to HBsAg. A serum or plasma sample added to the micro-well, together with a secondary anti-body conjugated with horseradish peroxidase (HRP), and directed against different epitopes of HBsAg. During incubation, the specific immune-complex formed in the presence of HBsAg in the sample captured in the solid phase. After washing to remove sample serum protein and unbound HRP-conjugate, chromogen solution containing Tetra-methyl Benzedrine (TMB) and urea peroxidase added to the walls. In the presence of the antibody-antigen-antibody (HRP) sandwich immune-complex, the colorless chromogen hydrolyzed by the bound HPR conjugate a blue-colored product. The blue color turns to yellow after stopping the reaction with sulphuric acid. The amount of measured color is proportional to the amount of antigen in the sample [66].

Anti-HCV rapid test principle

All sera were tested for the screening of HCV by anti-HCV antibody rapid test kit. The test kit detects antibodies to HCV through visual interpretation of color development in the internal strip. Recombinant HCV antigen immobilize on the test region of the membrane. During testing, the specimen reacts with recombinant HCV antigen conjugated to colored particles and pre-coated onto the sample pad of the test. The mixture then migrates through the membrane by capillary action and interacts with reagents on the membrane. If there were sufficient HCV antibodies in the specimen, a colored band will form at the test region of the membrane. The presence of this colored band indicates a positive result, while its absence indicates a negative result. The appearance of colored band at the control region serves as a procedural control, indicating that the proper volume of specimen has been added and membrane wicking has occurred [66].

Anti-HCV ELISA test principle

All sera that were positive by screening anti-HCV antibody were further analyzed by sandwich anti-HCV antibody ELISA tests. The sandwich ELISA method uses polystyrene micro-well stripes that were pre-coated with recombinant, highly immune-reactive antigens corresponding to the core and non-structural regions of HCV. During the first incubation step, anti-HCV specific antibodies, if present, was bound to the phase pre-coated HCV antigens. The wells washed to remove unbound serum proteins, and rabbit antihuman IgG antibodies (anti-IgG) conjugated to HRP added. During the second incubation step, these HRP conjugated antibodies was bound to any antigen- antibodies complexes previously formed and the unbound 18 HRP-conjugate removed by washing. Chromogen solutions containing Tetra-methyl Benzedrine (TMB) and urea peroxidase added to the wells and in presence of the antigen-antibody-anti-IgG (HRP) immune-complex; the colorless chromogens hydrolyzed by the bound HRP-conjugated to a blue colored product. The blue color turns to yellow after stopping the reaction with sulphuric acid. The amount of color measured and is proportional to the amount of antibody in the sample [66].

5.8 Data Quality Assurance and Quality Control

After completed each questionnaire, cross-check was performing among data collectors and PI to assure the completeness of the information gathered. Daily negative and positive control was run every morning before run participant's blood sample according to manufacturer's guidelines.

In this study, the results were reported and kept confidentially and stored in secured place until exported to statistical software.

5.8.1 Pre analytical phases

The collected whole blood specimens were checked for proper labeling. Generally, standard operating procedure was applied for every sample collection, transportation, preparation and storage.

5.8.2 Analytical phases

The working reagents, kits and the methods were evaluated with known negative and positive control materials. Then the test was performed based on SOP.

5.8.3 Post analytical phases

The result of each individual was documented on registration logbook with participant's identification number and the result was checked again before released for further analysis.

5.8.4 Data entry

Data was coded and entered into Microsoft Excel, then cleaned and cheeked. The data was analyzed by using SPSS version 23. Results of the data was managed and summarized in terms of frequencies, reported by using tables. Binary logistic regression and chi square test was used to determine association between dependent and independent variables. A p-value of <0.05 was considered as statistically significant.

5.9 Operational definition

HBV positive: Serum positive for HBsAg by rapid test and ELISA method.
HBV negative: Serum negative for HBsAg by rapid test method.
HBsAg low: (<2%), intermediate (2-8%) and high (>8%) according to WHO.
HCV positive: Serum positive for anti-HCV by rapid test and ELISA method.
HCV negative: Serum negative for HCV antibody by rapid test technique.
Syndromic STI: A group of the symptoms a patient complains.

5.10 Ethical considerations

Before conducting the study, ethical clearance was taken from the Department of Research and Ethical Review Committee (DRERC) of Department of Medical Laboratory Sciences College of Health Sciences Addis Ababa University and Addis Ababa Public Health Research and Emergency Management Directorate. In addition to this, formal and official letter of cooperation was given from Department of Medical Laboratory Sciences to the Addis Ababa Health bureau to the study sites. Formal consent was received from each study Participant prior to conducting the study. Participants were informed their result is confidential, not exposed to others. Only positive test result communicated with their physician to receive treatment.

5.11 Dissemination of results

At the end of this study is finalized, the result was submitted to Addis Ababa University department of Medical Laboratory Science and would be submitted to Addis Ababa Public Health Research and Emergency Management Directorate and concerned bodies or stake holders. It would be also available in the library to serve as a reference for students, researchers, experts or policy makers for intervention. This result will be also sent for publication in peer reviewed local and international journals and reported in related conferences.

6. Results

6.1 Socio demographic characteristic's

Among the 355 study subjects, 53.2 % (n=189) represents female participants with a female to male ratio of 1.1:1. The age category was 20 to 78 years with a mean age of 40. Most of the study participants were in the age group of 20 to 29 years of age which represents 31.3 % (n=111) followed by age group of 30-39 years which represents 25.9 % (n=92) and the lowest age group was 70+ years represents 5.4 % (n=19). Most of the participants were found to be married, 58.3% (n= 207) followed by single, 29.6 % (n=105) and widowed one account 6.2 % (N=22) the lost was divorced with the value of 5.6 % (n=20). In relation to residency, 99.3% (n=322) of the study participants were urban dwellers. About 39.7 % (n=141) of the study subjects were high school educational levels, 22.8 % (n=81) primary school and the 20.0 % (n=71) have first degree in their respective field (table 1).

Table 1 Socio-demographic characteristics among STI syndromic diagnosed patients investigated for HBV and HCV in selected government health facility (January to May 2024).

Variables	Socio-demographic	Frequency (N)	Percent (%)
Sex	Male	166	46.8
	Female	189	53.2
	Total	**355**	**100.0**
Age(in years)	20-29	111	31.3
	30-39	93	26.2
	40-49	66	18.6
	50-59	36	10.1
	60-69	30	8.5
	70+	19	5.4
	Total	**355**	**100**
Marital status	Single	106	29.9
	Married	207	58.3
	Divorced	20	5.6
	Widowed	22	6.2
	Total	**355**	**100**
Education	Illiterate	8	2.3
	Primary School	81	22.8
	Secondary School	141	39.7
	Diploma	39	11.0
	First Degree	71	20.0
	Second Degree	10	2.8
	PhD	5	1.4
	Total	**355**	**100**
Residence	Urban	322	90.7
	Rural	33	9.3
	Total	**355**	**100**
Occupation	Self-employee	56	15.8
	Civil servant	70	19.7
	Student	47	13.2
	House wife	136	38.3
	Total	**355**	**100**

Source: own computation (2024)

6.2. Magnitude of HBV and HCV

In this study 8.7 % (n=31) study participants were seropositive for either HBV or HCV. From total positive samples 5.6 % (n=20) were positive for HBsAg, while 3.1 % (n=11) were serologically positive for Anti- HCV antibody. About 1.7 % (n=6) of the study subjects had HBV and HCV co infection (table2).

Table 1: Socio-demographic characteristics among STI syndromic diagnosed patients investigated for HBV and HCV in selected government health facility (January to May 2024).

Variables		**HBsAg positive N (%)**	**p-value**	**HCV Ab Positive N (%)**	**p-value**
Age Category	20-29	10`/111 (9.00)	**0.169**	6/111 (5.4)	**0.684**
	30-39	3/93 (3.2)		3/93 (3.2)	
	40-49	4/66 (6.06)		1/66 (1.5)	
	60-69	2/30 (6.6)		1/30 (3.3)	
Residence	Urban	19/322 (5.9)	**0.497**	10/322 (3.1)	**0.981**
	Rural	1/33 (3.0)		1/33 (3.0)	
Sex	Male	7/166 (4.2)	**0.279**	7/166 (4.2)	**0.256**
	Female	13/189 (6.8)		4/189 (2.1)	
Marital Status	Single	5/106 (4.7)	**0.580**	2/106 (1.8)	**0.357**
	Married	12/207 (5.8)		8/207 (3.8)	
	Divorced	3/22 (13.6)		1/20 (5.0)	
Occupation	Self-employee	7/136 (5.1)	**0.943**	3/136 (2.2)	**0.302**
	Civil servant	1/55 (1.8)		1/55 (1.8)	
	Student	2/47 (4.3)		00(0.0)	
	House wife	6/70 (8.6)		4/70 (5.7)	
	Unemployed	4/47 (8.5)		3/47 (6.4)	
Educational Level	Primary School	2/81 (2.4)	**0.780**	2/81 (2.4)	**0.523**
	Secondary School	13/141 (9.2)		5/141 (3.5)	
	Diploma	2/39 (5.1)		2/39 (5.1)	
	First Degree	2/71 (2.8)		1/71 (1.4)	
	PhD	1/5 (20.0)		1/5 (20.0)	

Source: own computation (2024)

6.2.1. Magnitude of Hepatitis B virus infection by socio demographic characteristics

The burden of HBV in clinically diagnosed with STI patients was 5.6% (20/355). Based on sex the gender specific prevalence rate was higher in women 6.8% (13/189) than men 4.2 % (7/166) but the variation was not statistical significance (P=0.279). The magnitude of HBV was highest 9.0 % (10/111) in 20-29 years followed by specific age group 40-49 years had magnitude of seropositivity 6.0% (4/ 67) and no positive result at age group of 70+ years were recorded. But, the difference was not statistically significant (p=0.169).

Higher prevalence of HBSAg was found in study subjects with an education status of high school accounting 9.2 % (13/141) followed by diploma and first degree 5.1% (2/39) and 2.8% (2/71) respectively and the least was found in primary school educational levels which accounts 1.2% (1/81), however the variation was not statically significance (P=0.780). About 3.0% (1/33) sero-positive for HBsAg study participants were outside of urban area and 4.0% (13/322) were urban inhabitants (p = 0.497). Concerning to marital status the highest percent of positivity was obtained in divorced 5.0 % (1/20) followed by married 4.8 %(10/207) and the lowest seropositivity was observed in single 2.8% (3/105) study subjects (p = 0.580) (table 2).

6.2.2. Magnitude of Hepatitis C infection by socio demographic characteristics

In this study up to 3.1 % (11/355) of the study subjects were positive for anti-HCV antibody test. The gender based prevalence rate was highest among male 4.2 % (7/166) than female 2.1% (4/189), however the difference was not statistical significance (p=0.256). The burden of HCV by specific age group was higher 5.4% (6/111) in 20-29 years followed by specific age categories 3.3 % (1/30) in 60-69 the difference was not statistical significant. The highest seropositivity rate for anti-HCV Ab was seen among married participants with the amount of 3.0 % (6/207) followed by single 2.8 % (3/105) (P= 0.357) (table2).

6.3. Risk factors of HBV

In this finding, history of STI (p=0.001), blood transfusion (p=0.00) and shaving by barber (p=0.020) had association with HBV infections. In relation to history of STI, 54.9 % of STI sydromic diagnosed participants had history of STI in their overall life time. From those 3.7% (7/183) were positive for HBsAg (OR= 8.034; 95% CI: 1.835- 35.170, P= 0.001. Concerning

shaving by barber 63.9% (227/355) of STI sydromic diagnosed patients had history of shaving by barber. From those 7.4 % (18/227) were positive for HBsAg (OR= 4.894; 95% CI: 1.116-4.485, P= 0.020). Those who had history of blood transfusion in STI syndromic diagnosed participants were 21.4%. From those 3.2% (2/62) were positive for HBsAg (table 3).

Table 2: Bivariate Assessment of associated factors for hepatitis B infection at selected government health facility (January to May 2024)

Variables	Frequency (%)	No.of HBsAg positive (%)	p-value
Blood transfusion (yes)	62(17.4)	2(3.2)	0.000*
Dental extraction(yes)	108(30.4)	6(5.3)	0.966
Tattooing on gum(yes)	51(14.4)	4(7.3)	0.487
Tattooing on body(yes)	60(16.9)	3(4.8)	0.414
Shaving by barber*(yes)**	227(63.9)	18(7.4)	0.020*
Ear piercing(yes)	176(49.6)	12(6.4)	0.339
Contact with jaundice patient(yes)	64(18.0)	6(8.6)	0.141
Circumcision(yes)	178(50.1)	7(3.6)	0.164
Hospital admission(yes)	96(27.0)	5(5.0)	0.896
Surgical procedure(yes)	89(25.0)	4(4.4)	0.739
Alcohol consumption(yes)	119(33.5)	6(4.8)	0.732
History of STI/STD(yes)	183(51.5)	7(3.7)	0.001*
Abortion(yes)**	51(14.4)	4(7.3)	0.200
Body piercing for venous(yes)	302(85.0)	15(4.8)	0.331

= stands for female *= stands for male *=statically significance ($p \leq 0.05$).

Source: own computation (2024)

6.4. Associated factors of HCV virus infection

In this study blood transfusion was associated with HCV infection with (p=0.000). In this study, 21.4% (76/355) of blood transfused, STI syndromic diagnosed patients with evidence of blood transfusion. From those 8.4 % (7/76) were seropositive of anti-HCV (OR= 6.975; 95% CI: 1.985-24.503, P= 0.000). The participants with previous history of blood transfusion were 6.9 times more chances for contracting HCV infection than those who had not and the variation was statistically significant ($p<0.05$).

Table 3: Bivariate Assessment of associated factors for hepatitis C infection at selected government health facility (January to May 2024)

Variable	Frequency (%)	No of HCVAb positive (%)	p-value
Blood transfusion (yes)	76(21.4)	7(8.4)	0.000*
Dental extraction(yes)	108(30.4)	4(3.6)	0.700
Tattooing on gum(yes)	51(14.4)	1(2.0)	0.897
Tattooing on body(yes)	60(16.9)	1(1.6)	0.591
Shaving by barber*(yes)**	227(63.9)	8(3.4)	0.325
Ear piercing(yes)	176(49.6)	1(3.7)	0.698
Contact with jaundice patient(yes)	64(18.0)	1(2.8)	0.798
Circumcision(yes)	178(50.1)	3(1.7)	0.375
Hospital admission(yes)	96(27.0)	4(5.9)	0.113
Surgical procedure(yes)	89(25.0)	6(3.3)	0.964
Alcohol consumption(yes)	119(33.5)	2(2.1)	0.951
History of STI/STD(yes)	183(51.5)	2(2.2)	0.740
Abortion(yes)**	51(14.4)	6(4.8)	0.372
Body piercing for venous(yes)	302(85.0)	6(3.2)	0.408

= Concerning female *=concerning male *=Statically Significance ($p \leq 0.05$).

Source: own computation (2024)

6.5. Multivariate Analysis of risk factor for hepatitis B infection

In multivariate analysis of HBV infection, history of blood transfusion (COR= 10.274; 95% CI: 3.8-27.8, P=0.000), shaving by barber (COR=4.8; 95%CI: 1.1-21.4, p=0.020) and history of STI (COR=8.0; 95% CI; 1.8-35.2, p=0.001) had significant association. Those with ($p \leq 0.2$) were incorporated in multiple logistic regression analysis for controlling confounders and to investigate the influence of risk factors on HBV history of STD statically significant with (AOR 7.4; 95 % CI: 1.6-32.9, P= 0.009) but contact with jaundice was not statically significant with (AOR 1.6; 95%CI: 1.6-32.9, p=0.335) (Table 5).

Table 4: Multivariate analysis of risk factor for hepatitis B infection at selected government health facility (January to June 2024)

Characteristics	OR(95%, CI	p-value	AOR (95%, CI)	p-value
History of STD Yes	8.0(1.8-35.2)	0.001*	7.4(1.6-32.9)	0.009*
Contact with jaundice patient Yes	2.0(0.8-5.6)	0.141	1.6(0.6-4.6)	0.335
Circumcision Yes	0.5(0.2-1.3)	0.164	0.5(0.2-1.2)	0.877
Abortion Yes**	1.5(0.5-4.8)	0.200	0.9(0.3-3.0)	0.197

**= concerning female, *statically significant (P≤0.05).

Source: own computation (2024)

6.6. Multivariate Analysis of risk factor for hepatitis C infection

In multivariate analysis, it was investigated that of significance risk variables for HCV infection among participated STI syndromic diagnosed patients were history of blood transfusion (OR = 6.9; 95%CI: 1.9-24.5, p=0.003) had significance association with HCV. Those with p value equal or less than 0.2 were involved in multiple logistic regression to manage confounders and for assessing the effects of risk variables on HCV infection and history of blood transfusion had statically significant association with (AOR of 7.4; 95%CI: 1.9-28.8, p=0.003) (Table 6).

Table 5: Multivariate analysis of risk factor for hepatitis C infection at selected government health facility (January to June 2024)

Characteristics	OR (95%;CI)	P-value	AOR (95%;CI)	P-value
History of blood transfusion Yes	6.9(1.9-24.5)	0.000*	7.4(1.9-28.8)	0.003*
Contact With Jaundice Patients Yes	2.8(0.8-9.7)	0.113	1.8(0.4-6.6)	0.389
Abortion Yes**	1.3(0.3-6.4)	0.054	0.5(0.1-2.8)	0.451

**= concerning female,*=statically significant (P≤0.05).

Source: own computation (2024)

7. Discussion

In this study, which involved 355 syndromic cases of STI patients, 5.6% and 3.1% of patients were tested and positive for HBsAg and HCV antibodies, respectively. Comparable results from studies conducted on similar subjects in various countries were reported: the prevalence of HCV was 3.2% in Japan [28], 2.1% and 12.4% for HBV and HCV in Brazil [31], 12.1% for HBsAg in Mexico [43], 3% for anti-HCV positive individuals in Barcelona,Spain [44], 7% and 8% for HCV and HBV in Baltimore City [45], and 8% and 8% for HCV and HBV in North India [47]. Comparable research subjects in earlier studies had lower seropositivity than the current study. 1.36% for HBV and 0.45% for HCV in the USA [46], 2.5% for HBsAg and 1% for HCV [49], 3.2% for HBVsAg in Brazil [50], 1.4% for HBsAG and 0.3% for the HCV antibody [48], 3.4% for HBVsAg in Central India [51], 4.9% of patients in Chaina were HBVsAg seropositive [52], and 4.8% for HBVsAg in another study conducted in Canada [53]. These differences may arise from changes in the research populations, living conditions, and behavioral aspects related to HBV and HCV infection.

Comparing our findings with those of other studies conducted in Ethiopia on various study populations, other studies reveal varying and comparable rates of HBV and HCV. An investigation was conducted on the prevalence of HBV and reported 4.11% HBV and HCV 0.6% in pregnant women attending antenatal clinics in Bahir Dar health institutions [38]. The overall prevalence of HBsAg and anti-HCV among refugees in Gambella was 7.3% and 2.0%, respectively [10], the prevalence of HBV 3.57% and HCV 1.59% in cleaners in Addis Abeba [54], the magnitude of HBsAg and anti-HCV in blood donors in Dire Dewa 4.67% and 0.96%, respectively [39], and the community in Addis Ababa had 7% HBsAg [40]. In Addis Ababa, a cross-sectional investigation of people with presumed PTB revealed a 3.6% prevalence of HBsAg [41]. A subsequent retrospective investigation was carried out at the Aïoun hospital in Mauritania revealed a prevalence of 0.2% for HCV and 11.8% for HBV [36].

This variance may be caused by individuals who have serious STDs and engage in high-risk behaviors include injecting drugs and drinking, having several sexual partners, using condoms inappropriately, having same-sex relationships, and bartering sex for money [55]. In contrast to our investigation, earlier reports from Ethiopia among blood donors revealed higher seropositivity, with 14.9% HBsAg and 8.6% HCV Ab seropositivity [56]. These reports were

based on research conducted in our nation in various patients. The current study's findings were contrasted with findings from other research subjects in other nations, which were lower than 40.8% HBsAg and 30% HCVAbs among liver disease patients in Malaysia [57], and 33% HBsAg and 30% anti- HCV among HCC patients globally in 2015 [58]. The disease itself may be brought on by these viruses, which could explain the increased sero-burden in CLD patients compared to STI syndromic diagnosed patients in these various categories. The study participants' age distribution of STI syndromic diagnosed patients revealed that every single case of STI syndromic diagnosed patients was older than 20. 33.3% of patients were between the ages of 20 and 29, roughly 26.2% were between the ages of 39 and 49, 18.6% were in their fourth decade, 10.1% were in the 50–59 age groups, and the lowest age range seen was 70+.

In Gonder, a similar high age distribution (48.5%) of participants with STI syndromic diagnoses who were between the ages of 25 and 34 was noted [59]. This could be because the majority of STI diseases affect people over the age of 20. In this study, with the age range of 20-29 had higher magnitude of HBV, with a prevalence of 50% (10/20), and HCV, with a prevalence of 36.4% (4/11). Comparably, a study conducted on STI patients at the North Indian Clinic Attendees of Tertiary Care Hospital revealed that 44.1% of HbsAg positive participants were between the ages of 20 and 29 [60]. The hospital is situated in the Central Indian tribal zone. Young age groups accounted for 11.3% of HbsAg positive participants [51].

Approximately 1.7% (n=6) of the research participants co-infected with HBV and HCV. In contrast, 7.18% of patients in the University of Gonder, Ethiopia, had co-infection [61]. In the USA, the percentage is between 10% and 15% higher [62].The same source of transmission with sexual intercourse, contact with contaminated blood or body fluids, and breastfeeding may be to blame for this [55].

These findings suggest that female is more likely than male to be seropositive for HBV and have a lower prevalence of HCV Ab in women. According to comparatively fewer reports to this study, in Gambella, Ethiopia, 6.8% and 1.4% of females and 9.6% and 4.8% of males, respectively, tested positive for HBsAg and anti-HCV [10]. In the current study, cases of HCV had significant association with STI patients was lower than the HBV associated cases and the highest HBV-HCV pattern is mostly reported in Ethiopia for the past some decades as many

reports have shown on a variety of study subjects, such as in Dire Dawa Blood Bank HBV and HCV was 4.67% and 0.96% [39], in Gonder showed that the prevalence of HBV and HCV was 5.20% and 0.93%, respectively [63], Ukrainian regions, the prevalence of HBV and HCV was 60.2% and 23.3% among prisoners released within six months[34]. In diverse research populations, multiple studies found that the prevalence of HCV was higher than that of HBV. Geographical diversity and based on evidence the HCV has a higher morbidity for producing liver illness than the HBV may account for the highest burden of HCV infection compared to HBV infection in the another reports. Seropositivity results for HCV (2.10%) and HBV (1.6%) were reported from rest locations utilized by the miners in Brazil [31]. History of blood transfusion was found to be a significance risk factor for HCV infected among STI patients, with an (OR 6.9, 95% CI: 1.9-24.5, p=0.000), despite the fact that other researchers have reported different risk factors for HCV infection. One possible explanation for this could be misuse of blood transfusions. 13.2% of HIV patients with MSM in Germany tested positive for HCV [64]. A community-based study conducted in China revealed a general population prevalence rate of HCV infection of 0.79% [32].

The reasons for the difference in magnitude were sampling variability, testing methodological differences, and regional variation. The epidemiology of hepatitis in these various nations, knowledge of the route of transmission, and the need of health professionals to put universal procedures in place to stop the transmission is other factors contributing to the difference. The start of national immunization programs in other nations may characterize this nonconformance.

Though the studies used in this review were conducted by using immunosorbant assay-based detection kits with a roughly the same principle of antibody screening, the other possible cause of the nonconformance could be the possible variation in sensitivity and specificity of the commercially available test kits used in many studies. Many men get their hair trimmed by barbers in order to maintain their attractive appearance. If shaving tools are used on multiple people without being sterilized, the intimate contact of the tools with blood and other fluids could spread viral diseases. Other risk factors that were not linked to HBV infection were dental extractions at medical facilities, circumcision, surgery, blood transfusions, hospital admissions, ear piercings, uvuloctomy, gum tattoos, contact with patients who were jaundiced, alcohol intake, and abortions. Similar to the current study, the following investigations found no

significant associations between blood donors and HBV/HCV prevalence: in Ukrainian regions, HBV/HCV prevalence was 60.2%, and among inmates released within six months, it was 23.3% [34]. Multivariate investigation regarding HCV revealed a correlation between prior STD exposure and HCV infection. 54.5% of STI syndromic diagnosed patients with a history of blood transfusions had an overall prevalence of HCV. Individuals with a history of blood transfusion were more likely than their counterparts to be infected with HCV, and this difference was statistically significant (P=0.000). According to a research, the prevalence of HCV among blood donors in Mombasa, Kenya, was 12.5% [65].The improper use of blood transfusions and the inadequate specificity of blood donor screening tests may be examples of this.

8. Strength and Limitations of the study

8.1. Strength of the study

To underline this, we evaluated the sero-burden of HBV and HCV infection among the patients with STI-diagnosed syndromes for the first time.

8.2. Limitations of the study

This study did not assess the prevalence of that viral hepatitis at the population level because our focus was mainly on measuring the prevalence of HBV and HCV in STI syndromic identified patients.

Since the study participants were patients with STI diagnoses, there may be some bias in the information we have.

9. Conclusion and recommendation

9.1 Conclusion

According to the current study, the prevalence of HBV and HCV in patients with STI-syndromic diagnoses was 5.6% and 3.1%, respectively. Regarding socio-demographic characteristics, there was no statistically significant variation in the harboring of HBV and HCV infections; however, a history of blood transfusion, barber-shaved hair, and a history of STIs were significance risk variables for HBV infection, and previous history of blood transfusion was also associated with HCV infection. Patients with STI syndromic diagnoses and those with a history of blood transfusions and STI have a 10.2 and 7.4 times higher risk of HBV infection, respectively, than those without such diagnoses. HBV infection is common in these populations. Blood transfusions carry a 6.9-fold increased risk of HCV infection compared to people who do not have history of STI. To prevent viral infection, individuals with STI syndromic diagnoses must be informed about the hepatitis B and hepatitis C viruses' route of transmission.

9.2 Recommendations

HBV and HCV are common health incidences in STI patients, the following recommendations were mandatory.

- All STI syndromic diagnosed patients would be considered for testing for HBV and HCV virus with evidence of possible associated factors.
- Vaccine against HBV is fundamental in the control of HBV infection, and is advisable for all newborns and the population who are at elevated risk for infection especially STI syndromic diagnosed clients who were in acute phase of disease.
- Awareness of Patients of STI syndromic diagnosed patients on the ways of transmission of the HBV and HCV is vital for the control of viral infection.
- Additional wide scale studies are needed to aware more on the incidence and ways of transmission of the viral in STI syndromic diagnosed individuals.
- To learn more about the prevalence and mode of virus transmission in patients with STI syndromic diagnoses, larger-scale research is necessary.

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11. ANNEX

Annex I: Participant Information Sheet (English version)

My name is Beyene Demil. I am a laboratory technologist and postgraduate student at Addis Ababa University. Now I am conducting a study entitled Prevalence of HBV and HCV and associated risk factors among sexually transmitted infection syndromic diagnosed patients attending at selected government health facility, Addis Ababa, Ethiopia. The patient you were invited to participate in this study. Please read the following statements and ask any unclear points before you agree to your patient participate. If you agree to be included in this study, I will like to ask you to sign on a document to show your agreement; participate accordingly, and give clinical specimen (the patient you were). The topic of this study is sero-burdun HBV and HCV and associated risk factors among sexually transmitted infection syndromic diagnosed patients attending at selected government health facility in Addis Ababa, Ethiopia. Since HBV and HCV is one of the major health problems in our country, the result of the study can be helpful in planning and intervention to solve the problem.

Participation in this study is exclusively voluntarily. If you were not interested to participate or if you once decide to participate and withdraw your patient at any time, there was no consequence and your patient will get all the services provided in the hospital with no problem. If you decide to make your patient participate, you have to sign on the assent/ permission template form and you may obtain a copy of this information sheet. Expected from participant/ guardians as a participant of this study, patient is expected to give blood. Being asked to give sample does not necessarily mean that he/she has the disease? When he/she is found to be positive for the HBV/HCV, he/she was informed by the health worker and receives proper treatment.

You need to know that your patient results might be discussed with other appropriate individual out of this hospital. But his/her name, address will not be disclosed rather an identification code was used in such conditions. Time required for participating, your patient will spend 10-15 minutes until the specimen is collected and permission form is signed. Risks of participant Specimen collection will not have serious effect and she/he will not get any risk as the sample was collected by well trained professionals. But he/she may fill minor temporary pain during sample collection. Confidentiality The information in his/her records is strictly confidential. All

information that you give and the results from his/her specimen was used for this study only. Only limited numbers of professional will have access the information. The information was encoded in a computer and saved with password protection. Benefits of participation by participating, he/she will get no financial benefits. Even though there is no direct benefit due to participation in this study, the findings of the study is useful for better understanding of the problems of HBV and HCV. You will also obtain all the results of the analysis for free and communicated to his/her physician for the appropriate management. Rights of participants His/her participation is completely voluntary, and you can refuse to participate or withdraw from the study at any time. Refusal to participate will not result in loss of medical were provided or any other benefits. He/she can get his/her results of the analysis. Communication In case if you have any questions, unclear ideas and doubt about the project, contact addresses were:

Investigator: Beyene Demil (BSc), +251910166134

Email- beyenedemi292009@gmail.com

For additional information, please contact Addis Ababa University, College of Health Sciences and Department of Medical Laboratory Sciences at: Telephone +251112755170 40 Your signature below indicates that you have read /or listened, and understand the information provided for you about the study. Before you sign, please understand purpose of the study, procedure, risks and benefits of participation, right to refuse or withdraw, confidentiality and privacy, and who to contact if you have question. I have read /or listened to the description of the study and I understand what procedures were and what will happen to my patient in the study.

Participants name ----------Signature---------- Date: ----------

Data collector name: ---------- Signature: ---------- Date: ----------

2. Participant Information Sheet (Amharic version)

ጥናቱ መረጃና ተሳታፊነት መግለጫ ቅጽ የጥናቱ ዓላማ ሄፓታ ይተስ “ቢ” እና ሄፓታይተስ “ሲ”ቫይረሶች የዓባላዘር በሽታ ህሙማን መካከል ያለዉን ስርጭት ለማጥናት የታቀደ ነዉ፡፡ በጥናቱ ስለመሳተፍ፡- በዚህ ጥናት መሳተፍ በሙሉ ፈቃደኝነት ሊይ የተመሰረተ ነዉ፡ ስለሆነም በጥናቱ እንዲሳተፉ ፈቃደኛነትዎን እንጠይቃሆን ፡ለመሳተፍ ከፈቀዱ ፤5ሚሊ ሌትር የደም ናሙና ከክንዱ/ዷ ተወስዶ የሊቦራቶሪ ምርመራ ይደረግለታል/ላታል ፡፡ የሊቦራቶሪ ምርመራውም ሄፓታይተስ “ቢ ” እና “ሲ” ቫይረስን በደም ዉስጥ መኖርና አሆመኖር ማረጋገጥ ይሆናል ፡፡ ደም ከመወሰዱ በፊት እና ከውጤት በሁዋ በሰለጠነ ባለሙያ የምክር አገሌግልት ያገኛሉ፡ የደም ናሙናዉም የሚወሰደዉ ንጽህናዉ በተጠበቀ አዲስ እና በታሸገ መርፌ ና ስሪንጅ ነዉ፡፡ በጥናቱ ሊከሰቱ የሚችሉ ተያያዥ ችግሮች የደም ናሙናዉን ለመዉሰድ መርፌ ሲገባ ከሚፈጥረዉ የቅጽበት ህመም

ስሜት በስተቀር የጎላ ችግር አያመጣም ነገር ግን ምቾት ካሌተሰማው/ት ሀኪም እንዲያይሌዎት ይደረጋል ። በጥናቱ በመሳተፍ የሚገኝ ጥቅም የደም ናሙና የላብራቶሪ ዉጤት ምንም አይነት ችግር ካሳየ የመድሃኒት ትእዛዝና የባለሙያ ምክር ይሰጠዋል/ጣታል ። የጥናቱ መረጃዎች ሚስጥራዊነት በጥናቱ ውስጥ የተሰበሰቡ ማናቸዉም ግላዊ መረጃዎች ሚስጥራዊነታቸዉ የተጠበቀ ይሆናል ። ከማንነትዎ ጋር በቀጥታ ተያያዥነት ያላቸዉ መረጃዎች በሙሉ በዋና ተመራማሪዉ ሚስጥራዊ በሆነ የመረጃ ጥንቅር ዘዴ ከተቀየሩ በኃላ ብቻ ለምርምር ሂደቱ የሚዉሉ ይሆናል ። የጥናቱን ዉጤት ስለማሳወቅ የዚህ ጥናት ዉጤት በተለያዩ የህትመት ውጤቶች የሚቀርብ ሲሆን ይህ ከማንነቱ/ዋ ጋር የተያያዘ ምንም አይነተ መረጃን አያካትትም። ስለዚህም የጥናቱን ዉጤት በሪፖርት እናቀርበዉ ዘንድ ፈቃዶን እነጠይቃለን ። ከጥናቱ ስለመዉጣትና ስለማቋረጥ፡- ይህ ጥናት በፈቀደኝነት ላይ የተመሰረተ እንደመሆኑ መጠን በማንኛዉም ወቅት በፈቃድዎ ከጥናቱ መውጣት ይችላሉ ። ከጥናቱ ቢያስወጡትም እንኳን የተለመደዉን የህክምና እርዳታ በጤና ተቋሙ ዉስጥ በማንኛዉም ጊዜ የማግኘት መብት አለው/ላት

Annex II: Consent form (English version) I undersigned the purpose of this study. I have been informed there is no harm except little discomfort during sample collections. I have been informed that other people will not know my results. I understand that there is no benefit to me personally apart from clinical service I get from these results .I have been told that participation in this study is voluntary and I may refuse to be in the study. The study has been explained to me in the language I understand. I give consent to participate after a clear understanding of the objectives and conditions of the study.

Participants name ----------Signature----------Date: ----------

Data collector name: ----------Signature: ---------- Date: ----------

2. Consent form (Amharic version) ስለ ስምምነቱ ማረጋገጫ ፊርማ እኔ ስሜ ከታች የተገለፀዉ የጥናቱ ተሳታፊ ስሆን 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 የሚችለ አደጋዎች በሚገባ የተረዳሁና ከጥናቱ በማንኛዉም ደረጃ ለመሰረዝ ብወስን ተገቢ የሆኑ ህክምናዎችና እገዛዎች ሁሉ እነደማይነፍጉበኝ በማመን ነዉ። እነዚህ መረጃዎች ሁሉ በሚገባ በምረዳዉ ቋንቋ የተገለጸልኝ መሆኑን በፊርማዬ አረጋግጣለሁ ።

የተሳታፊው ሙሉ ስም----------ፊርማ----------

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

የምስክር ሙሉ ስም ----------ፊርማ---------- ይህን ጥናት በተመሆከተ ጥያቄ ቢኖርዎት ወይም ከዚህ ጋራ በተዛመደ መልኩ ስለሚያጋጥመዎት ድንገተኛ ችግር በሚከተለዉ አድራሻ ይጠቀሙ። ሞባይሌ+251910166134

ኢ-ሜይሌ፤ beyenedemi292009@gmail.com የሕክምና ላብራቶሪ ሳይንስ ትምህርት ክፍል፤ የጤና ሳይንስ ኮሌጅ፤ አዲስ አበባ ዩኒቨርሲቲ ለተጨማሪ መረጃ፡ አዲስ አበባ ዩኒቨርሲቲ ፤ የሕክምና ሊብራቶሪ ሳይንስ ት/ክፍል ይጠይቁ፡፡ ስሌክ+251112755170 44

Annex III: Questionnaires (English version)

ADDIS ABABA UNIVERSITY COLLEGE OF HEALTH SCIENCE DEPARTMENT OF MEDICAL LABORATORY SCIENCES

For data collectors: For each question please put a cross clearly inside one box 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 in years A.20-29 B. 30-39 B. 50-69 D.70+

1.4. Types of STI cases A. unusual discharge around genital area B. Rash C. Ichy genitals and anus Warts around your genital and anus

2. Back ground information

2.1. Residence A. Urban B. Rural

2.2. Current occupational status A. Self-employed B. Civil servant C. House Wife D. Student F. Other specify

2.3. Marital status A. Married B. single C divorced D. widowed

2.4 Educational Status A. Illiterate B. read and writes C. 1_8 C. 9_12 D. >12 Has she/he have or ever practiced the following?

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

3.2. Blood transfusion A. Yes B. No

3.3. Abortion A. yes B. No

3.4. Dental extraction at health facility A. yes B. No

3.5. Circumcision A. yes B. No

3.6. Hospital admission A. yes B. No

3.7. Surgical procedure A. yes B. No

3.8. Venous or body piercing for treatment A. yes B. No

3.9. Delivery by TBA A. yes B. No

3.10. Ear piercing A. yes B. No

3.11. Nose piercing A. yes B. No

3.12. Uvuloctomy A. yes B. No

3.13. Tattooing on body A. yes B. No

3.14. Tattooing on gum A. yes B. No

3.15. Shaving A. yes B. No

3.16. Contact with jaundiced patient A. yes B. No

3.17. Frequent alcohol consumption A. yes B. No

2. Questionnaires (Amharic version) አዲስ አበባ ዩኒቨርስቲ የህክምና ፋኩሊቲ የሚዲካል ላቦራቶትሪ ትምህርት ክፍል ለመረጃ ስብሳቢዎች ፤ ጥያቄዉን ከጠየቃችሁ በኋላ መሌሱን በተሰጠዉ ሳጥን ውስጥ ከተሰጡት አማራጮች አንዱን የኤክስ ምልክት ይጻፉ ፡፡ ጠቅላላጥያቄ 1.1. ኮድ----------

1.2. ፆታ ሀ. ወንድ ለ . ሴት

1.2. እድሜ ሀ.18-34 ለ.35-50 ሐ. 5ነ-69 መ. 70-87

1.3. መኖሪያ አካባቢ ሀ. ገጠር ለ . ከተማ

1.4. ሥራ ሀ. የግል ለ . ሹፌር ሐ. የቤት እመቤ ት መ. ተማሪ ሠ. ገበሬ ረ. ሌላ (ይገለጽ)---

1.5. የጋብቻ ሁኔታ ሀ. ያገባ /ች ለ. ያላገባ /ች ሐ. የፈታ /ች መ. የሞ ተበት /ባት

1.6 የዓባላዘር በሽታው አይነት ሀ. ፈሳሽ ለ. ማሳከክክ ሐ.ብልትና ፊጢጣ አካባቢ ቁስለት

2.ሄፓታይተስ “ቢ ” እና “ሲ”ን በተመለከተ ጥያቄዎች ከዚህ በታች ያለዎትን በህይወት/ዋ አድርጎ /ጋ ያውቃል/ታውቃለች ?

2.1 ያባላዘር በሽታ ሀ. አዎ ለ .አይደለም

2.2 ከአንድ በላይ የትዳር ጓደኛ ሀ. አ ዎ ለ. አይደለም

2.3 ደም መቀበል ሀ. አዎ ሇ. አይደለም

2. 4 ማስወረድ ሀ. አዎ ለ. አይደለም

2.5 ጤና ድርጅት ጥርስ ማስነቀል ሀ. አዎ ለ. አይደለም

2.6 ግርዛት ሀ. አዎ ለ. አይደለም

2.7 ሆስፒታል መተኛት ሀ. አዎ ለ. አይደለም

2.8 ማንኛዉም አይነት ቀዶ ጥገና ሀ. አዎ ለ. አይደለም

2.9 ሞኝ ባገኝ መበጣት ሀ. አዎ ለ. አይደለም

2.10 በልምድ አዋላጅ መዉለድ ሀ. አዎ ለ. አይደለም

2.11 ጆሮ መበሳት ሀ. አዎ ለ. አይደለም

2.12. አፍንጫ መበሳት ሀ. አዎ ለ. አይደለም

2.13. እንጥል ማስቆረጥ ሀ. አዎ ለ. አይደለም

2.14 ሰዉነት መነቀስ ሀ. አዎ ለ. አይደለም

2.15. ድድ መነቀስ ሀ. አዎ ለ. አይደለም

2.16 ፀጉር ቤት ፂም መላጨት ሀ. አዎ ለ . አይደለም

2.17 የወፍ በሽታ ከያዘዉ ሰዉ ጋር ንክኪ ሀ. አዎ ለ . አይደለም

2.18. አዘውትሮ መጠጥ መጠጣት ሀ. አዎ ለ. አይደለም

Annex V: Blood sample collection procedure

1) Syringes

2) Blood Collection Tubes.

3) Tourniquets. Single use, disposable, latex-free tourniquets

4) Antiseptic. Individually packaged 70% isopropyl alcohol wipes.

5) 2×2 Gauze

6) Sharps Disposal Container. An OSHA acceptable, puncture proof container marked "Bio hazardous".

7) Cotton

8) PPE's was worn at all times.

9) Wash hands in warm, running water with a appropriate hand washing product,

10) A lab coat or gown must be worn during blood collection procedures.

11) Place a sheathed needle or butterfly on the syringe.

12) Remove the cap and turn the bevel up.

13) Pull the skin tight with your thumb or index finger just below the puncture site.

14) Holding the needle in line with the vein, use a quick, small thrust to penetrate the skin and vein in one motion.

15) Draw the desired amount of blood by pulling back slowly on the syringe stopper. Release the tourniquet.

16) Place a gauze pad over the puncture site and quickly remove the needle.

17) Immediately apply pressure. Ask the patient to apply pressure to the gauze for at least 2 minutes.

18) When bleeding stops, apply a fresh bandage, gauze or tape.

19) Transfer blood drawn into the appropriate tubes as soon as possible using a Blood Transfer Device, as a delay could cause improper coagulation.

20) Gently invert tubes containing an additive 5-8 times.

21) Dispose of the syringe and needle as a unit into an appropriate sharps container.

Sexually Transmitted Diseases

Sexually transmitted diseases (STDs), also known as sexually transmitted infections (STIs), were very common. Millions of new infections occur every year in the world.

STDs pass from one person to another through vaginal, oral, and anal sex. They also can spread through intimate physical contact like heavy petting, though this is not very common.

STDs don't always cause symptoms or may only cause mild symptoms. Therefore, it is possible to have an infection and not know it. That is why getting an STD test is important if you were having sex. If you receive a positive STD diagnosis, know that all were treatable with medicine and some were curable entirely.

There were dozens of STDs. Some STDs, such as syphilis, gonorrhea, and chlamydia, were spread mainly by sexual contact. Other diseases, including Zika, Ebola, and mpox, can be spread sexually but were more often spread through ways other than sex.

STDs were preventable. If you have sex, know how to protect yourself and your sex partner(s) from STDs.

Bacterial Vaginosis

BV is a common, treatable, vaginal condition which can increase your chance of getting an STD.

Chlamydia

Chlamydia is a common, but treatable, STD. If left untreated, chlamydia can make it difficult for a woman to get pregnant.

Gonorrhea

Gonorrhea is a common STD that can be treated with the right medication. If left untreated, gonorrhea can cause very serious health problems.

Hepatitis

Viral hepatitis is the leading cause of liver cancer and the most common reason for liver transplants.

Herpes

Genital herpes is a common STD, but most people with the infection do not know they have it. While there is no cure, there were medicines available that can prevent or shorten outbreaks. These medicines also can make it less likely to pass the infection on.

HIV

HIV/AIDS & STDs People who have STDs were more likely to get HIV, when compared to people who do not have STDs.

Human Papillomavirus (HPV) Infection

HPV is the most common STI in the world, but most people with the infection have no symptoms. HPV can cause some health effects that were preventable with vaccines.

Mycoplasma genitalium (Mgen)

Mycoplamsa genitalium, or Mgen is an STD that can be treated with antibiotics. People receiving treatment for Mgen should take all of the medication as prescribed.

Syphilis

Syphilis can have very serious problems when left untreated. It is simple to cure with the right treatment.

Trichomoniasis

Most people who have trichomoniasis do not have any symptoms.

Annex IV: Laboratory method

1. HBsAg EIA Test

HbsAg ELISA is used for the qualitative determination of Hepatitis B surface antigen (HBsAg in human serum or plasma. This test is indicated for the screening of blood and blood products to be used for transfusion and an aid for the diagnosis of existing or previous hepatitis B infection.

HBsAg is one of the earliest markers that appear in blood following infection with Hepatitis B virus (HBV). Hepatitis B surface antigen (HBsAg) appears 1-7 weeks before biochemical evidence of liver disease or jaundice. Three weeks after the onset of acute hepatitis almost half of the patients will still be positive for HBsAg. In the chronic carrier state, the HBsAg persists for long periods (6-12 months) with no seroconversion to the corresponding antibodies. Therefore, screening for HBsAg is highly desirable for all donors, pregnant women and people in high-risk groups [66].

PRINCIPLE OF THE TEST

The HBsAg EIA is a solid-phase simultaneous sandwich immunoassay, which employs monoclonal antibodies and polyclonal antibodies specific for HBsAg. Microtiter well is coated with monoclonal antibodies specific for HBsAg. A serum specimen is added to the antibody coated Microtiter wells together with enzyme conjugated polyclonal antibodies.

HBsAg, if present, will form an antibody-HBsAg-antibody-enzyme complex. The plate is then washed to remove unbound material. Finally, a solution of substrate is added to the wells and incubated. A blue color will develop in proportion to the amount of HBsAg present in the specimen. The enzyme-substrate reaction can be stopped and the result is visualized by naked eye or read by EIA plate reader for absorbance at the wavelength of 450 nm [66].

SPECIMEN COLLECTION AND PREPARATION

No special preparation of the patient is required prior to blood collection. Blood should be collected by approved medical techniques. Remove serum or plasma from the clot or blood cells as soon as possible to avoid hemolysis. Grossly hemolytic, lipidic or turbid samples should not be used. Plasma samples containing EDTA, heparin or oxalate may interfere with test procedures and should be avoided. Specimen with extensive particulate should be clarified by centrifugation prior to use. Covered specimens may be stored for up to 48 hours at 2°-8°C prior to assaying. Specimens held for a longer time can be frozen at -20°C for mix prior to testing. Avoid repeated

freeze thaw. At least, two wells of negative and positive controls each should be run in every assay [66].

PRECAUTIONS

1. Caution: Some components of this kit contain human serum. No known test method can offer complete assurance that products derived from human blood will not transmit infectious agents. Therefore, all blood derivatives should be considered potentially infectious. It is recommended that these reagents and human specimens be handled using established good laboratory working practices.
2. Wear disposable gloves while handling kit reagents and specimens and thoroughly wash hands afterwards.
3. Dispose of all specimens and materials used to perform the test as if they contained infectious agents.
4. Do not mix reagents from kits with different lot numbers.
5. Cross contamination between reagents will invalidate the test results.
6. All reagents and components except the conjugate must be equilibrated at room temperature prior to use [66].

STORAGE OF TEST KITS AND INSTRUMENTATION

Unopened test kits should be stored at 2°-8°C upon receipt. Micro titer plate, once opened, should be kept in a sealed bag with desiccants to minimize exposure to damp air. To remove the required number of strips from the micro titer plates, bring the sealed pouches to room temperature first and then open the pouches [66].

This is very important because absorbed atmospheric moisture by cold plates significantly reduces their shelf life. Opened test kits will remain stable until the expiration date shown in 4°C, provided it is stored as described above. A micro titer plate reader with a bandwidth of 10 nm or less and an optical density range of 0-2 OD or greater at 450 nm wavelength is acceptable for use in absorbance measurement [64].

WORKING REAGENT PREPARATION, STORAGE AND STABILITY

No reagent preparation is required except for wash buffer, which is supplied as a 20 X concentrate [66].

WORKING WASH BUFFER

Dilute the 20X wash buffer concentrate with deionized or distilled water 1:20. For example, 5ml of wash buffer concentrate should be diluted to a total volume of 100 mL with deionized or distilled water [66].

STABILITY OF OPENED KIT COMPONENTS AND DILUTED REAGENTS

The diluted wash buffer is stable for at least one week when stored at room temperature. Substrate is stable for the expiration date of the kit. The micro titer plates should be opened after they have been kept at room temperature for 20-30 minutes. After removing the required number of strips, the plates should be resealed in the foil pouch bags along with the desiccant and stored at 2°-8°C. Exposure of HBsAg plates to humidity drastically reduces the shelf life [66].

PROCEDURE

It is strongly advised to analyze each specimen and controls in duplicate. All the reagents should equilibrate to room temperature before use.

1. Dispense one drop (50ul) of Positive Control as well as Negative Control in duplicate into respective wells. Set one blank well as background control, and 50ul of serum or plasma samples into respective test wells
2. Add one drop (50 ul) of Enzyme Conjugate to each well. Mix it gently by swirling the microtiter plate on flat bench for 1 minute. Do not add Enzyme Conjugate to the blank well.
3. Place the micro titer plates into a humidified box, and incubate at 37°C for 30 minutes.
4. Wash each well 4 times by filling each well with diluted wash buffer, then inverting the plate vigorously to get all water out and blocking the rim of wells on absorbent paper for a few seconds.
5. Add one drop (50 ul) of Substrate Solution A (HRP-substrate) to each well, then add one drop (50 ul) of Substrate Solution B (TMB) to each well. Mix gently and incubate at 37°C for 15 minutes.
6. Add 1 drop (50ul) of Stop Solution to each well to stop the color reaction.Read O.D. at 450 nm with an EIA plate reader [66]

INTERPRETATION OF RESULTS

A. Calculate OD ratio Specimen OD ratio = OD Value of test sample/Average OD Value of Negative Control

If the OD value of the negative control is less than 0.05, it should be reported as 0.05. If it is more than 0.05, it should be reported as the actual OD value measured.

B. Interpretations

Specimen OD ratio

Negative < 2.1

Positive ≥ 2.1

• The negative result indicates that there is no detectable HBsAg in the specimen.

• The specimen with a positive result should be tested duplicate again and confirmed with Western blot or other tests.

EIA Reader at 450 nm (using the OD value of the blank well to correct all the OD reading from all wells, The positive control OD value should be ≥0.8, the negative control should be ≤0.10): [66].

Cut-off Calculations:

Take average OD values of Negative control and add 0.15:

1x NC + 0.15 = Cut-off.

Positive OD reading: ≥Cut-off value

Negative OD reading: < Cut-off value [66].

2. HCV ANTIBODY EIA

Hepatitis C virus is a single stranded RNA virus with some structural relations to the flavivirus family. Nucleic acid sequences of HCV cDNA clones provided the basis for the construction of recombinant peptides representing putative hepatitis C virus proteins.4,5

Antihepatitis C virus antibody screening of blood using synthetic or recombinant proteins, helped to identify apparently healthy blood donors with anti-HCV antibodies who otherwise might have transmitted the virus.

This is an enzyme linked immunosorbent assay using recombinant proteins derived from core regions of HCV virus to detect the presence of HCV antibodies in human sera [67].

PRINCIPLE

Multiple epitopes of HCV proteins (Core, NS3, NS4 and NS5) are bound to the microliters wells. When antibodies to HCV are present in the test sample, they react with recombinant proteins and

attach to the solid-phase. Non-reactive antibodies are removed with the wash buffer. Human IgGs bound to the antigen are reacted with goat-anti-human IgG peroxidase conjugate and visualized by subsequent reactions with a chromogenic substrate. Positive sample generates a medium to dark blue color. No color or very pale blue color indicates a negative reaction. The intensity of the reaction is photo metrically quantitated [67].

PRECAUTION FOR USERS

All human source material used in the preparation of this product was found to be negative for the presence of HIV-1/HIV-2 antibodies, as well as for the hepatitis B surface antigen, using a commercial licensed method. Nevertheless, because no test method can offer complete assurance of the absence of infectious agents, this product should be handled with caution.

1. Avoid contact of reagents with the eyes and skin. If that occurs, wash thoroughly with water.
2. Wear gloves.
3. Do not pipette by mouth.
4. Do not smoke.
5. Dispose all used materials in a suitable biohazardous waste container.

Remains of samples, controls, aspirated reagents and pipette tips should be collected in a container for this purpose and autoclaved 1-hour at 121°C or treated with 10% sodium hypochlorite (final concentration) for 30 min before disposal. (Remains containing acid must be neutralised prior addition of sodium hypochlorite).

6. Adjust washer to the plate used (flat bottom) in order to wash properly.
7. Do not mix reagents from different lots.
8. Do not use reagents after expiration date.
9. Extreme care should be taken to avoid microbial contamination and cross contamination of reagents.
10. Use a new pipette tip for each specimen and each reagent.
11. Soaps and/or oxidising agents remaining in containers used for the substrate-TMB solution can interfere with the reaction [67].

SPECIMEN COLLECTION AND PREPARATION

Serum should be prepared from a whole blood specimen obtained by acceptable medical techniques. Either serum or plasma can be used in this test. Remove serum or plasma from the

clot or blood cells as soon as possible to avoid hemolysis. Specimen with extensive particulate should be clarified by centrifugation prior to use. Specimen frozen at -20°C or colder may be used. Avoid repeated freeze thaw [67].

STORAGE OF TEST KIT

Unopened test kits should be stored at 2-8°C upon receipt and the microtiter plate should be kept in a sealed bag to minimize exposure to damp air. Use up the reagents as soon as possible after the kit is unpacked [67].

PROCEDURE

1. Dispense 100µl of specimen diluent into individual test wells.
2. Dispense 100µl positive control and negative control duplicate into individual wells.
3. Add 10µl of each test sample into duplicate test wells; vortex to mix.
4. Incubate for 30 minutes at 37°C
5. Wash each well 5 times by filling each well with diluted wash buffer, then inverting the plate vigorously to get all water out and blocking the rim of wells on absorbent paper for a few seconds.
6. Add 100µl of Enzyme Conjugate to each well. Mix it gently by swirling the microtiter plate on flat bench for 1 minute. Do not add Enzyme Conjugate to the blank well.
7. Incubate for 20 minutes at 37°C
8. Wash the plate 5 times as step 6.
9. Add one drop (50µl) of Substrate Solution A (HRP-substrate) to each well, then add one drop (50µl) of Substrate Solution B (TMB) to each well. Mix gently and incubate at 37°C for 10 minutes. .
10. Add one drop (50µl) of Stop Solution to each well to stop the color reaction. Read O.D. at 450 nm with an EIA reader [67].

RESULT INTERPRETATION

EIA Reader at 450 nm (using the OD value of the blank well to correct all the OD reading from all wells, The positive control OD value should be ≥ 0.8, the negative control should be ≤ 0.10):

Cut-off Calculations:

Take average OD values of Negative control and add 0.15:

1x NC + 0.15 = Cut-off.

Positive OD reading: ≥Cut-off value

Negative OD reading: < Cut-off value [67].

LIMITATIONS

1. As the other sensitive immunoassays, there is the possibility that non-repeatable reaction may occur due to inadequate washing. So do aspirate the well or get rid of entire content of wells completely before adding the washing solution.

2. As with all diagnostic tests, a definitive clinical diagnosis should not be made based only on the results of a single test. A complete evaluation by physician is needed for a final diagnosis.

3. Samples with positive or equivocal result must be reanalyzed in duplicate. If both retest values are lower than the cut-off, the final interpretation of the test is negative for HCV antibodies. If the result is repeatedly positive or equivocal, the sample should be further investigated with other methods.

4. Optimal assay performance requires strict adherence to the assay procedure described.

Deviation from the procedure may lead to aberrant results.

5. A negative result does not exclude the possibility of exposure or infection With HCV [67].

Declaration

Annex IV: Assurance of Principal Investigator

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 of the Investigator

Signature: _____

Date: _____

Approval of the primary Advisor

This thesis has been submitted for examination with my approval as University Advisor.

Name:- Mistire Wolde(MSc,PhD, Associate Professor)

Signature: _____

Date: _____

Name: - Asegedech Asmamaw (MSc)

Signature: _____

Date: _____