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Magnitude of Hepatitis B, Hepatitis C, Syphilis and Their Associated Factors among Emigrants come for medical checkup in Labco Advanced Clinical Laboratory, Addis Ababa, Ethiopia.

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Abbreviation

AAU	Addis Ababa University
Ab	Antibody
Ag	Antigen
Anti-HCV	HCV antibody
AOR	Adjusted odds ratio
CHB	Chronic hepatitis B
CHC	Chronic hepatitis C
CI	Confidence interval
CLD	Chronic Liver Disease
DNA	Dinucleotide antibody
ELISA	Enzyme-linked immune sorbent assay
EPHI	Ethiopian Public Health Institute
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HIV	Human immune virus
IgA	Immune globulin A
IgG	Immune globulin G
IgM	Immune globulin M
MTCT	Mother-to-child transmission
RNA	Ribosomal Nucleotide
RT	Rapid test
SD	Standard deviation
SOPs	Standard operating procedures
SPSS	Statistical Package for the Social Sciences
TPHA	Trepanoma palladium hem- agglutination test
WHO	World Health Organization

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Abstract

Background: Viral Hepatitis is a contagious liver disease mainly caused by hepatitis B virus and hepatitis C virus. Syphilis is an infectious venereal disease caused by the spiral-shaped bacterium *Treponema palladium*. Globally, Hepatitis B, Hepatitis C and syphilis are common conditions associated with high morbidity and mortality.

Objective: To assess the magnitude of Hepatitis B, Hepatitis C, Syphilis and Their Associated Factors among Emigrants come for medical checkup in Labco Advanced Clinical Laboratory, Addis Ababa, Ethiopia.

Methods: A cross-sectional study was conducted among 500 emigrants, from February until April 2021. A questionnaire was used to collect emigrants' socio-demographic and risk factors, of hepatitis B, hepatitis C and syphilis infections. Five milliliters of blood sample were collected from each participant and the serum was used for HBsAg, anti-HCV antibody and Syphilis screening rapid tests. Reactive samples were further tested and confirmed by ELISA method. Data was analyzed using SPSS version 26 software package. To identify associated factors, Bivariable and Multivariable binary logistic regression analyses were done. Statistical significance was considered at level of significance of 5%, and adjusted odds ratio (AOR) with 95% confidence interval (CI) was used to present the estimates of the strength of the association.

Result: In this study, a total of 500 emigrants were participated making a response rate of 96.8%. Among the total participants, 36.4% (n=182/500) were male and 63.6 % (n=318/500) were female resulting in a male-to-female ratio of 1:2. The overall magnitude of Hepatitis B, Hepatitis C, and Syphilis were 2.2%, 0.2%, and 0.6% respectively. The identified independent determinants of HBsAg were exchange of sharp materials and history of tooth extraction with [AOR= 7.89; 95% CI 1.75-34.95:].and [AOR= 24.92:95% CI (5.95-104.4):], respectively.

Conclusion: The magnitude of HBsAg was intimidate, anti-HCV and syphilis was low among Emigrants attending in Labco Advanced Clinical Laboratory according to WHO criteria. Exchange of sharp materials and history of tooth extraction are independent determinants of HBsAg.

Keywords: Hepatitis B, Hepatitis C, Syphilis, Emigrants, Addis Ababa, Ethiopia.

1. Introduction

1.1. Background

A different medical checkup had done for emigrants who travel abroad searching for jobs. They are testing for infectious diseases such as viral Hepatitis and syphilis. Viral Hepatitis is a contagious liver disease mainly caused by hepatitis B virus (HBV) and hepatitis C virus (HCV). It can be Transmitting through blood and body fluids products. The main risks of HBV and HCV transmission were associated Horizontally through unprotected sex, unsafe injection, and Traditional practice such as scarification, ear, nose piercing, tattooing, and vertically from infected Mother-to-child transmission (MTCT), before birth, during birth, and after birth. [1, 2].

According to a WHO report in 2015, the global Prevalence of HBV infection in the general population was 3.5%. Among those childhood-borne before the hepatitis B vaccine became accessible, the highest Prevalence of persons living with chronic HBV infection in Western Pacific regions (6.2%) and African (6.1%) [3]. The magnitudes of all immigrants chronically infected with HBV range from 3.7% to 9.7% in the different migrant-receiving countries [4]. In sub-Saharan Africa, 5–12% of patients who received blood transfusions are at risk of post-transfusion hepatitis. [5]. A study conducted in Addis Ababa, Ethiopia, showed that HCV antibody prevalence was 0.9%. [6].

WHO reports in 2013, 1.4 million deaths per year due to hepatitis-related liver cancer and cirrhosis. Viral Hepatitis was the seven leading cause of mortality in the world. In Africa 19, million adults are chronically infected with hepatitis C. [7, 8, 9].

Syphilis is a chronic inflammatory disorder caused by the spirochetal pathogen *Treponema palladium*. Over 12 million cases per year of infectious syphilis are estimated to occur worldwide *Palladium* is obligate pathogens of humans transmit, usually by sexual, with an actively infected partner, in case of congenital syphilis to transmitted from mother to baby, During pregnancy, or at birth. The vast majority are within the low-income country, especially in sub-Saharan Africa. The World Health Organization that in some nations, the syphilis rates among commercial sex workers are between 23% and 32% [10, 11, 12]. Also, Syphilis may raise the acquiring and transmission of human immune deficiency virus (HIV) infection [13]. While recently, the annual worldwide incidence of effective treatments accessibility reached more than 5.6 million cases [14].

Each year, there are an estimated 6 million new cases of syphilis globally in persons aged 15 to 49 years [+15]. Also Globally pooled data showed that the Prevalence of Syphilis is 1.11%, the Prevalence had inclined. However, the region of African is consistently the most affected area having a pooled prevalence of 3.04% [15]. The study conducted by Ethiopian Public Health Institute (EPHI) Surveillances reports showed a slight increment of syphilis prevalence from 1.0% in 2012 to 1.2 in 2014 [16]. However, in Ethiopia, among different groups of study populations over time and across geographical areas. The study showed syphilis prevalence ranges from 0.1 to 7.5% among blood donors, 1 to 5.1% among pregnant women, and 7.3 to 9.8% among HIV patients. [17]. Although this study is, aimed to assess the magnitude of Hepatitis B, Hepatitis C, and Syphilis and their associated factors for getting infections among people who travel to different countries for work.

1.2 Statement of the problem

HBV and HCV are the major leading causes of liver diseases including, acute and chronic liver diseases [18]. Globally 30% of the population, about 2: billion, persons have serologic evidence of HBV infection. While: Over 350 – 500 million people are carriers of chronic HBV worldwide [19].

Hepatitis C virus infection is a globally prevalent infection. Around: 3% of the world's population estimate >185 million individuals have chronic hepatitis C virus infection 3-4 million people are newly infected annually [20, 21]. The incidence of HCC is increasing rapidly and is currently the second leading cause of cancer death worldwide with, 20%-25% of HCC cases predicative to the development of liver Cirrhosis, Hepatocellular cancer, liver failure, and death, the highest anti-HCV prevalence recorded in South-Eastern Asia and Central Africa [22, 23, 24]. About 800,000 people die from acute or chronic HBV. While 70-90% of all HCC between chronic HBV infections And Cirrhosis. Estimated that 41.5% for chronically infected patients, with a cumulative risk of developing HCC of 21.7% annually [25].

In sub-Saharan Africa, which had a high prevalence of liver disease due to HBsAg, It is, reported that 12% of hospital admissions [18]. Although anti-HCV recorded has lower prevalence compared with the others areas [26].

In a different study done in Addis Ababa, Ethiopia, the prevalence of HBsAg was 6.1%. [27]. Also, HCV prevalence is 0.9% among the population and 1.3% among adults over 15 years of age [24]. Another study showed that the prevalence of HBV infection was 10–15 %, and 2–5 % HCV infection was reported [22].

Infectious diseases cause more than 13 million deaths a year and represent the leading cause of mortality in less developed countries, many of them located in tropical and sub-tropical areas [28]. Syphilis is a serious public health burden, especially in sub-Saharan Africa. The prevalence of active syphilis infection among African countries was 3.8% in Kenya and 12.8% in Tanzania [29].

In Ethiopia, more than 60 % - 80 % of chronic liver disease and hepatocellular carcinoma caused by HBV and HCV chronic infections and also cause 31% of the mortality in medical wards hospitals was due to CLD [18, 23].

Currently, there is an increased flow of emigrants from Ethiopia to abroad all over the world. Unless extended health education and preventive mechanisms are planned, different diseases can be transmitted, from emigrant to hosting country and vice versa [30].

Even though hepatitis B, hepatitis C, and syphilis are significant public health, Concern, however little is known about the Magnitude and risk factors hepatitis B, hepatitis C and syphilis in emigrants, especially in Ethiopia.

This study will assess the magnitude and their associated risk factors of hepatitis B, hepatitis C viruses, and syphilis infection.

1.3 Significance of the study

The findings from this study serve as input data for concerned bodies like the minister of health and Ethiopian public health institution in taking appropriate action based on the Finding Especially It influences planning and implementing mitigation activities. Moreover, this study will be, used to assist and provide baseline information for further research in this area.

2. Literature Review

2.1 Magnitude of Hepatitis B, Hepatitis C and Syphilis

The study conducted in 2015 by Monge-Maillo B, in Australia, sub-Saharan Africa immigrants' prevalence of Chronic HBV 14% (N=42/300), HCV 3.7% (N=11 /300), and Syphilis : 3% (N=11/ 367). A similar study conducted on Latin American immigrants' revealed that the magnitude of chronic HBV was 18.3%. HCV, 3% (11 of 367) and syphilis, 1.4 % (5 of 353) [31].

Another study conducted in 2008 by Batash S in New York City. Showed that from the total of 283. The mean age of the 283 subjects was 51.9±15.7 yr. And 116 (41.0%) were less than 50 yr of age. Overall, 151 (53.4%) of the 283 participants were female. prevalence of HCV antibody Seropositivity among the 283 subjects was 28.3% (95% CI 23.0–33.5%) and frequency of HCV antibody Sero-positivity was 28.5% [32].

A study conducted by Tafuri S, 2010 in Bari, Italy. A total of 529 immigrants', 442 males and 87 females, aged between 7 and 52 years (average = 23.9; SD = 6.7 years), 44 individuals (8.3%) were HBsAg positive and the prevalence of HBsAg positive subjects was significantly higher in males (9.7%; 95% CI = 7.2- 13.0) than in females (1.1%; 95% CI = 0-6.2; p = 0.008). A total of 24 (4.5%) individuals, 23 males (5.2%; 95% CI = 3.4-7.9) and 1 female (1.1%; 95% CI = 0-6.3) were anti-HCV positive. Four of the 269 tested (1.5%) were positive to the VDRL test [33].

A study conducted from January 2012 to June 2013 by Coppola N in southern Italy the result showed that from a total of 882, (median age: 34.5 years; range: 14–74), Mostly male (72%) and 78 (9%) were HBsAg positive, 35 (4%) anti-HCV positive and 638 were male, and 244 female. Compared with those who were male, female participants were generally older (38 years (SD: 12.2) vs: 34 years (SD: 9.0); p < 0.001), In addition, female participants had fewer serum markers of HBV infection (HBsAg positivity: 6% vs. 10%; p = 0.07, male participants [18].

A study conducted in 2019 by Cuomo G in Italy. The result showed that from the total of 304 migrants male (285/304, 93.8%), the median age was 21years (range16-44). HBsAg positivity was found in 37 patients (12.2%; CI 95% 0.08– 0.16). Other infections found were: HCVAb

positivity in 10 cases (3.3%; CI 95% 0.01–0.05), TPHA positivity in 2 cases (0.7%; CI 95% 0.00–0.02). [34].

Study conduct in Rome immigrant in 2020 by Marrone R. The prevalence of HBsAg positive was (2.5%) (22/879). Prevalence of HCV from the total 836 migrants was 1.1%: (9/836) positive, Syphilis positive was (0.4%): (3/692). [35].

Studies were conducted in Italy by El-Hamad I in 2015. The prevalence of hepatitis B, C, and Syphilis infection among 3,728 migrants. HBsAg: 6%. (224/3,728): positive: HCV-Ab: 3.65%, (134/3,704) positive, syphilis: 0.9% (34/3,626), VDRL positive. [36].

The study conducted in 2013 to 2015 migrant in Nederland by et .al Bil JP result showed that the prevalence of chronic HBV infection (HBsAg-positive) and HCV exposure (anti-HCV-positive) in Gelderland was 4.48% and 0.99%, [37].

In a cross-sectional study by Chernet A in 2017 in Switzerland, the result showed that from the total of: 107 immigrants aged <16 years, Most participants were males (89%) with a median age of 25 years. The result showed that from the total of while the prevalence of HCV was zero, two cases reactive for HBsAg and one is reactive for Syphilis was detected [38].

The study conducted in 2010 by Tessema B in north west Ethiopia from 2003-2007 the total 6361 blood donors participants the prevalence of HBV ,HCV and Syphilis 4.7%, 0.7% and 1.3%. [39].

2.2 Risk factors and socio-demographic

In a study conduct by Hladun O. in Barcelona in 2014, the result showed that from a total of 3,132 immigrants chronic HBV infections were detected in 2.6% of cases, respectively. Hepatitis C prevalence was 3.3%, with elevated hepatic transaminase levels as a risk factor (OR 26.1, CI 8.68 – 78.37). Syphilis was detected in 4.5% of patients and positive Syphilis in: 3.1% latent and active [25].

In another study in 2019 by Alhooda N in Libya, the result showed that a total of: 252 individuals from 19 countries attended. Of this 88%, were male, both mean and median age was

17 years (IQR 16–17.2). 55 (22%) from Afghanistan and 51 (20%) from Eritrea. 211 (84%) tested for hepatitis B and C of, whom 10 (4.8%) were positive for hepatitis B 1 (0.5%) for hepatitis C. Highest hepatitis B infection rates, were found in those from Sudan (15%) and Afghanistan (12%). [40].

Another Cross-sectional study conducted in Amhara National Regional State tooth extraction history positively predicts the occurrence of hepatitis B infection [AOR=4.5, 95% CI: 1.1–18] [2].

The magnitude of HCV was significantly lower than as compared to the reports from other parts of Ethiopia as well for instance that in south omo that account for and 1.9% respectively [41].

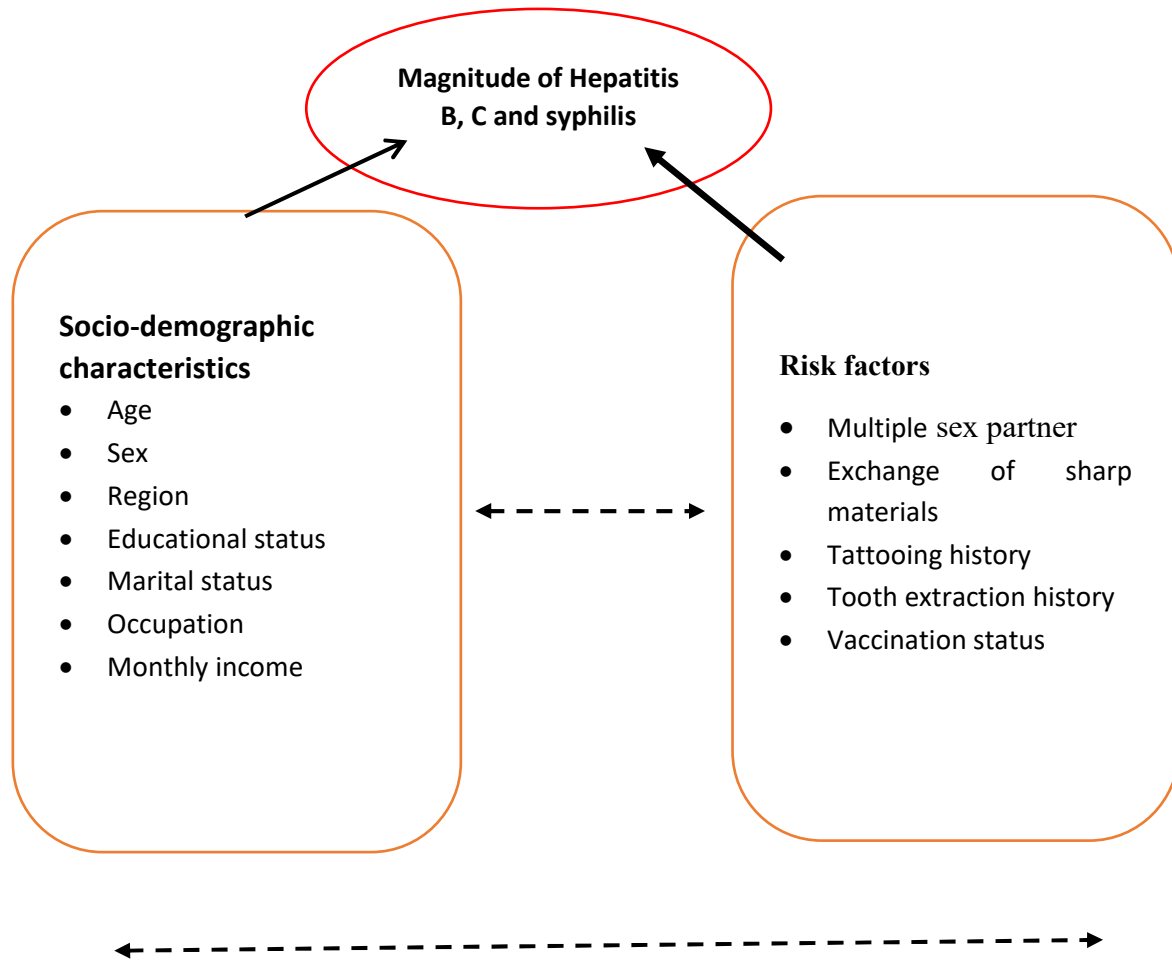
A cross-sectional study conducted in Jigjiga Town, Eastern Ethiopia Exchanges of Sharp material positively predicts the occurrence of hepatitis B infection with [AOR = 2.78, 95% CI (1.13– 6.83)] [42].

In another Cross-sectional study conducted in Southwestern Saudi Arabia, tooth extraction was a risk factor for developing hepatitis B infection [AOR=3.25, 95% CI: 1.0–8.7] [43].

In a cross-sectional study conducted in 2020 by Belay AS, a total of 612 eligible adults comprised 276 (45.1%) males and 336 (54.9%) females participated in the study. The mean age of the participants was 32.5 years. Almost half of the participants: 310 (50.7%) were in the age range of 25–34 years. The prevalence of HBV infection among adults in the community was 9.0% (95% CI: 5.03–12.96). Of the total HBV infected participants, 27 (49.1%) and 28 (50.9%) were males and females. Sharing of Sharp materials, Tattooing might contribute to the transmission of HBV [AOR (95% CI) 1.0 (0.04–2.5)] and [AOR (95% CI) 6.8 (1.1–43.1)] in Southwest Ethiopia [44].

A cross-sectional study had conducted in 2020 by Tegegne D in Addis Ababa with a total of 265 delivering women with a mean age of 25.8 years. 8 (3.0%); of mothers were positive for Hepatitis B Virus surface antigen. 6 (2.3%): of cord blood was positives for exposed infants with seropositive mothers. Only 11% of the mothers know their Hepatitis B Virus status. Possible assessed risk factors, 69 (26%) had only one type, while 161 (60.8%) had multiple exposure [45].

2.3 Conceptual Framework



-----> The broken line indicates the association between the explanatory variables

—> The solid line indicates the association between the outcome variable and explanatory variables

Figure1: Conceptual framework (source: prepared by the principal investigator after revising different literatures)

3. Objective

3.1. General objective

- To assess the magnitude of Hepatitis B, Hepatitis C, Syphilis and Their Associated Factors among Emigrants come for medical checkup in Labco Advanced Clinical Laboratory, Addis Ababa, Ethiopia.

3.2. Specific objectives

- To determine the Magnitude of Hepatitis B among emigrants attended medical checkup in Labco Advanced Clinical Laboratory.
- To determine the Magnitude of Hepatitis C among emigrants attended medical checkup in Labco Advanced Clinical Laboratory.
- To determine the Magnitude of Syphilis among emigrants attended medical checkup in Labco Advanced Clinical Laboratory.
- To identify associated factors with Hepatitis B virus among emigrants attended medical checkups in Labco Advanced Clinical Laboratory.
- To identify associated factors with Hepatitis C virus among emigrants attended medical checkups in Labco Advanced Clinical Laboratory.
- To identify associated factors with Syphilis infection among emigrants that attended a medical checkup in Labco Advanced Clinical Laboratory.

4. Method and Materials

4.1. Study site

This study was conducted in Labco Advanced Clinical Laboratory. Labco was established with a basic mission to become an acknowledged leader in diagnostic testing by the Sudanese Prof. Dr. Eltom Sirageldin in 2016, who had more than thirty a long time of involvement in research facility administrations in Germany. The diagnostic center has thirty staff, Labco Advanced Clinical Laboratory wants to construct and run a standard lab diagnostics center to provide more cutting-edge innovation here in Ethiopia, Addis Ababa (the capital city).

4.2. Study design and period

- An institution based cross-sectional study was conducted from February 01, 2021 - April 30, 2021 in Labco Advanced Clinical laboratory, Addis, Ababa.

4.3. Population

4.3.1. Source population.

- All customers' visited Labco Advanced Clinical Laboratory to get different laboratory test serves.

4.3.2. Study population

- Emigrants visited Labco Advanced Clinical Laboratory from February to April 2021 for medical checkup and voluntaries to participate in this study..

4.4. Inclusion and exclusion criteria

4.4.1 Inclusion criteria

- All emigrants attended Labco Advanced Clinical Laboratory

4.4.2 Exclusion criteria

- Any emigrant who did not accept to participate in the interview and collection of blood samples form.
- All emigrants age below 18 years
- Other people traveler they came for a medical checkup.

4.5. Study Variables

4.5.1. Outcome variable

- The magnitude of Hepatitis B, Hepatitis C, Syphilis.

4.5.2. Explanatory variables

- Demographic and socio-economics factors
 - Age, sex, region, educational status, marital status, occupation, monthly income.
- Risk factors
 - Multiple sex partner
 - Exchange of sharp materials
 - History of tattooing,
 - History of tooth extraction
 - Vaccination status

4.6. Sample size calculation and Sampling methods

4.6.1. Sample size calculation

The sample size was determined using single population proportion formula considering a confidence level of 95%, marginal error of 5%, and proportion of 0.09 %. (This obtained from a study conducted by Southwest, Ethiopia). The sample size was determined: 516.

Where: n = sample size P = proportion of femoral fraction q = 1-p d = desired degree of precision (2%) Z= is the standard normal value at 95% confidence level

$$n = \frac{p(1-p) \left(Z_{\frac{\alpha}{2}} \right)^2}{d^2}$$

$$n = \frac{(1.96)^2 \times 0.09 (1-0.09)}{0.02^2} = 469 \longrightarrow 469 + 10\% = 516$$

4.6.2. Sampling method

- A random convenient sampling technique had used to enroll the study participants.in Labco Advanced Clinical Laboratory 500 individual was selected.

4.7. Measurement and Data collection

4.7.1. Data Collection Procedures

A structured questionnaire had used to capture individual socio-demographic characteristics and risk factors (age, sex, region, educational status, marital status, occupation, monthly income, multiple sex partner, exchange of sharp materials, history of tattooing, history of tooth extraction, Vaccination status): And routinely collected venous blood from emigrants in blood collection tubes upon donating blood each blood sample collected from each emigrant. All Samples had collected during people came for a checkup. Sterile serum separated Tubes with the capacity volume containing jells, Separated, about 5 ml of blood. The blood had used to screen for hepatitis B, hepatitis C, and syphilis on the same day at the time of sample collection from each emigrant in Labco Advanced Clinical Laboratory Addis Ababa. February – April 2021 the samples had collected processed immediately and every, reactive result is repeated by RT. The serum had separated from all Samples and store at -20 0C. The reactive Result was Analysis by enzyme-linked immunesorbent assay (ELISA) test in Labco Advanced Clinical Laboratory Addis Ababa, Ethiopia.

4.7.2. Laboratory Analysis

After obtaining the Participants, written consent, 5 ml of the blood samples was collected from each participant of emigrant for routine work. Each, blood sample was allowed to clot 30 minutes and, serum was separated by centrifuging at 3000 rpm for 5 min. All serum samples had tested for HBsAg, anti-HCV, and syphilis rapid test strips (CTK BIOTECH), followed by the manufacturer's instruction. Every reactive test repeats by a Rapid test. And the reactive samples were further tested with Enzyme-Linked Immunesorbent Assay (ELISA) at Labco Advanced Clinical laboratory. During testing, standard operating procedures (SOPs) were followed for each laboratory analysis and known positive and negative serums samples for HBV, HCV, and Syphilis tests were utilized as a control to dodge untrue positive and negative results.

4.8. Data Quality Assurance:

The participants' blood sample quality was ensured by strictly following standard operating procedure and it was collected by professional laboratory personnel. A quality assurance was followed in Pre analytic, analytical and post analytical phases by taking the following in measures.

Pre-analytic phase

- The patient's blood sample quality was ensured by strictly following standard operating procedures. It had collected by professional laboratory personnel.
- Completed questionnaires was checked for completeness by the principal investigator
- Quality assurance followed in Pre analytic, analytical, and post-analytical phases by taking the following in measures.
- After proper collection, samples were properly labeled, with their identification name or ID number.
- Samples had been checked to whether they are in the acceptable criteria like; hemolysis, clotting, volume, and collection time.
- The functionality of the kit and instrument will check before use. Unexpired kit and reagent use and manufacturers' instructions regarding test procedures and SOP used strictly followed.

Analytical phase

- Before analysis, samples were homogenized by inverting 8-10 times and check-free hemolysis.
- The performance of ELISA (enzyme-linked immune sorbent assay analyzer was check by running calibration Normal/Negative and Positive control.

Post analytical

- To avoiding any clerical error, printout results of the machine used.
- The result recently has been checked before reporting.
- The result registered properly with hard copies and soft copies.

4.9. Principles of each Laboratory analysis

4.9.1 .Hepatitis B Surface Antigen Rapid Test Kit (RT) (Immunochromatography, CTK Biotech)

Test Principle: the sample mixing up colloidal- gold monoclonal antibody sandwich method and gold immunochromatographic assay, which is a positive result. Unreacted markers move

forward continually to combine with anti-mouse antibody and form a control line. If the test line does not appear, it is a negative result.

4.9.2. Hepatitis C virus Antibody Rapid test kit (Immunochromatography, CTK Biotech)

Test principle: The test utilizes antibodies including a recombinant protein mixed HCV antigen and rabbit anti –HCV antibody on the nitrocellulose membrane with colloidal gold marked mixed HCV antigen as a mark tracer. The reagent is used to detect the HCV antibody, in serum/plasma according to the principle of double antigen sandwich method and gold immunochromatography assay. The sample mixing up HCV antigen- markers move along the membrane to the T line, and from the T line when the sample contains HCV antibody, which is a positive result. Conversely, it is a negative result.

4.9.3. Syphilis rapid test cassette (Immunochromatography , CTK Biotech)

Principle: The syphilis rapid test cassette (serum/plasma) is qualitative membrane-based immunoassay for the detection of TP antibodies (IgG and IgM) in serum or plasma. In this test procedure recombinant syphilis antigen is immobilized in the test line region of the test. After the specimen is added to the specimen well of the test, It reacts with syphilis antigen coated particles in the test. This mixture migrates chromatographically along the length of the test and interacts with the immobilized syphilis antigens. If the specimen contains TP antibodies, a colored line will appear in the test line region indicating a positive result. The double antigen test can detect both IgM and IgG in specimens. If the specimen does not contain TP antibodies, a colored line will not appear in this region indicating a negative result. To serve as procedural control a colored line will always appear in the control line region, indicating that proper volume of specimen has been added and membrane wicking has occurred.

Directions for use: allow the test, specimen, and/or controls to reach room temperature (15-30°C) prior to testing.

4.9.4. ELISA (enzyme-linked immunosorbent assay Wantai AiD™)

Is a technique to detect the presence of antigens in biological samples? An ELISA like other types of immunoassays relies on antibodies to detect a target antigen using highly Specific antibody- antigen interaction. In an ELISA assay, the antigen is immobilized to a solid surface this is done either directly or via the use of a capture antibody itself immobilized on the

surface. The antigen is then complexed to a detection antibody conjugated with a molecule amenable for detection such as an enzyme or a fluorophore.

4.9.5. Hepatitis B Surface Antigen ELISA (enzyme-linked immunosorbent assay, Wantai AiD™)

HBsAg ELISA is sandwich direct enzyme immunoassay method. The wells of the micro titration plate are coated with anti- HBsAg guinea pig antibody (anti- HBsAg) acting as a capture antibody. The sample is incubated in one well. If it contains HBsAg. It will form a complex with the antibody bound to the plate. The unbound material is removed by washing. Then the anti-HBs goat antibody conjugated to peroxidase is added, which will bind to the antibody-antigen preformed complex. The unbound conjugate is removed by washing. Subsequently, a solution containing tetramethylbenzidine and hydrogen peroxide is added. In cases in which HBsAg is present in the sample a light-blue color is developed which becomes yellow when the reaction is stopped with sulfuric acid.

4.9.6. Hepatitis C virus Antibody ELISA (enzyme-linked immunosorbent assay, Wantai AiD™)

This kit is a two-step incubation enzyme immunoassay, which uses polystyrene micro well strips pre-coated with recombinant HCV antigens expressed in E.coli (recombinant core and NS3/4/5). Patient's serum or plasma sample is added together with biotin-conjugated HCV Antigens. During the first incubation step, the specific HCV antibodies ,if present, will be captured inside the wells as a double antigen "sandwich" complex comprising of the coated' and the biotin conjugated HCV antigens. The micro wells are then washed to remove unbound conjugate, and chromogen solutions are added to the wells. In wells positive for HCV antibodies, the colorless chromogens are hydrolyzed by the bound HRP conjugate to a blue colored product. The blue color turns yellow after stopping the reaction with sulfuric acid. The amount of color intensity can be measured and is proportional to the amount of antibodies captured in the wells, and to the sample respectively. Wells containing samples negative for anti-HCV remain colorless.

4.9.7. Syphilis ELISA (enzyme-linked immunosorbent assay, Wantai AiD™)

The Aria syphilis Ab ELISA kit is a solid phase enzyme -linked immunoabsorbent assay based on the principle of the double antigen sandwich technique for the detection of antibodies to TP in human serum or plasma.

The Aria Syphilis Ab is ELISA kit is composed of two key components

1. Solid micro wells pre-coated with recombinant TP antigens;
2. Liquid conjugate composed of recombinant TP antigens conjugated with horse reddish peroxidase (HRP-TP conjugates)

During the assay, the test specimen and HRP-TP conjugates are incubated simultaneously with the coated microcells'. Antibodies (IgA, IgM, or IgA) to TP If present in the specimen reacts to the TP antigens coated on the microcells surface as well as the HRP-TP conjugates forming sandwich complex conjugates.

Unbound conjugates are then removed by washing. The presence of complex conjugates is shown by a blue color upon additional incubation TMB Substrate the reaction is sopped with stop solution and absorbance are read using an electrophotometer at 450/620 -690 nm .

4.10. Data Analysis and interpretation

Data were coded, entered, and analyzed by SPSS software version 26. Descriptive statistics which performed to describe the frequency of categorical variables such as the socio-demographic characteristics, risk factors to estimate the magnitude or proportion of HBV, HCV, and syphilis infections. The associations of potential risk factors of HBV, HCV, and syphilis infections, assessed by using binary logistic regression, all variables a P-value< 0.25 in the bivariate analysis and the multivariate logistic regression model to look if the association existed after controlling against all the rest of the variables, a cut-off value less than 0.25 support by the literature. Odds ratio (OR) at 95% confidence interval (CI), was calculated and a p-value <0.05 was considered as statistically significant.

4.11. Operational Definition

- **Emigrants:** - emigrant refers to a person who has moved from one country to another, usually in a permanent or semi-permanent manner. A person who is emigrating or has emigrated can be called an emigrant.

4.12. Ethical consideration

Ethical clearance had obtained from the Departmental Research and Ethics Review Committee (DRERC) of the Department of Medical Laboratory Sciences of Addis Ababa, University. A letter of permission is obtaining from the office of the Administration of Labco Advanced Clinical laboratory in Addis Ababa, Ethiopia. The purpose and procedure of the study had explained to the study participants in the study site. Written and informed consent had obtained after briefly explained about the objective of the study. In addition, the clinical specimens collected during the study period were used only for the stated object. The test comes about were kept private by utilizing one-of-a-kind codes given to each consider member. All positive come about was communicated, to the going to members.

5. Results

5.1 Socio-demographic characteristic of emigrant in Labco Advanced clinical laboratory

In this study, a total of 500 emigrants were participated, making a response rate of 96.8%. Among the total participants, 36.4% (n=182/500) were male, and 63.6 %: (n=318/500) were female resulting in a male-to-female ratio of 1:2. The mean reported age (\pm SD) of participants was 29.94 (\pm 6.4) years. More than half, 55.4 %: (n=277/500) of the participants were between the age group of 18 to 29, followed by n=188/500 (37.6%) were 30 to 41years, and 7.0% (n=35/500) were greater than 41 years. Most 33.8% (n=169/500) of the participants were from Oromia, followed by 25.8% (n=129/500) were from SNNP. About one-third of the study participants, 32.8% (n=164/500), were secondary high school students, whereas 28.4% (n=142/500) were Primary students. Concerning marital status, more than half of 55.4% (n=277/500) of the participants were single whereas, 32.8 %: (n=205/500) were married. The majority, 61.4%: (n=307/500) of study Participants, were Unemployed. About two-thirds of 63.4% (n=317/500) of the participants had reported average monthly income less than 500 birrs, whereas 36.0% (n=180/500) of the participants reported monthly income greater than 1000 birr. The socio-demographic characteristic of the study population is shown: in *table 1*.

Table 1: Socio-demographic characteristics of the emigrants in Labco Advanced clinical Laboratory, Addis Ababa, Ethiopia February to April 2021.

Variables		Frequency	Percent %
Sex	Male	182	36.4%
	Female	318	63.6%
	Total	500	100.0%
Age	18-29	277	55.4%
	30-41	188	37.6%
	> 41	35	7.0%
	Total	500	100.0%
Region	Oromia	169	33.8%
	SNNP	129	25.8%
	Amhara	75	15.0%
	Addis Ababa	62	12.4%

	Other regions	65	13 %
	Total	500	100%
Educational status	illiterate	41	8.2%
	Primary	142	28.4%
	Junior	77	15.4%
	Secondary high school	164	32.8%
	University	76	15.2%
	Total	500	100.0%
Marital Status	Single	277	55.4%
	Married	205	41.0%
	Divorced	18	3.6%
	Total	500	100.0%
Occupation	Unemployed	307	61.4%
	Employed	70	14.0%
	Self-Employed	123	24.6%
	Total	500	100.0%
Monthly Income	<500	317	63.4%
	500-1000	3	0.6%
	>1000	180	36.0%
	Total	500	100.0%

*SNNP: Southern nation national people.

5.2. The magnitude of Hepatitis B Hepatitis C and Syphilis

In this study, Out of 500 Participants, 2.8%, 0.2%, and 1.0 % were positive for HBsAg, HCV, and Syphilis using rapid tests, respectively. In the case of HCV, the magnitude of the rapid test was almost the same as the confirmatory test (with ELISA) result which, was 0.3% (n=1/500). However, it shows some variation in case of HBsAg and syphilis that accounts 2.2% (n=11/500) and 0.6% (n=3/500) respectively.

The magnitude of HBsAg, HCV and syphilis in female participants was 1.6% (n=5/318), 0.3% (n=1/318) and 0.3% (n=1/318) respectively, whereas it was 3.3% (n=6/182), 0% (n=0/182) and 1.1% (n=2/182) among males respectively. A slightly higher proportion of HBsAg 2.9%: were

detected among aged groups of >41 years. The positivity rate was higher in married participants in both HCV, 1(0.5), and syphilis, 3(1.5%) in the case of HBsAg Divorce participants had a higher positivity rate 1 (5.6%). The details are shown: in *table 2*.

Table :2 Prevalence of HBV, HCV and syphilis with ELISA among study participants in Labco Advanced clinical Laboratory, Addis Ababa, Ethiopia February to April 2021 (n = 500)

Variables		HBsAg		HCVAb		Syphilis		Total
		Positive (%)	Negative (%)	Positive (%)	Negative (%)	Positive (%)	Negative (%)	
Sex	Male	6(3.3%)	176(96.7%)	0(0.0%)	182(100.0%)	2(1.1%)	180(98.9%)	182
	Female	5(1.6%)	313(98.4%)	1(0.3%)	317(99.7%)	1(0.3%)	317(99.7%)	318
	Total	11	489	1	499	3	497	500
Age	18-29	7(2.5%)	270(97.5%)	0(0.0%)	277(100%)	0(0.0%)	277(100%)	34
	30-41	3(1.6%)	185(98.4%)	1(0.5%)	187(99.5%)	2(1.1%)	186(98.9%)	188
	> 41	1(2.9%)	34(97.1%)	0(0.0%)	35(100%)	1(2.9%)	34(97.1%)	35
	Total	11	489	1	499	3	497	500
Educational status	illiterate	0(0.0%)	41(100%)	0(0.0%)	41(100%)	0(0.0%)	41(100%)	41
	Primary	3(2.1%)	139(97.9%)	1(0.7%)	141(99.3%)	1(0.7%)	141(99.3%)	142
	Junior	0(0.0%)	77(100%)	0(0.0%)	77(100%)	1(1.3%)	76(98.7%)	77
	Senior Secondary	7 (4.2%)	157(95.7%)	0(0.0%)	164(100%)	1(0.6%)	163(99.4%)	164
	University	1(1.3%)	75(98.7%)	0(0.0%)	76(100%)	0(0.0%)	76(100%)	76
	Total	11	489	1	499	3	497	500
Marital Status	Single	6(2.2%)	271(97.8%)	0(0.0%)	277(100%)	1(0.0%)	277(100%)	277
	Married	4(2.0%)	201(98.0%)	1(0.5)	204(99.5%)	3(1.5%)	202(98.5%)	205
	Divorced	1(5.6%)	17(94.4%)	0(0.0%)	18(100%)	0(0.0%)	18(100%)	18
	Total	11	489	1	499	3	497	500
Occupation	Unemployed	6(2.0%)	301(98.0%)	1(99.7%)	306(0.3%)	1(0.3%)	306(99.7%)	307
	Employed	1(1.4%)	69(98.6%)	0(0.0%)	70(100%)	1(1.4%)	69(98.6%)	70
	Self-	4(3.3%)	119(96.7%)	0(0.0%)	123(100%)	1(0.8%)	122(99.2%)	123

	Employed							
	Total	11	489	1	499	3	497	500
Monthly Income	<500	7(2.2%)	310(97.8%)	1(0.3%)	316(99.7%)	1(0.3%)	316(99.7%)	317
	500-1000	0(0.0%)	3(100%)	0(0.0%)	3(100%)	0(0.0%)	3(100%)	3
	>1000	4(2.2%)	176(97.8%)	0(0.0%)	180(100%)	2(1.1%)	178(98.9%)	180
	Total	11	489	1	499	3	497	500
Multiple Sex Partner	No partner	2(1.5%)	135(98.5%)	0(0.0%)	137(100%)	1(0.0%)	137(100%)	137
	One partner	9(2.5%)	350(97.5%)	1(0.3%)	358(99.7%)	2(0.6%)	357(99.4%)	359
	Two Partners	0(0.0%)	3(100%)	0(0.0%)	3(100%)	1(33.3%)	2(66.7%)	3
	More than three	0(0.0%)	1(100%)	0(0.0%)	1(100%)	0(0.0%)	1(100%)	1
	Total	11	489	1	499	3	497	500
Exchange Of Sharp Materials	Yes	4(10.8%)	33(89.2%)	0(0.0%)	37(100%)	0(0.0%)	37(100%)	37
	No	7(1.5%)	456(98.5%)	1(%)	462(99.8%)	3(0.6%)	460(99.4%)	463
	Total	11	489	1	499	3	497	500
History of Tattooing	Yes	3(9.7%)	28(90.3%)	0(0.0%)	31(100%)	0(0.0%)	31(100%)	31
	No	8(1.7%)	461(98.3%)	1(0.2%)	468(99.8%)	3(0.6%)	466(99.4%)	469
	Total	11	489	1	499	3	497	500
History of tooth extraction	Yes	8(17.8%)	37(82.2%)	1(2.2%)	44(97.8%)	0(0.0%)	45(0.0%)	45
	No	3(%)	452(99.3%)	0(0.0%)	455(100%)	3(0.7%)	452(99.3%)	455
	Total	11	489	1	499	3	497	500
Vaccinated	Yes	0(0.0%)	1(100%)	0(0.0%)	1(100%)	0(0.0%)	1(100%)	1
	No	11(%)	488(97.8%)	1(0.2%)	499(99.8%)	3(0.6%)	496(99.4%)	499
	Total	11	489	1	499	3	497	500

5.3. The magnitude of HBsAg, anti-HCV, and Syphilis infection of ELISA positive test

In this study, a total of 500 emigrants participated, the Magnitude of ELISA test result which was positive of HBsAg, anti-HCV and Syphilis the details are shown: in *Figure 2*.

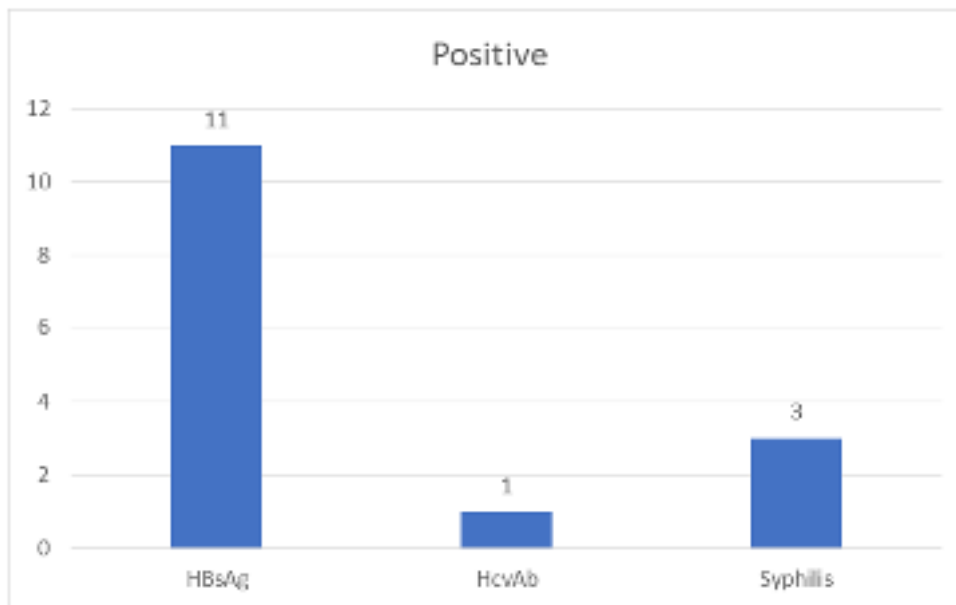


Figure 2 : Magnitude of HBsAg, HCVAb and Syphilis infection in Labco Advanced clinical Laboratory diagnostic center, Addis Ababa, Ethiopia February to April 2021.

5.4. Risk factors associated with prevalence of HBsAg

In this study, explanatory variables such as age, sex, educational status, marital status, occupation, monthly income, multiple sex partners, exchange of Sharp materials, history of tattooing, history of tooth extraction, and vaccination status had been analyzed first by based on the p-value of the bivariable analysis. Three variables this identified as candidate variables for the multivariable analysis in the Case of HBsAg: - Exchange of Sharp materials, History of tattooing, and History of tooth extraction. Whereas no significant association with HCV and Syphilis.

The result of the multivariable analysis revealed that the exchange of Sharp materials and the history of tooth extraction are independent determinants of HBsAg. (Table 3)

The odds of HbsAg were 8: times higher in people who exchange Sharp material when compared to the counter group [AOR= 7.89; 95% CI 1.75-34.95:].

The odds of HbsAg were 25 times higher in those who had tooth extraction history when compared to the counter group: [AOR=24.9; 95% CI (5.95-104.43):]. The details are shown: in table 3.

Table 3: Magnitude of HbsAg with ELISA test associated with Risk factors emigrants in Labco Advanced clinical Laboratory diagnostic center, Addis Ababa, Ethiopia, February to April 2021 (n = 500)

Explanatory Variable		HBsAg		Bivariate analysis (COR)	Multivariate analysis (AOR)	P-value
		Positive	Negative			
Exchange Of Sharp Materials	Yes	4	33	7.89(2.19-28.35)	7.82(1.75-34.95)	0.007
	No	7	456	1	1	
Tattooing History	Yes	3	28	6.17(1.55-24.55)	1.63(0.12-21.40)	0.14
	No	8	461	1	1	
Tooth Extraction History	Yes	8	37	32.5(8.29-128.017)	24.92(5.95-104.4)	0.00001
	No	3	452	1	1	

6. Discussion

This study was a one-site cross-sectional study of the magnitude and associated risk factors of HBsAg, HCV, and Syphilis among Emigrants in Labco Advanced clinical Laboratory, diagnostic center in Addis Ababa, Ethiopia.

The finding of this study indicates that the Magnitude of HBsAg was 2.2%. It was comparable with a study were done on Rome immigrants (which reported that the magnitude of HBsAg was: 2.5%) [35]. And it was comparable that with a study done in Switzerland on infectious diseases among newly arrived Eritreans (reported that the magnitude of HBsAg was 1.87%) [38]. But it is significantly lower than that of the Reports in Canada, ill travelers, and migrants. The magnitude of HBsAg accounted for 13.7 % [32]. While in Libya illegal immigrant population accounted for 4.8% [40]. However, HBsAg was significantly lower than the reports from other parts of the country. Community-based study in the northwest and south Omo Zone Ethiopia, The seroprevalence of hepatitis B infections 4.7% and 8.0% [41]. Whereas, a study conducted in Southwest Ethiopia, the prevalence of hepatitis B surface antigen (HBsAg) was 9.0% [44]. The magnitude of HBsAg was intermediate according to the "WHO criteria" ranges between 2-8% [46].

The possible justification for intermediate in the Magnitude of HBsAg, among other studies, could be explained reason might be due to a lack of knowledge about transmission and prevention of the infection and a large number of study participants were not vaccinated, unsafe tooth extraction and exchange of Sharp materials the risk source of infections. In addition, this variation of the study settings is due to a geographical area.

Regarding HCV, the finding of this study indicates that the magnitude of anti-HCV was 0.2%. It is comparable with a study in Libya, northwest, Ethiopia, and Rome that account for 0.5, 0.7 1.1% respectively [40, 39 35]. However, it is significantly lower when compared to a study conducted in New York City on the prevalence of hepatitis C virus infection among immigrants from the former Soviet Union that accounted for: 13.7 % [32]. The magnitude of HCV was significantly lower from the reports of other parts of Ethiopia instance, northwest and south omo Ethiopia account for 1.3 and 1.9%: respectively [39, 41].

Low Magnitude of HCV among other studies may be due to variation of potential risk factors and geographical area.

In the case of Syphilis, the finding of this study indicates that the magnitude of Syphilis was 0.6%. It is comparable with a study conducted in Switzerland and Rome that accounts for 0.5 and 0.4%: respectively [38, 35]. However, it is significantly lower than a Study conducted in northwest Ethiopia and Barcelona, immigrants Syphilis was can be detected in 1.3 and 4.5% respectively [39, 25].

The exchange of Sharp materials had identified as one of the significant determinants of HBsAg. The odds of hepatitis B infection were 8: times higher in people who exchange Sharp materials Compared to the counter group: [AOR= 7.89; 95% CI 1.75-34.95:]. That is in line with the cross-sectional study conducted in Jigjiga Town, Eastern Ethiopia, and a community-based study Bench Maji zone in Southwest Ethiopia [AOR = 2.78, 95% CI (1.13– 6.83)] and [AOR (95% CI) 1.0 (0.04–2.5)]. Which exchange Sharp material was at a higher risk of developing hepatitis B infection [42, 44].

History of tooth extraction was another identified determinant of HBsAg. The odds of hepatitis B infection were 24 times higher in those who had tooth extraction history when compared to the counter group: [AOR=24.92; 95% CI 5.95-104.43:]. Which, in line with the cross-sectional study conducted in south-western Saudi Arabia and pregnant women in Amhara National Regional State: [AOR=3.25, 95% CI: 1.0–8.7] and [AOR=4.5, 95% CI: 1.1–18] and which tooth extraction history was at a higher risk of developing hepatitis B infection [43, 2].

Tattooing history not associated with hepatitis B virus infection the odds of hepatitis B infection were 2: times lower in people who had tattooing history, compared to the counter group: [AOR= 1.63(0.12-21.40):]. Which is not in line with study finding Southwest Ethiopia and Addis Ababa [AOR (95% CI) 6.8 (1.1–43.1)] and [AOR (95% CI) 1.2 (0.3-2.4)] [44, 45].

7. Conclusion

This study has determined the magnitude of HBV, HCV, and Syphilis infections among emigrants and found intermediate HBV infection. Anti-HCV and Syphilis were low emigrants who attended Labco Advanced Clinical Laboratory compared to report literature. Exchange of Sharp materials and history of tooth extraction are independent determinants of HBsAg. It calls us for interventional strategies.

8. Limitations of the study

Limitation of literature the magnitude of HBV, HCV, and syphilis infections among Emigrants to discussed to others study in Ethiopia.

9. Recommendations

Based on the findings of our study, the following recommendations were, forwarded for the respective organs:

Government higher officials: Since for implemented prevention and screening health policy for immediate action, HBV is a vaccine-preventable disease. Target to achieve awareness creation about the infection and risk factors such as exchange of Sharpe material and unsafe tooth extraction. This indicates that the need for health policy to do implemented and integrated with other health services.

Researchers: Further, study in a large number of sample sizes in the different backgrounds. To do all diagnostic markers of hepatitis, this would have been helpful to distinguish chronic infection from acute infections and determine viral load.

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Lists of Annex

Annex 1. Information sheet in English Version

I. English version of participant information sheet

Department of Medical Laboratory Science, college of Allied Health Sciences, Addis Ababa University, Addis Ababa, Ethiopia, 2021.

Title of the Research Project: Magnitude of Hepatitis B, Hepatitis C Syphilis and their associations risk factors Emigrants come for medical checkup in Labco Advanced clinical Laboratory diagnostic center in Addis Ababa, Ethiopia.

Principal Investigator: Rahel Bogale (BSc, MSc candidate)

Name of the Organization: Department of Medical Laboratory Sciences, College of Health Sciences, Addis Ababa University

Introduction

You are invited to participate as a study subject in a research conducted by MSc candidate, from medical Laboratory Sciences department, Addis Ababa University. Your participation is voluntarily. The research teams will include one principal investigator, two advisors from Addis Ababa University microbiological department. Please take as much time as you need to read or listen in the information sheet.

Purpose of the Research Project

We are asking you to take part in this study because we will try to study the Magnitude of Hepatitis B, Hepatitis C, Syphilis and their association's risk factors Emigrants come for medical checkup in Labco Advanced clinical Laboratory diagnostic center in Addis Ababa, Ethiopia.

Purpose of the research:

The health laboratory plays an indispensable role in the health care system. It supports diagnosis, monitoring of response to treatment, epidemiological surveillance, prevention as well as research. Especially there is a shortage of information about the risk factors of hepatitis B, Hepatitis C and Syphilis that's the reasons for Emigrant visit diagnosis centers, but knowledge of the transmission and risk factor and of these infection cases is also limited. Therefore, the purpose

of this proposed study is to assess the exact causative agents and risk factors of hepatitis B, Hepatitis C and Syphilis in Addis Ababa, Ethiopia. You have been chosen for this study. Therefore, we invite you to take part in this study and contribute to the establishment of indigenous reference values. The values are needed for providing quality laboratory service. Thus, result from this study is anticipated to improve the health status of the emigrant at large in Ethiopia.

Procedures and the expected participation

If you are willing to participate, you need to understand the purpose of the study and give your consent. Not only this but also specimen collect from you will be used for the research purpose, and the results of the sample will be exposed to some concern professional staffs as it is needed. You are request to give your consent to the sample collector. There will be a face-to-face interview for additional questions.

Procedures: After agreeing that you can take part, one or more of our research staff will ask you some questions which will take up to 5 minutes. Your age, sex, marshal status will be write and register. You will be asked to provide blood sample on a particular container we provide. We will conduct laboratory examination to determine different Serological parameters.

Confidentiality

We respect your privacy and confidentiality. Any information that identifies you will not be shared with anyone else outside the study team. The information we will collect from you as part of the study will be kept in a lock file cabinet, or be protect by a password on the computer only accessible to personnel involve in the study. There is no sensitive issue that you will be ask related with your social desirability but any information that is obtain in connection with this study and that can be identified with you will remain confidential.

Potential benefits to subjects and/or to the society

You will not receive any payment for your participation in this research study as compensation. However, based on the diagnosis result you will be treated in view of that. In addition, the result of the study will be beneficial for the decrease the morbidity and mortality rate which is caused by Hepatitis B, Hepatitis C and syphilis. Hence, you are indirectly benefiting other patients and

the society in this respect.

Participation and Withdrawal from the Study

The participation is voluntary and you have the right not to participate in this study. You may withdraw at any time and place without consequences of any kind. You may also reject to give any sample. You can ask any questions regarding to this study and you have a right to get a laboratory diagnosis result free.

Contact information

If you have any questions about this study you can contact the following principal investigators and advisors for further information.

Name Rahel Bogale

Phone: +251911893017

E-mail: rahelbo@yahoo.com

Annex 2. Amharic version of information sheet

የተሳታፊዎች ፈቃድና መተማመኛ ቅፅ

በአዲስ አበባ ዩኒቨርሲቲ ጤና ሳይንስ ኮሌጅ የሕክምና ላቦራቶሪ ሳይንስ ት/ክፍል በማስተርስ ድግሪ ተማሪ የመመረቂያ ጥናት ላይ እዲሳተፉ ተጋብዘዋል። እባክዎ በዚህ ጥናት ለመሳተፍ ከመስማማትዎ በፊት ከዚህ ቀጥሎ የሚገኘውን ምንባብ በጥሞና ያንብቡና ግልጽ ያልሆነልዎትን ማንኛውም ሃሳብ ይጠይቁ።

መግቢያ

የጥናቱ ርዕስ “ከ አስራስምት አመት በላይ ያሉ አዋቂ የሚያጋጥሟቸው የጉበት ባይረስ ቢ፡ሲ እና ቂጥኝ መንስኤውን ለይቶ ለማወቅና ስላለው ስርጭት ምክንያት ለማወቅ የሚል ነው”.

የእርስዎ በዚህ ጥናት ላይ የሚኖርዎት ተሳትፎ ሙሉ በሙሉ በበጎ ፈቃደኝነት ላይ የተመሰረተ ነው። በዚህ ጥናት ውስጥ ላለመሳተፍ ወይም ለመሳተፍ ከወሰኑ በኋላ ለማቋረጥ የሚወስኑ ቢሆንም እንኩዋ በዚህ ሆስፒታል የሚሰጠው ማንኛውም አገልግሎት አይቋረጥም። በጥናቱ ለመሳተፍ የሚስማሙ ከሆነ የስምምነት ቅጹ ላይ በጽሁፍ ወይም በጣት ፈርማ ማስቀመጥ ይጠበቅዎታል።

የጥናቱ ተሳታፊ ለመሆን የሚጠበቅበዎት ምንድን ነው?

በዚህ ጥናት ለመሳተፍ የሚስማሙ ከሆነ ናሙናዎ ለጥናቱ እንዲሟወድ መስማማት ይጠበቅብዎታል። ከተወሰደው ናሙና ላይ የሚገኙ መረጃዎች ከዚህ ላቦራቶሪ ውጭ ለሚገኙና ለስራው አግባብነት ላላቸው ሰዎች ቢነገር የማይቃወሙ መሆኑን መስማማት ይጠበቅብዎታል። ይሁን እንጂ ይህ አይነቱ መረጃ የርስዎን ማንነት የሚገልጹ መረጃዎችን ማለትም ስም፣ አድራሻና የስልክ ቁጥር የመሳሰሉትን መረጃዎችን አይጨምርም። ይልቁንም ለዚህ አገልግሎት ብቻ የሚወድ እርስዎን ለማወቅ የሚያስችል መለያ ቁጥር ጥቅም ላይ እንዲወድ ይደረጋል። በተጨማሪም ስለ እርስዎ አጠቃላይ የጤና ሁኔታ ለሚቀርቡ አንዳንድ ተጨማሪ ጥያቄዎች መልስ መስጠት ይኖርብዎትዎታል።

በዚህ ጥናት መሳተፍ የሚያስከትላቸው ችግሮች ምንድን ናቸው?

ናሙና በሚሰበሰብበት ወቅት ምንም አይነት የከፋ ችግር አያጋጥምዎትም።

የህክምና መረጃ በሚሰጥረ ተጠብቆ መቆየት የሚችለው እንዴት ነው?

ስለራስዎ የሰጡት ማንኛውም መረጃና ከተወሰደው ናሙና ላይ የተገኘው የላቦራቶሪ ውጤት የሚወለደው ለጥናቱ አላማ ብቻ ነው። ይህን ማህደር ሊያገኙ የሚችሉት የተወሰኑ የጥናቱ ተባባሪ ሰዎች ብቻ

ናቸው።ከዚያም በላይ ስለ እርስዎ ያለውን ማንኛውንም መረጃ የተለየ የይላፍ ቃል ባለው የኮምፒውተር የመረጃ ማህደር ውስጥ እንዲቀመጥ ይደረጋል ።

በዚህ ጥናት መሳተፍ የሚያስገኛቸው ጥቅሞች ምንድን ናቸው ?

ይህ ጥናት የማስተርስ ዲግሪ መመረቂያ እንደመሆኑ መጠን በዚህ ጥናት በመካፈልዎ በገንዘብ የሚያገኙት ጥቅም ባይኖርም ከጥናቱ በሚገኘው ውጤት ግን ተጠቃሚ ነዎት።

በዚህ ጥናት ተሳታፊ የመሆንዎ መብቶች ምንድን ናቸው ?

በዚህ ጥናት መሳተፍ ሙሉ በሙሉ በእርስዎ ፈቃደኝነት የተመሰረተ በመሆኑ በማንኛውም ሰዓትና በታ የማቋረጥ ሙሉ መብት የተጠበቀ ከመሆኑም በላይ እራስዎን ከጥናቱ በማግለልዎ ምክንያት የሚቀርብዎት ምንም አይነት የላቦራቶሪ አገልግሎት አይኖርም ።ከዚህም በተጨማሪ ጥናቱን በተመለከተ ማንኛውንም አይነት ጥያቄ የመጠየቅና ገለጻ የማግኘት መብት አለዎት።የላቦራቶሪ ምርመራ ውጤቱንም በገጸ ማግኘት ይችላሉ። ነገር ግን እርስዎ በሚሰጡን መረጃ የችግሩን ስፋት ለመከላከል እና ለመቆጣጠር ጠቃሚ ስለሆነ ለሚቀርብልዎት ጥያቄ ቀጥተኛ መልስ ይሰጡን ዘንድ በታላቅ አክብሮት እንጠይቃለን።

ጥያቄ ካለኝ ወይም ችግር ቢያጋጥመኝ ምን ማድረግ ይገባል?

ይህንን ጥናት በተመለከተ ወይም ከዚህ ጥናት ጋር በተዛመደ መልኩ ስለሚያጋጥሙ ድንገተኛ አደጋዎች ወይም ጥያቄ ካለዎት በሚመለከተው አድራሻ ይጠቀሙ።

ራሂል ቦጋለ

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Annex 3. Informed consent form in English version

ID no.....

I have been informed that the objective of this study is to know the causative agents and risk factors of Hepatitis B, Hepatitis C and Syphilis. The results of this study have an importance to know the Magnitude of Hepatitis B, Hepatitis C Syphilis and their association's risk factors among Emigrants, and to be used as an input for the future development of strategies or guidelines for diagnosing of Hepatitis B, Hepatitis C Syphilis in Ethiopia. I had been also informed about the confidentiality of this study. The principal investigator requested me to participate in the study that would require my willingness to provide the required data that include blood sample, and filling questionnaire. Therefore, with full understanding of the importance of the study, I agreed voluntarily to provide the requested samples and my benefit will be only from the free laboratory investigation results.

I _____ hereby give my consent for providing the requested information and specimens as the participant find best for me.

Signature: _____ Date _____

Annex 4. Informed consent form in Amharic version

የተሳታፊዎች ስምምነት ማረጋገጫ

የሚስጥር ቁጥር -----

የተሳታፊው ስም -----

እኔ ስሜ ከላይ የተጠቀሰው ተሳታፊ “ከ አስራስምት አመት በላይ ያሉ አዋቂ የሚያጋጥማቸው የጉበት ባይረስ ቢ፣ሲ እና ቁጥኝ መንስኤውን ለይቶ ለማወቅና ስላለው ስርጭት ምክንያት ለማወቅ የሚል ነው”። ጥናት ላይ በቂ ገለጻ ተደርጎልኛል። ለጥናቱም የደም ናሙና እንደሚያስፈልግ ተገልጿል። የጥናቱንም አላማዎችም ተረድቻለሁ።

በቃለ መጠይቁ ላይ የገለጽኳቸው መረጃዎች በሙሉ በሚስጥር የተጠበቁ እንደሚሆኑ ተነግሮኛል ። በጥናቱ ላይ ያለመሳተፍና ማንኛውንም መረጃ ያለመስጠት እንዲሁም በማንኛውም ጊዜ ከጥናቱ ራሴን የማግለል መብቴ የተጠበቀ እንደሆነ ተገልጿል።

ስለዚህ ለዚህ ጥናት መረጃና የስምምነት ቃሌን የሰጠሁት በአጠቃላይ ሁኔታውን በመረዳትና በፍጹም ፍቃደኝነት ነው። በተጨማሪም ጥያቄ ለመጠየቅ ተፈቅዶልኝ ለማወቅ የፈለኩትን ያህል ማብራሪያ አግኝቻለሁ ። የዚህ ጥናት ተሳታፊ በመሆኔ የማገኘው ጥቅም የሁሉንም ምርመራ ውጤት በነጻ ማግኘት እንደሆነ ተረድቻለሁ።

በአጠቃላይ እኔ ከላይ በመተማመኛ ቅፅ የተጠቀሱትን ሁሉ በሚገባና በተረጋጋ መንፈስ አንብቤዋለሁኝ። ስለዚህ በዚህ ጥናት ለመሳተፍ ፈቃደኛ መሆኔን በፊርማዬ አረጋግጣለሁ።

ፊርማ----- ቀን ----/---/-----

(የስምምነት ቅጹን ማንበብ ለማይችሉ ተሳታፊዎች)

የአማካሪ ላብራቶሪ ቴክኒሻን ስም -----

ፊርማ -----

ቀን-----

Annex 5: English version of Questionnaire

I. Demographic, Socio-Economic Information and Risk factors association with Hepatitis B, Hepatitis C and syphilis infection

1. Identification number:
2. Address: Region.....
3. Your age:years.
4. Sex (Put \checkmark in the applicable box) Male Female
5. Education: 1. Illiterate
 2. Primary
 3. Senior secondary
 5. University
6. Marital status: 1. Single
 2. Married
 3. Divorced
 4. Widowed
 5. Separated
7. Occupation: 1. Unemployed
 2. Employed
 3. Self-employed
8. Your income per month: 1. < 500 Birr
 2. 500-1000 Birr
 3.> 1000 Birr
9. Multiple sex partners 1. Yes 2 .No
10. Exchange of sharp materials 1. Yes 2. No
11. Tattooing history 1 .Yes 2 .No
12. Tooth extraction history 1. Yes 2 .No
13. Vaccinated 1 .Yes 2. No

Annex 6: Amharic version of Questionnaire

“ከ አስራ ስምት አመት በላይ ያሉ አዋቂ የሚያጋጥሟቸው የጉበት ባይረስ ቢሲ እና የቂጥኝ መንስኤውን ለይቶ ለማወቅና ስላለው ስርጭት ምክንያት ለማወቅ በሚል የተዘጋጀ ቃለ መጠይቅ ።

የተሳትፎ መሙያ :

1. መለያ ቁጥር.....

2. አድራሻ.....

3. እድሜ.....

4. ጾታ ወ ሴ

5. የት/ት ደረጃ
1. ማብብ ና መጻፍ የመማይቸል
 2. መጀመርያ ደረጃ
 3. ሁለተኛ ደረጃ
 4. ዩኒቨርሲቲ

6. የጋብቻ ሁኔታ:
1. ያገባ
 2. ያላገባ
 3. ፍቺ የፈጸመ
 4. በሞት የተለየ
 5. ተለያይተዉ የሚኖሩ

7. የስራ ሁኔታ:
1. የለም
 2. ተቀጣሪ
 3. የግል

8. የወር ገቢ
1. <500
 2. 500-1000
 3. >1000

- | | | |
|------------------------------|-----------|-------------|
| 9. ምን ያህል የጾታ ጎደኛ አለዎ? | 1. አንድ | 2. ከአንድ በላይ |
| 10. ስለት ያለዉ ነገር ይጋራሉ? | 1. እጋራ ለሁ | 2. አልጋራም |
| 11. ጥርስ አስነቅለዉ ያውቃሉ? | 1. አዉቃለሁ | 2. አላቅም |
| 12. ንቅሳት ተነቅሰዉ ያውቃሉ? | 1. አዉቃለሁ | 2. አላቅም |
| 13. የጉበት ባይረስ ቢ: ክትባት ተከትባል? | 1. ተከትቤለሁ | 2. አልተከተብኩም |

Annex 7: Test procedure

Sample for Hepatitis B Hepatitis C and syphilis Rapid test

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 5 minutes at 3000 rpm. Remove serum and assay immediately or aliquot and or store samples at ≤ -20 °C.

Sample may be stored at 2-80c.

Transport and Storage: 2-8 0c

Supplies

- ✓ Serum separate tube test tube (SST)
- ✓ Disposable glove
- ✓ Tourniquet
- ✓ 70% Ethanol alcohol
- ✓ Syringe 5cc / Vacutainer
- ✓ Cotton Swab
- ✓ Dry gauze

Hepatitis B Surface Antigen Test Kit (Immunochromatography)/RT/, CTK. Biotech Made in USA

Test procedure: Instructions must be read entirely before taking the test. Allow the test device controls to equilibrate to room temperature for 30 minutes (20°C- 30°C) prior to testing. Do not open the inner packaging until ready, it must be used in one hour if opened (humidity: 20%- 90% temp: 10°C-50°C)

Cassette:

- 1). Take off the outer packing; put the cassette onto the desk with the sample window up.
- 2). Using the pipette drop 2 drops of serum or plasma (80u/l-100 u/l) vertically into the sample well of the cassette.
- 3). read test results immediately within 10-15 minutes; the result is invalid over 20 minutes

Result judgment

Positive; two distinct red lines appear. One line should be in the control region (c) and the other line should be in the test region (T).

Negative: only one red line appears or in the control lines no apparent line on the test (T) region.

Invalid: a total absent of color in both regions, indicating that the operator error or reagent failure. Verify the test procedure and repeat the test with a new testing device.

Hepatitis C virus Antibody Rapid test kit (Immunochromatography), CTK. Biotech Made in USA

Test procedure

Instructions must be read entirely before taking the test. Allow the test device controls to equilibrate to room temperature for 30 minutes (20°C- 30°C) prior to testing. Do not open the inner packaging until ready, it must be used in one hour if opened (humidity: 20%- 90% temp: 10°C-50°C)

Cassette:

1). Take off the outer packing; put the cassette onto the desk with the sample adding area of the sample window of the cassette up.2).Dispense 1 drops of Serum/plasma (25u/l) serum/plasma vertically into the sample adding area of the sample well of the cassette. Add 2 drops (80-100u/l) of sample buffer into the sample adding area of the sample hole of cassette.3). Observe the test results immediately within 10-20 minutes; the result is invalid over 20 minutes.

Result judgment:

Positive; two red lines appear on both section. One line should be in the control region (C) and the other line should be in the test region (T).

Negative: one red line appears in the control line region (C) no apparent red or pink line appears in the test region (T).

Invalid: no red lines appear or control line fails to appear, indicating that the operator error or reagent failure. Verify the test procedure and repeat the test with a new testing device.

Syphilis rapid test cassette (serum/ plasma), CTK. Biotech Made in USA

Test procedure

1). Remove the test cassette from the package foil pouch and use it as soon as possible.2). Place the test cassette on a clean and flat surface. Hold the dropper vertically and transfer 2 full drops

of serum or plasma (approximately 80u/l) and start the timer. Avoid air bubbles must be prevented.3). Wait for the colored lines (s) to appear. Read the results in 10 minutes. Do not interpret the result after 20 minutes.

Interpretation of result

Positive: two colored lines appear. One colored line should be in the control line (C) region and another apparent colored line should be in the test line(T) region .

Note: the intensity of the color in the test line region (T) will vary depending on the concentration of TP antibodies present in the specimen. Therefore, any shade of color in the test line region (T) should be considered positive.

Negative: single red colored line appears in the control line region (C). No red line appears in the test line region (T).

Invalid: control line fails to appear. Insufficient specimen volume or incorrect procedural techniques are the most likely reasons for control line failure. Review the procedure and repeat the test with a new test. If the problem persists, discontinue using the test kit immediately and contact your local distributor.

Annex 8: Standard Operating Procedure for Biobase ELISA Microplate Reader

ELISA (enzyme-linked immunosorbent assay)

Procedure

1. Antigens to coat the microliter plate wells.
2. Blocking reagents for unbound sites to prevent false positive results:
3. Antibodies
4. anti-(species) IgG conjugated to an enzyme: and
5. Substrates that react with the enzyme to produce a colored product (indicate a positive reaction).



BIOBASE EL-10A Elisa micro plate reader, equipped with LCD touch screen display, able to monitor timely and print testing results directly

- **Method:** End point method, two point method, dynamics, and single /dual wavelength test mode.
- **Analysis Modes:**
 - ✓ Measurement mode: automatic single- wavelength, dual-wavelength end point methods.
 - ✓ Calculation mode: open type qualitative determination and quantitative analysis

➤ **Reagent**

- ✓ ELISA Diluent solution
- ✓ ELISA reagent kit
- ✓ ELISA buffers
- ✓ Micro plate coating
- ✓ Plate coating buffers
- ✓ Calibrators
- ✓ ELISA P Control
- ✓ ELISA N Control
- ✓ Stop solution
- ✓ Wash buffer

➤ **Microplate Washer**

- ✓ Wash Solution A
- ✓ Wash Solution B
- ✓ Distilled Water
- ✓ Waste Water

Supplies

- ✓ Serum separate tube test tube (SST)
- ✓ Disposable glove
- ✓ Tourniquet
- ✓ 70% Ethanol alcohol
- ✓ Syringe 5cc / Vacutainer
- ✓ Cotton Swab
- ✓ Dry gauze

ELISA Sample Preparation & Collection Guide

- ✓ The ELISA sample collection and storage conditions listed below are intended as general guidelines. Specific protocols may vary by cell line or tissue type. If storing sample for extended length of time, testing sample stability is recommended. Avoid repeated freeze-thaw cycles for all sample types.
- ✓ **Serum** - Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C

Annex 9: Daily Blood sample Collection form

Date	Lab .no	Test			Client name	Age	Sex	Tele phone	Sample collection Time	Sample analysis Time	Result delivery Time	Remark
		TPSAC	HCV/ab	TPHA/VVD RL								
	1											
	2											
	3											
	4											
	5											
	6											
	7											
	8											
	9											
	10											
	11											
	12											
	13											
	14											
	15											
	16											
	17											
	18											
	19											

Sample collected by: _____

Done By: _____ Signature: _____

Annex 10: Declaration

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

M.Sc. candidate:

Rahel Bogale (B.Sc.)

Signature:

Date of submission:

This thesis has been submitted with our approval as advisors.

Advisor:

Melese Hailu (MSc, PhD candidate)

Signature:

Date:

Place:

Addis Ababa, Ethiopia.

Advisor:

Regasa Diriba (BSc, MSc)

Signature:

Date:

Place:

Addis Ababa, Ethiopia.