ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCE

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DEPARTMENT OF MEDICAL LABORATORY SCIENCES

Magnitude of Hepatitis B and Hepatitis C infection and Their Associated Risk Factors Among Psychiatric Patients at Amanuel Mental Specialized Hospital, Addis Ababa, Ethiopia

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Table of Contents

List of Tables

Abbreviations

Ab	Antibody
Ag	Antigen
AMSH	Amanuel Mental Specialized Hospital
CHB	chronic hepatitis B infection
CLD	Chronic liver disease
DNA	Deoxy Ribose nucleic acid
EIA	Enzyme immunoassay
ELISA	Enzyme linked immune sorbent assay
HAV	Hepatitis A virus
HBIG	Hepatitis B immune globulin
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B Virus
HCC	Hepatocellular carcinoma
HCV	Hepatitis C Virus
HRQOL	Health-related quality of life
IDU	Injection drug users
OPD	Outpatient department
RNA	Ribose nucleic acid
SOPS	Standard operating procedures
TBA	Traditional birth attendant
US	ultrasound

Abstract

Background: Hepatitis B (HBV) and Hepatitis C (HCV) viruses are hepatotoxic viruses that spread mainly through contaminated blood and blood products, sexual contact and contaminated needles. HBV and HCV infection share similar transmission routes and therefore co-infection is common. These viruses are prevalent in different parts of the world including Ethiopia. Five and three point three percent of world population have been infected with HBV and HCV respectively. The prevalence of HBV is 2.0% in USA, 8.0-20.0% in South- East Asia, the prevalence of HCV 4.8% in Pakistan, 3.2% in china and 22% in Egypt. In Ethiopia, there is lack of data on the prevalence of HBV and HCV and globally concerning to psychiatric patients.

Objective: The aim this research was to determine the Magnitude of HBV and HCV infection among psychiatric patients.

Methods: A Cross-sectional study was conducted in the study site among psychiatric patients. Structured questionnaires were used to collect information pertaining to socio demographic factors including history of mental illness, and associated factors for HBV and HCV infection. Blood specimen was collected and serum was separated to determine hepatitis B surface antigen (HBSAg) and anti-HCV antibody by Enzyme linked immunosorbent Assay (ELISA). Data was analyzed using SPSS version 20 software. We have used descriptive statistics for some variables. Moreover, bivariate and multivariate analysis was applied. The Odds ratio with 95 % CI was used to assess the risk factors. P value less than 0.05 was taken as statistically significant.

Result: A total of 384 participants who have Psychiatric problems were participated in this study, where 208 (54.2%) of them were males. The age distribution ranges form 20-70 years. The overall burden of HBsAg and HCV antibody was 20.3% and 14.3 % respectively. History of tattooing of body and blood transfusion were found to be statistically significance with HBV virus infection and history multi sexual partners with HCV infection.

Conclusion: Magnitude of HBV and HCV is high among Psychiatric patients and special efforts should be taken by the hospitals and other stakeholders to investigate the real source of HBV and HCV infection among the psychiatric and other mentally ill people in order to address appropriate intervention measures.

Key words: HBV, HCV, Magnitude

1. Introduction

1.1. Background

Hepatitis B and C are common cause of liver disorders accounting for up to 75% of all cases of liver diseases worldwide making it a disease of global concern. HBV and HCV viruses are hepatotoxic virus whose primary replication occurs in the liver. Infection with HBV and HCV affects the liver and results in a broad spectrum of disease outcomes particularly cirrhosis and hepatocellular carcinoma [1]

HBV is a member of the family Hepadnaviridae which contain a unique deoxyribonucleic acid (DNA), while HCV is a member of family Flaviviridae under RNA viruses. An infection with HBV may spontaneously resolved and lead to protective immunity, chronic infection and, in rare cases, acute liver failure with a high risk of death. In contrast to HBV, an infection with HCV becomes chronic in most cases. People with chronic HBV and/or HCV infection remain infectious to others and are at risk of serious liver disease such as liver cirrhosis or hepatic cell carcinoma (HCC) later in life [2].

According to the most recent WHO report, approximately 2 billion people worldwide have been infected with HBV, an estimated 360 million individuals have been infected with HBV each year and an estimated 600,000 people die every year due to the acute or chronic consequences of HBV. At any given time, more than 350,000 people die from hepatitis C-related liver diseases each year. HCV which was discovered in 1989 as a global disease has a worldwide prevalence of 3.3% with about 170-200 million infected individuals [3, 4]

The modes of transmission of HBV and HCV include sexual contact, exposure to infected blood, body secretions, and breast feeding. Reports have indicated higher rate of HBV transmission compared with HIV as a result of the ability of the virus to remain viable and infectious for more than a week in the environment [5].

HCV exhibits high genetic diversity, characterized by regional variations in genotype prevalence. This poses a challenge to the improved development of vaccines and pan-genotypic treatments, which require the consideration of global trends in HCV genotype prevalence [6].

Although the incidence and HBV infection has been markedly reduced after mass vaccination programs, HCV infection remains a worldwide public health concern [7]. The statistics suggests that there is a further need to prevent, screen and intervene early cases of HBV and HCV infections. The prevalence of chronic HBV infection is about 5% worldwide, but this figure varies substantially between regions. Infection rates are low (2.0%) in the United States and Western Europe, intermediate (2.8%) in Mediterranean countries and Japan, and high (8.0-20.0%) in South-East Asia and the sub-Saharan regions. HCV infection is of epidemic proportions worldwide. Countries with high rates of chronic infection are Egypt (22%), Pakistan (4.8%), and China (3.2%) [8].

Higher infection rate with HBV and HCV in specific groups, such as parenteral drug users, male homosexuals,immunosuppressed patients, those with hemophilia, and patients undergoing dialysis. A higher incidence of hepatitis has also been identified in healthcare workers, ambulance personnel, teachers, and staffs in institutions such as retirement homes and institutions for the intellectual disability and those suffering serious mental illness [9].

About 240 million people have a serious mental illness, with a broadly similar distribution worldwide. Serious mental illness is defined as a diagnosis of mental illness (e.g., schizophrenia and schizoaffective disorders, bipolar disorder, or psychosis) that is persistent, disabling, and requiring specialized psychiatric treatment as an outpatient or inpatient admission. The point prevalence of serious mental illness is 4.6 cases per 1000 people, and 4.0 % of people have a serious mental illness at some point during their life [10]

Associated with their psychiatric impairment, persons with severe mental illness are at an increased risk for several co-morbid conditions, including substance use disorders. They are also likely to be over presented in high risk categories for infections such as HBV and HCV. However, few studies have focused on HBV and HCV infection rates among institutionalized patients with severe mental illness, especially in a country with a high prevalence of HBV [9, 10].

The natural history and clinical course of HBV differs from that of HCV infection. However, similarities may be drawn in the context of chronic disease and public health burden. Chronic infection by these viruses leads to slow progressive liver disease that over a period of up to 30 years may result in cirrhosis, chronic liver failure and hepatocellular carcinoma (HCC) [11].

Chronic diseases are associated with a significant burden of psychosocial problems including impaired health-related quality of life (HRQOL), depression, anxiety, and other psychological impairments. In cases where these chronic conditions stigmatize the patient, psychological impairment becomes more pronounced. This is particularly seen in infectious diseases associated with high risk behaviors, highly contagious diseases, and psychiatric disorders. Chronic HBV, as one of the most common causes of liver disease, is associated with a high psychosocial burden. Moreover, complications of advanced liver disease may be associated with poorer HRQOL irrespective of the cause of the liver involvement [12].There is a lack of information on the prevalence of HBV and HCV among psychiatric patients globally.

In Ethiopia large number of research done and it indicated that prevalence of liver disease due to HBV and HCV is high. It accounts for 12% of the hospital admissions and 31% of the mortality in medical wards of Ethiopian hospitals [13].

1.2. Statement of the problem

People with severe mental illness appear to have increased rates of sexually transmitted diseases (STDs), and they are likely to engage in high-risk behaviors such as using injection drugs, having multiple sexual partners and high-risk partners, infrequently using condoms, engaging in same-sex sexual activity, trading sex for money or drugs, and engaging in sex while using psychoactive substances [14].

People with serious mental illness have significant health inequalities, increasing prominence has been given to physical health screening, health education, and improving access to treatment in primary and secondary care. Sexual health has largely been neglected by researchers and policy makers involved in mental health. Failure to address this shortcoming could increase morbidity and mortality of blood-borne virus infections (HBV and HCV) [14, 15].

Many researchers have investigated prevalence rates of HBV and HCV infections in various groups (health care workers, blood donors, medical waste handlers, and others); however there is lack of information concerning the epidemiology of HBV and HCV infection in psychiatric patients in Ethiopia particularly in the study site. Therefore, cross sectional study was conducted in order to determine the Magnitude of HBsAg and anti HCV antibody at Amanuel mental specialized hospital among psychiatric patients.

1.3. Significance of the study

As HBV and HCV is a silent killer viruses, diagnosis, treatment and prevention are very important [1]. Hence the results of this study will, if further explored, benefit the psychiatric patients for diagnosis, treatment and prevention purpose.

This study could provide information on the pattern of HBV and HCV as well as associated factors among psychiatric patients. This study could also be useful for health managers and planners to develop appropriate preventive services, allocate resources, decide on priorities and target certain populations.

To the best of our knowledge there is no study done on burden of HBV and HCV among psychiatric patients in Ethiopia. Hence this study can be used as a baseline information for further studies.

2. Literature review

Studies from various countries have found an increased prevalence of two- to three-fold with HBV and two- to eight-folds with HCV in patients with psychiatric illness compared to those without [16]. In a study on psychiatric patients chronically hospitalized at Oregon State Hospital, found 20.3% seropositivity for HCAb and 24.7% for HbsAb [17]. A study by Dinwiddie et al. on psychiatric patients in the Chicago area found 27.8% with HBsAb and 8.5% with HCV anti-body [18].

In Japan, Sawayama et al. studied on neuropsychiatric patients and found that 10.2% of the patients were HCAb positive compared to 1.5% of controls and 44.4% were HBsAg positive compared to 20.5% of controls [19]. Nakamura et al. studied on psychiatric patients in Japan and found 9.1% positive for anti-HCV antibody compared to an estimated 1.2% anti-HCV ant-body positivity rate in the general Japanese population [20].

In a Brazilian study, 22.4% of the psychiatric patients studied were positive for HBV infection [21]. A study done in Spain showed that HBV seropositivity in mentally handicapped adults was 81.3% [22]. In Northern Ireland 4.5% of the residents of an institution for the mentally handicapped carried any HBV marker [23]. Study in US, the prevalence of hepatitis B and C infection among psychiatric patients were 23.4% and 19.6% respectively and these were approximately 5 and 11 times the overall estimated population rates for these infection [24]. In Taiwan a sero-prevalence of hepatitis B infection was 18.1% among institutionalized psychiatric patients and the disease was higher in male than in female psychiatric patients and 13.4% for anti-HCV anti-bodies [25].

Furthermore, a study performed on psychiatric inpatients in Hamedan showed that 1.8% were positive for HBsAg, 1.2% were positive for HBV surface antibody (HBsAb), and 69% were positive for HBV core antibody [26]. The study done Turkey among psychiatric patients over all seroprevalence was 4.08% for HBsAg, 42.19% for anti-HBs and 0.69% for anti-HCV [27].

Other studies from the USA reported that 10.5% of inpatients with psychosis were positive for HCV and 20.3% of long-term psychiatric in-patients and 9.9% of in-patients with schizophrenia were positive for HCV [28]. Study from Singapore showed prevalence of 12.7% for HBsAg, 63.4% for surface antibody and as high as 69% for HBV core antibody which is a more sensitive test of infectivity [29].

In a psychiatric hospital of Mexico by Esquivel et al. 7.1% were positive for HBsAg [30]. A study done by Shittu et.al 2010 among mentally ill patients attending a tertiary hospital in Nigeria the prevalence of Hepatitis B and C among patients with mental illness was 10.0% and 12.6% respectively as compared to a sero-prevalence of 10.9% and 1.1% in the blood donors [31], study in Portugal, 1.6% of psychiatric Patients were positive for HBAg [32], the study done to know Prevalence of HBV, HCV, and HIV Infections Among Patients in a Psychiatric Hospital in Greece,2% tested positive for having the HBsAg, 9% for the HCV antibody[33].

The prevalence of HCV among multi-transfused patients varies from one area to another and depends on the endemicity of viral hepatitis in different regions. The highest reported prevalence was in Egypt, where 75% of patients with homozygous β-thalassaemia are infected with HCV, while the prevalence ranges from 33–67.3% [34].

Taiwan is among the countries where the infection is highly prevalent, with 15-20% of the general population suffering from chronic HBV infection and an estimated 4.4% of the general population with chronic HCV infection [35]. The study in Oman 41% of investigated β-thalassaemia patients had been infected with HCV [36], in Jamaica reported 41% of 90 hemophilia patients are seropositive for HCV [37] while the rates of positive HCV among Brazilian hemophiliacs vary from 39% to 64.3% [38].

The study done in Egypt among multi-transfused thalassemic patients 19.5%, were anti-HCV positive, 29.0%, were HBsAg positive [39]. The study conducted to assess the prevalence of Human immunodeficiency virus (HIV) among hemophilia and thalassemia patients HCV 10.8% and HBV 11% in Iran was recorded [40]. Another study in Lagos among children and adults with Sickle Cell Anaemia (SCA) using third generation Enzyme Immuno sorbent Assay (EIA) kit showed 5% prevalence [41].

Study among blood donors for HBV, from south Dar fur 6.25%[42], Tete Mozambique 10.6%[43], kano Nigeria 11.1% [44], Ibadan Nigeria 5.9% [45], Akure Nigeria 7.4% [46], Quảng Trị Vietnam 11.1% [47], 35.8% HBsAg and 22.5% HCV Ab seropositivity was reported previously from Ethiopia May 2013 among Chronic liver disease patients [48]. 44.6% HBsAg and 26% HCVAbs in India among CLD patients [49], 42.9% Gana among CLD [50], 36% HBsAg % and 24% HCV Antibody in Zimbabwe among HCC patients were recorded [51].

The study carried out in Vietnam, HBV and HCV prevalence among CLD was 47% and 23% respectively [52]. In Eastern India in blood transfused patents seropositivity of HCV (18.70% and HBV (3.33%) was recorded [53]. In Syria high seropositivity, HBsAg 24.5% in multi-transfused patients with was recorded [54].

A systematic review and meta-analysis in Ethiopia the overall pooled prevalence of hepatitis HBV was 7.4%, pooled prevalence among subgroups showed that 5.2% in human immunodeficiency virus (HIV) infected individuals, 8.0% in community based studies, 8.4% in blood donors, 11.0% in immigrants and 6.9% in other groups and the overall pooled prevalence of anti-HCV was 3.1% Unlike HBV, the anti-HCV prevalence in HIV infected individuals was higher [55].

Studies in blood donors has been reported from Ethiopia in Bahir Dar for HBV 4.11%, for HCV 2.1% [56], in Amhara and Tigray Regional states among blood donors 6.2% for HBV and HCV 1.7% [57]. HBV and HCV was detected in 3.57% and 1.59% respectively in Addis Ababa among cleaners [58]. In Ethiopia on ANC patient sero-burden of 3 % and 3.6 % for HbsAg and anti-HCV, respectively [59]. In Shashemene General Hospital HBsAg(5.7%), among visitors voluntary counseling and testing center[60], in Addis Ababa HBsAg (5.7%), among VCT clients [61].

Another community based sero-epidemiological survey that addresses the transmission dynamics and control of HBV in Addis Ababa, reported HBsAg prevalence of 7.0% from the general population [62]. Three point seven percent (3.7%) HBsAg prevalence was also reported among pregnant women in Jimma [63].

3. Objective of the study

3.1. General Objective

To assess the Magnitude of HBV and HCV infection among psychiatric patients at Amanuel Mental Specialized Hospital in Addis Ababa, Ethiopia.

3.2. Specific Objectives

To determine HBV Magnitude among psychiatric patients

To determine HCV Magnitude among psychiatric patients

To identify associated factors of HBV and HCV among psychiatric patients

4. Hypothesis

There is no difference in the prevalence of HBV and HCV in Psychiatric patients and the general population.

5. Materials and Methods

5.1. Study area

The study was conducted at Amanuel Mental Specialized Hospital, Addis Ababa; the capital city of Ethiopia, established in 1878. A population of 3,384,569 (2007 census) located in the geographic center of the country which lies at an altitude of 7,546 feet (2,300 meters) with a grass land biome. The hospital has 20 department including diagnostic laboratory and 270 beds. It is the only referral hospital that provides psychiatric specialty services to an average of 253(110 female/143 male) patients daily including private wing. 15 doctors 250 nurses 16 laboratory professionals and 27 pharmacy professionals are serving the hospital. Many people having psychiatric problems are referred from all over the country to this hospital.

5.2. Study Design and period

Cross-sectional study design was conducted from October 2016 to February 2017 at Amanuel Mental Specialized Hospital.

5.3. Study Variables

Dependent variables: Sero-buredn of Hepatitis B and Hepatitis C viruses

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

5.4. Population

5.4.1. Source population

All patients who visited Amanuel mental specialized hospital during the data collection period was the source population.

5.4.2. Study population

All psychiatric patients whose parents or guardians volunteer to participate in the study were our study population.

5.5. Eligibility criteria

5.5.1. Inclusion criteria

All individuals who visited OPD and Ward assumed to be as psychiatric Patients by psychiatrist during the study period were included.

5.5.2 Exclusion criteria

Individuals who assumed to be as psychiatric Patients by psychiatrist during the study period but whose parents or legal guardians refuse to give informed consent were excluded.

5.6. Sample size estimation and Sampling technique

5.6.1. Sample size estimation

Since there is no study conducted on the prevalence of HBC and HCV in Ethiopia among psychiatric patients, we use p=50 %(0.5) with 95% confidence interval and 5 % margin of error, the minimum sample size was estimated as follows. Sample size is derived using the following formula:-

$$n = \frac{(Z\text{-}\alpha/2)^2 P (1-P)}{d^2}$$

$$\frac{(1.96)^2(0.5x0.5)}{(0.05)^2}$$

Where: n = the required sample size

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

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

P= Proportion in the target population to have HBV.

According to the formula the calculated sample size is 384

5.6.2. Sampling technique

Convenient sampling technique was used.

5.7. Data Collection and Processing

5.7.1. Data collection

Clinically diagnosed psychiatric patients were sorted by the clinical history on their patient history sheet. All parents or guardians of outpatients and inpatient, whose clinical history reported as psychiatric (schizophria, bipolar and depression) patient were approached for consent. Socio-demographic characters such as, Residence, marital status, ethnicity, educational Status data and other associated risk factors such as multiple sexual partners, blood transfusion, abortion, dental extraction at health facility, circumcision, hospital admission, Surgical procedure, venous or body piercing for treatment delivery by TBA, ear piercing, nose piercing, uvuloctomy, tattooing on body, tattooing on gum, shaving, contact with jaundiced patient, frequent alcohol consumption, factors were collected using a structured questionnaire

5.7.2. Sample collection and processing

Five ml of venous blood was collected aseptically by vacuntainer needle in serum separating tube; samples were left at room temperature for 1 hour to facilitate clotting and were centrifuged at 3000 rpm for 5 minutes to get clear serum for serological analysis. Serum samples was kept in nuc tubes and stored at -20 degree centigrade until it was processed.

5.8. Serological Test Principle

5.8.1. HBsAg EIA Test

HBsAg ELISA test is third generation immunoassay for determining HBsAg(DIALAB CHEMISCH-TECHNISCHEN, JOAQUINCOSTA,PLANTA, GESELLSCHAFT, CHINA). It is based on a one step “Sandwich principle”. Antibody to HBsAg coupled to horseradish peroxidase is served as conjugate with 3, 3′, 5, 5′- Tetramethylbenzidine and peroxidase as a substrate. Upon completion of the test, a color developed which is directly proportional to the amount of the HBsAg in the sample [64].

5.8.2. HCV Antibody EIA

HCV Antibody ELISA Serological test is third generation immunoassay for determining anti-HCV anti-body (HUMAN BIOCHEMICA UND DIAGNOSTICA, MBH, GERMANY). This assay based on formation of stable Ag-Ab complex when a positive sample is added into a

well coated with recombinant Ag representing HCV epitopes. The conjugate is a rabbit antihuman IgG labeled with horseradish peroxidase. When Enzyme substrate added, a blue color developed. This color changes to yellow after blocking the reaction with sulphuric acid. The intensity of color is proportional to the anti-HCV concentration that present in the sample [65].

5.9. Data Processing and Analysis

Data was entered, cleaned and analyzed by using the statistical software SPSS version 20.The Magnitude for HBV and HCV were expressed in percentages for the entire study group. Results obtained were presented in tables. Descriptive statistical methods were used to summarize data on socio-demographic and clinical characteristics. The chi-square test and binary logistic regression were used for statistical analysis of data. A 'P' value less than 0.05 was considered as statistically significant.

5.10. Operational Definitions

Psychiatric patients: Patients who have problem of schizophrenia/bipolar/depression

5.10. Ethical Considerations

Before starting the research work, ethical clearance was obtained from the Departmental Research and Ethics Review Committee (DRERC) of Addis Ababa University College of Health Sciences, School of Allied Health Sciences, and Department of Laboratory Sciences. Then a letter informing to Amanuel Mental Specialized was obtained from Amanuel Mental Specialized Hospital to access data from study population. Patient's parents or guardians were informed about the study and if they are willing they were asked for consent.

All personal information obtained from the study participants was coded to maintain confidentially. When the patient's result is identified to be positive for either HBV or HCV they were linked to the hospital's medical OPD with an internal referral form of the hospital.

6. Results

A total of 384 patients were included in the final analysis, Out of these, 35.9 % (n=138) were schizophrenic, 34.1% (n=131) were bipolar, and the remaining 29.9% (n=115) were depressed (table1).

6.1. Socio demographic characteristics

Among the 384 clients, 54.2 % (n=208) represents male participants with a male to female ratio of 1.2:1. The age range was 20 to 70 years with a median age of 36 and range of 50 years. Most of the study subjects were in the age group of 30 to 39 years of age which represents 44 % (n=169) followed by age group of 40-49 year which represent 23.4 % (n=90) and the list age group was ≥ 60 years represents 2.3 % (n=9).

Most of the clients were found to be married, 58.1% (n= 223) followed by single, 33.3 % (n=128) and widowed one accounted 4.7 % (N=18). Concerning ethnicity, majority 34.9 % (n=134) of participants were Amhara followed by 33.6 % (n=129) Oromo and the least ethic group was Wolita accounting 1.6 % (n=6).

On the religious background, most of the clients were Christians, 74.5% (n=286). In relation to living area, 77.6% (n=298) of the study participants were urban dwellers. About 22.4 % (n=86) the participants were illiterate, 23.2 % (n=89) could read and write and the 10.4 % (n=40) have diploma in their respective filed (table1).

Table1 Socio-demographic characteristics of participants with psychiatric problem investigated for HBV and HCV in AMSH (October 2016- February 2017)

Characteristics		No.	(%)
Age (years)	20-29	76	19.8
	30-39	169	44
	40-49	90	23.4
	50-59	40	10.4
	≥60	9	2.3
Sex	Male	208	54.2
	Female	176	45.8
Ethnicity	Amhara	134	34.9
	Oromo	129	33.6
	Guragie	63	16.4
	Tigre	32	8.3
	Wolayta	6	1.6
	Others	20	5.2
Marital status	Married	223	58.1
	Single	128	33.3
	Divorced	15	3.9
	widowed	18	4.7
Religion	Christian	286	74.5
	Muslim	98	25.5
Residence	Urban	298	77.6
	Rural	86	22.4
Educational status	Illiterate	86	22.4
	Read and write	89	23.2
	Primary school	59	15.4
	Secondary school	49	12.8
	Diploma	40	10.4
	1st Degree & above	61	15.9
Psychiatric cases	Schizophrenic	138	35.9
	Bipolar	131	34.1
	Depressed	115	29.9

6.2. Magnitude of HBV and HCV

In this study 28.9 % (n=111) study subjects were seropositive for one of the viruses. From overall positive samples 20.3 % (n=78) were positive for HBsAg, while 14.3 % (n=55) seropositive for Anti- HCV antibody. About 5.7 % (n=22) of the study subjects had HBV and HCV co infection (table2).

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

The magnitude of HBsAg in clinically diagnosed with Psychiatric (schizophrenia, bipolar and depression) patients were 20.3 % (78/384). The sex specific prevalence rate was higher in females 22.2% (39/176) than males 18.8 % (39/208) but the difference did not reach statistical significance (P=0.408). The magnitude of HBV by specific age group was highest 22.5 % (9/40) in 50-59 years followed by specific age group 60 years and above accounting seropositivity of 22.2 % (2/ 9) in ≥60 and the lest positive result ,17.8 % (16/90) found in 40-49 years age group However, the difference was not statistically significant (p=0.921)

Highest magnitude of HBSAg was found in study participants with an education level of diploma accounting 25.0 % (10/40) followed by grades 9-12 and illiterate 22.4% (11/49) and 22.1% (19/86) respectively and the least was found in degree and above which accounts 16.4% (10/61) and the difference was not statically significance (P=0.900).

About 22.1% (19/86) sero-positive for HBsAg study participants were rural dwellers and 19.8% (59/298) were urban dwellers (p = 0. 0.641). In relation to marital status the highest percent of positivity was observed by single 24.2 % (5/15) followed by divorced 20.0 % (3/15) and married 18.4% (41/223) respectively and the least prevalence was observed in widowed 16.7% (3/18) subjects (p = 0.600) (table2).

6.2.2. Magnitude of Hepatitis C infection by socio demographic characteristics

About 14.3 % (55/384) of the study participants were positive for anti-HCV Ab. The sex specific prevalence rate was higher among male 14.4 % (30/208) than female 14.2% (25/176) the difference was not statistical significance (P=0.951). The magnitude for HCV by specific age group was highest 22.5% (30/169) in 30-39 years followed by specific age groups 22.2 % (2/9) in ≥60 the difference was not statistical significance and 15.6%(14/90) in (40-49) respectively and the list positive result was recorded specific age group 15% (6/40) in 50-59 and the difference was not statistically significance (P=0.307). The highest seropositivity rate for anti-HCV Ab was observed among married subjects accounting 11.9 % (14/117) followed by single 4.2 % (5/117) (P= 0.809) (table2).

Table.2 Magnitude of HBV and HCV participants with psychiatric problem investigated for HBV and HCV in AMSH (October 2016- February 2017)

Variables		HbsAg Positive No. (%)	P-value	HCV PositiveNo. (%)	P-value
Age group	20-29	14/76(18.4%)	0.921	6/76(7.9%)	0.307
	30-39	30/169(17.8%)		37/169(21.9%)	
	40-49	16/90(17.8%)22.5		12/90(13.3%)	
	50-59	9/40(22.5)		5/40(12.5%)	
	=/>60	2/9(22.2)		2/9(22.2%)	
Sex	Male	30/208(14.4%)	0.408	30/208(14.4%)	0.951
	Female	39/176(22.2%)		25/176(14.2%)	
Residence	Urban	59/298(19.8%)	0.641	39/298(13.1%)	0.198
	Rural	19/86(22.1%)		16/86(18.6%)	
Marital Status	Married	41/223(18.4%)	0.600	31/223(13.9%)	0.809
	Singe	31/128(24.2%)		18/128(14.1%)	
	Divorced	3/15(20.0%)		2/15(13.3%)	
	Widow	3/18(16.7%)		4/18(22.2%)	
Occupation	Self-employee	25/151(16.6%)	0.106	24/151(15.9%)	0.935
	Student	3/24(12.5%)		3/24(12.5%)	
	House wife	23/90(25.6%)		10/90(11.1%)	
	Farmer	6/27(22.2%)		5/27(10.3%)	
	Unemployed	5/25(20.0%)		5/45(11.1%)	
Education	Illiterate	11/86 (12.8%)	0.900	4/86(16.0%)	0.925
	Read and write	17/89(19.1%)		12/89(13.5%)	
	Elementary	11/59(18.6%)		7/59(19.9%)	
	Secondary	11/49(22.4%)		9/49(18.4%)	
	Collage	10/40(25.0%)		6/40(15.0%)	
	Degree and Above	10/61(16.4)		10/61(16.4%)	

6.3. Risk factors of HBV

In this study, history of blood transfusion (p=0.002) and tattooing (p=0.001) on the body was associated with HBV infection. On the other hand, history of STD/STI, dental extraction at health facility, circumcision, surgical procedure, hospital admission, ear piercing, uvuloctomy, tattooing on gum, contact with jaundiced patient, alcohol consumption, abortion, shaving by barber, and multiple sexual partners were not associated with HBV infection.

In relation to blood transfusions, 6.2 % of psychiatric patients had history of blood transfusions. From those 18.6% (11/24) were positive for HBsAg (OR= 3.7; 95% CI: 1.588-8.620, P= 0.002). Those who had history of blood transfusions were 3.7 times more likely to have HBV infection than those who had not and the difference was statistically significant (P < 0.05).

Concerning tattooing on the body 53.1% (204/384) of psychiatric patients had history of tattooing on the body. From those 18.6 % (56/204) were positive for HBsAg (OR= 2.717; 95% CI: 1.581-4.671, P= 0.001). Those who had history of tattooing on the body were 2.7 times more likely to have HBV infection than those who had not and the difference was statistically significant (P < 0.05) (table3).

Table 3 Bivariate Assessment of risk factor for hepatitis B infection at Amanuel Mental Specialized Hospital (October 2016- February 2017)

Variable	Frequency	No of HbsAg positive	p-value
Tattooing on gum	89	22	0.117
Tattooing on body	204	56	0.001*
Shaving by barber* **	162	34	0.779
nose piercing	21	4	0.882
Uvuloctomy	120	27	0.473
Ear piercing	127	29	0.388
Contact with jaundice patient	19	3	0.615
Circumcision	328	64	0.345
Hospital addimition	212	41	0.599
Blood transfusion	24	11	0.002*
Dental extraction	127	24	0.628
Surgical procedure	21	3	0.480
Alcohol consumption	63	11	0.538
History of STI/STD	12	3	0.682
Multi sexual partner	31	9	0.208
Delivery by TBA**	8	30	0.386
Abortion**	12	41	0.135
Venous piecing	0	6	0.213

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

6.4. Risk factors of HCV virus infection

In this study, history of Multiple sexual partner was associated with HCV infection (p =0.015) other risk factors like, history of blood transfusion, tattooing on body, STD/STI, dental extraction at health facility, circumcision, surgical procedure, hospital admission, ear piercing, uvuloctomy, tattooing on gum, contact with jaundiced patient, alcohol consumption, abortion, and shaving by barber were not statistically associated with HBV infection.

In this study, 8.1% (31/384) of psychiatric patients had multiple sexual partners. From those 29.0 % (9/31 were positive for ant-HCV (OR= 2.7; 95% CI: 1.184-6.294, P= 0.018). Those who had history of Multiple sexual partner were 2.7 times more likely to have HCV infection than those who had not and the difference was statistically significant (P<0.05).

Table 4 Bivariate Assessment of risk factor for hepatitis C infection at AMSH (October 2016- February 2017)

Variable	Frequency	No. of anti-HCV positive	p-value
Tattooing on gum	87	17	0.117
Tattooing on body	204	20	0.820
Shaving by barber* **	162	21	0.516
nose piercing	3	21	0.996
Uvuloctomy	120	13	0.191
Ear piercing	127	20	0.575
Contact with jaundice patient	19	1	0.320
Circumcision	328	48	0.674
Hospital addmition	212	27	0.324
Blood transfusion	24	4	0.735
Dental extraction	127	15	0.323
Surgical procedure	21	2	0.518
Alcohol consumption	63	7	0.424
History of STI/STD	12	0	0.682
Multiple sexual partner	31	9	.018*
Delivery by TBA**	30	4	0.872
Abortion**	41	5	0.681
Venous piercing	6	0	0.213

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

5.5. Multivariate Analysis of risk factor for hepatitis B infection

In bivariate analyses, for HBV infection, blood transfusion (COR= 3.7; 95% CI: 1.6-8.6, P= 0.002) and tattooing body (COR=2.7; 95%CI: 1.6-4.7, p=0.002) had significant association. Those with p value equal or less than 0.2 were included in multiple logistic regression analyses for controlling confounders and for evaluating the effects of risk variables on HBV both variables remained statically significance with (AOR of 3.2; 95 % CI: 1.3-8.0, P= 0.011) for blood transfusion and (AOR of 2.6; 95%CI: 1.5-4.6, p=.001) for tattooing body.

Table5 Multivariate analysis of risk factor for hepatitis B infection at AMSH (October 2016- February 2017)

Characteristics	OR (95%, CI)	p-value	AOR (95%, CI)	p-value
Blood transfusion Yes	3.7(1.6-8.6)	0.002*	3.2(1.3-8.0)	0.011*
Tattooing body Yes	2.7(1.6-4.7)	0.001*	2.6(1.5-4.6)	0.001*
Tattooing on gum Yes	1.4(0.8-2.6)	0.117	1.2(0.7- 2.3)	0.461
Abortion Yes**	1.7(0.8-2.5)	0.135	1.1(0.5-2.5)	0.772

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

6.6. Multivariate Analysis of risk factor for hepatitis C infection

In bivariate analysis, it was found that significant risk factors for HCV infection among studied Psychiatric patients was having multi sexual partner (COR= 2.7; 95% CI: 1.2-6.3, P= 0.018). Those with p value equal or less than 0.2 were included in multiple logistic regression for controlling confounders and for evaluating the effects of risk variables on HCV infection and having multiple sexual partner remained statically significant (AOR of 2.7; 95% CI: 1.1-6.6, P= 0.024).

Table6 Multivariate analysis of risk factor for hepatitis C infection at AMSH (October 2016- February 2017)

Characteristics	OR(95%, CI)	p-value	AOR (95%, CI)	p-value
Multiple sexual Partner Yes	2.7(1.2-6.3)	0.018*	2.7(1.1-6.6)	0.024*
Tattooing gum Yes	1.6(0.88-3.1)	0.117	1.8(0.94-3.6)	0.078
Uvuloctomy Yes	0.64(0.33-1.24)	0.191	0.6(0.3-1.1)	0.116

*statically significant (P≤0.05).

7. Discussion

In this study conducted in 384 clinically diagnosed cases of psychiatric patients 20.3% (n=78) were positive for HBsAg, 14.3% (n=55) were positive for HCV antibodies. The study done in similar subjects in different countries comparable result was recorded, Oregon State Hospital, (20.3%) for HCV anti-body and 24.7% for HBV [17], Japan (10.2%) of the patients HCV ant-body[19], for HBsAg Brazili 22.4% [22], USA for HBV (23.4%) and for HCV (19.6%) [28], another study USA for HBV (20.3%) [24].

Studies conducted in similar study subjects were showed lower seropositivity than the present study. Tiwan 10.4% [25], Mexico 12.1% for HBsAg [30], USA HCV (9.9%) [18], Greece 2% for HBsAG, 9% for the HCV antibody [32], Chicago 8.5% for HCV anti-body [18], Japan (10.2%) of the patients HCV ant-body [19], another study in Japan (9.1%) for HCV anti-body [20].

Variation in magnitude was due to geographical variation, difference in methodology, and sampling variability. Another reason for the difference in hepatitis epidemiology in these countries, awareness of the routes of transmission, efforts made to implement universal precautions by health professionals and preliminary benefits due to the initiation of national programs of immunization in other countries might explain these discrepancies. The other source of the discrepancy might be due to the potential variability in sensitivity and specificity of the commercially available test kits used in each study, although the studies used in this review were based on an immunoassay based screening kits with more or less similar principle of antibody detection.

Many Studies had done in Ethiopia within different study subject reports low percentages of HBV and HCV when compared to our findings. Study done on blood donors in Bahir Dar for HBV 4.11%, for HCV 2.1% [46], Amhara ,and Tigray Regional states 6.2% for HBV [47], among cleaners in Addis Ababa HBV and HCV 3.57% and 1.59% respectively [48], among ART patient in Addis Ababa of 3 % and 3.6 % for HbsAg and anti-HCV, respectively [49], among visitors at Shashemene General Hospital HBsAg(5.7%) [60], among VCT clients in Addis Ababa HBsAg (5.7%) [61]. Another community based sero-epidemiological survey that addresses the transmission dynamics and control of HBV in Addis Ababa, reported HBsAg prevalence of 7.0% from the general population [62],among pregnant women in Jimma HBsAg (3.7%) prevalence was also reported [63].

Similarly the finding of this study was in dissimilar with studies done in different subjects (blood donors) in other countries concerning to HBV, lower burden was reported from south Dar fur 6.25%[42], Tete Mozambique 10.6%[43], kano Nigeria 11.1% [44], Ibadan Nigeria 5.9% [45], Akure Nigeria 7.4% [46], Quảng Trị Vietnam 11.1% [47].

This difference may be due to people with severe mental illness appear to have increased rates of sexually transmitted diseases (STDs), and they are likely to engage in high-risk behaviors such as using injection drugs, having multiple sexual partners and high-risk partners, infrequently using condoms, engaging in same-sex sexual activity, trading sex for money or drugs, and engaging in sex while using psychoactive substances [14].

In contrast with our study, studies done in our country in different subjects, higher seropositivity, 35.8% HBsAg and 22.5% HCV Ab seropositivity was reported previously from Ethiopia May 2013 among Chronic liver disease patients [48].

When the finding of the current study were compared with results reported from different study subjects of other countries higher, 44.6% HBsAg and 26% HCVAbs in India among chronic liver disease patients [49], 42.9% Gana among CLD [50], 36% HBsAg % and 24% HCV Antibody in Zimbabwe among HCC patients [51]. The higher sero-burden in CLD than psychiatric patients in these different groups may be due to disease itself may cause by these viruses.

Age distribution of psychiatric patients in our study population showed that all of psychiatric patients' cases were above twenty years of age. 19.8% of patients were up to 39 years of age, about 44.0 in third decade and 23.4% in fourth decade and 10.4% in fifth decade of life. A comparable high age distribution (88.8%) of psychiatric participant age ≥20 years was observed by Mexico by Esquivel et al [30]. This may be due to most psychiatric disease occurred above 24 years old [15].

A high prevalence of HBV was recorded in the age group 50-59 with a prevalence of 22.5% (9/40) and HCV it was 22.2% (2/9) in the age group =/>60. Similarly the study in a psychiatric hospital of Mexico by Esquivel et al showed 58.3% HbsAg positive participant was 45 years and older [30], Ibadan Nigeria 69% showed HbsAg positive participant was 40 years and older [45] in Zimbabwe among HCC patients 77% showed HbsAg positive participant was 35 years and older [51]. One reason may be chronic infection with hepatitis

viruses leads to slow progressive liver disease. It may end up in cirrhosis, chronic liver failure and hepatocellular carcinoma over a period of up to 30 years [20].

About 5.7 % (n=22) of the study subjects had HBV and HCV co infection. Comparably the study done by Abel in Addis Ababa,Ethiopia dual infection was observed in 2.5% patients[48], in Mexico 4.8%[30], in USA 3.5%[24] ,in Japan 2.8[20]. This may be due to the similar modes of transmission (sexual contact, exposure to infected blood, body secretions, and breast feeding) [5]

In relation to sex specific prevalence in the present study HBV and HCV prevalence was 18.8% for males and 22.2 for females (P=0.408) and 14.4% for males and 14.2% for females (P=0.951) respectively indicating higher proportion in females than males in seropositivity for HBV. In agreement with our study, the study cared out in Shashemene General Hospital voluntary counseling and testing center Male (7.7%) and Female (3.8%) [38].

In present study, cases of HCV associated psychiatric patients was less common than the HBV associated cases and this higher HBV to HCV pattern is well recognized in Ethiopia for the last few years as many studies have shown on different study populations, in Gondar HBV to HCV was 4.1% and1.3% [44], Amhara ,and Tigray regional state showed that the prevalence of HBV and HCV was 4.1% and 1.7%, respectively [35], Vietnam, the prevalence of CLD due to HBV and HCV was 47% and 23% [46], Eastern India in blood transfused patents seropositivity of HCV (18.70% and HBV (3.33%) were recorded [47].

Different studies reported higher prevalence of HCV than HBV in different study populations the higher prevalence of HCV to HBV infection in the other studies could be explained by geographical variation and with the fact that Hepatitis C virus has a higher propensity for causing liver disease than HBV [20].

Although different risk factors are reported by different researchers for HBV infection, blood transfusion was a significant risk factors for HBV infection among the psychiatric patients with OR of 3.7, 95 % CI: 1.588-8.620, p=0.002 and tattooing on body , with OR of 2.7, 95 % CI: 1.581-4.671, p=0.001. This might be explained by poor screening of blood donors. Correspondingly, this finding agreed with a study conducted in among Hemodialysis Patients in Gaza Strip, Palestine the risk of infection increased sharply if patient received more than 15 blood units for HBV infection, and 10 blood units for HCV infection [48], in Syria high seropositivity, HBsAg 24.5% in multi-transfused patients with was recorded [49], in Egypt

among multi-transfused thalassemic patients, 29.0%, were HBsAg positive with [32], in Eastern India in blood transfused patents was found, HBsAg [27], in the U.S.A. a study performed on people with severe mental illness showed a 23.4% prevalence rate of HBV infection among transfused patients [18] .

In relation to tattooing body might be explained by Tattooing and body arts have become more prevalent in recent years, their popularity increasing among young adults. Such close contact of the tattoo instruments with blood and bodily fluids may cause transmission of viral and bacterial infections if the instruments are used on more than one person without being sterilized. In North America, most cases of hepatitis B infection occur via blood or sexual contact [50].

Other risk factors like, history of STD/STI, dental extraction at health facility, circumcision, surgical procedure, hospital admission, ear piercing, uvuloctomy, tattooing on gum, contact with jaundiced patient, alcohol consumption, abortion, shaving by barber and multiple sexual partners were not associated with HBV infection. Similar to present study in the following studies there were not significantly associated ,in Addis Ababa, Ethiopia, among cleaners [58], Addis Ababa in public hospital Ethiopia in 2013 among Chronic liver disease patients [48], Ibadan, Nigeria among blood donors [45], Brazili, 22.4% among psychiatric patients [22], in Greece among psychiatric patients[32], in Chicago among psychiatric patients [18].

Concerning to HCV multivariate analysis showed a strong association between Multi sexual partner and HCV infection. The overall prevalence of HCV among psychiatric patients who had history Multi sexual partner was 29.0% (COR= 2.7; 95% CI: 1.2-6.3, P= 0.018). Those who had history of Multi sexual partner were 2.7 time more likely to have infection with HBV than their counter parts and the difference was statistically significant (P=0.018).

In agreement with present study, in the study done Oregon State Hospital, (20.3%) for HCV [17], Japan (10.2%) of the patients HCV anti-body with [19], USA for HCV (19.6%) [28] were recorded. Surveillance data also indicate that 15%–20% of persons reported with acute Hepatitis C infection have a history of sexual exposure in the absence of other risk factors [66]. This might be explained by unsafe sex in psychiatric patients.

8. Strength and Limitations of the study

8.1. Strength of the study

To the best of our knowledge, we did for the first time to assess the sero-burden of HBV and HCV infection among the psychiatric patients.

8.2. Limitations of the study

Our study was only set and able to measure the prevalence of HBV and HCV in clinically diagnosed psychiatric patients (in general term) and therefore this study didn't measure the prevalence of these viruses in community level. Being the study groups are psychiatric patients, the information generated from the guardian could have some bias.

9. Conclusion and recommendation

9.1. Conclusion

This study showed that HBV and HCV burden among clinically Psychiatric patients was 20.3% and (n=78), 14.3 %(n=55) respectively. There was no statistical significance difference in acquisition of HBV and HCV infection with respect to socio- demographic characteristics but history of blood transfusion and tattooing on the body was significant risk factors for hepatitis B virus infection and multi sexual partner for hepatitis C virus infection.

HBV and HCV infection is high among Psychiatric patients and those psychiatric patients who had history of blood transfusion and tattooing on the body had 3.7 and 2.7 times higher risk of acquisition of HBV infection than their counter parts. Concerning to HCV, those who had history of multiple sexual partners is 2.7 times higher risk of acquisition of HCV infection than those do not have.

9.2. Recommendation

Since HBV and HCV are major health problem in psychiatric patients, the following recommendations were forwarded:

- All clinically diagnosed psychiatric patients would be considered for testing for HBV and HCV virus with evidence of possible risk factors.
- Vaccination against HBV is instrumental in the prevention of HBV infection, and is recommended for all newborns and individuals who are at increased risk for infection especially psychiatric patients who are active in acute phase of disease towards multiple sex partners.
- Awareness of Patients and guardians of psychiatric patients on modes of transmission of the hepatitis B and hepatitis C viruses is important to prevent viral infection.
- Further large scale studies are required to understand more on the burden and mode of transmission of the viruses in psychiatric patients.

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Annex I: Participant Information Sheet

1. **Participant Information Sheet (English version)**

My name is Endalew Asnake. I am a laboratory technologist and postgraduate student at Addis Ababa University. Now I am conducting a study entitled ***Prevalence of HBV and HCV among psychiatric patients at Amanuel Mental Specialized Hospital, Addis Ababa, Ethiopia***

The patient you care is 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 would like to ask you to sign on a document to show your agreement; participate accordingly, and give clinical specimen (The patient you care).

The topic of this study is *sero-burdun of HBV and HCV among psychiatric patients at amanuel mental specialized hospital Addis Ababa, Ethiopia*. Since *HBC 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 are not interested to participate or if you once decide to participate and withdraw your patient at any time, there will be no consequences 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 neccessarly mean that he/she has the disease.When he/she is found to be positive for the HBV/HCV, he/she will be informed by the health worker and receive 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 will be 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 will be 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 will be used for this study only. Only limited numbers of professional will have access the information. The information will be 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 care 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 are: Investigator: Endalew Asnake (BSc), +251912661769

Email- endo.asnake@gmail.com

For additional information, please contact Addis Ababa University, College of Health Sciences,

Department of Medical Laboratory Sciences at: Telephone +251112755170

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 are and what will happen to my patient in the study.

Guardians' name ----------

Relationship to participant: ----------

Signature---------- Date: ----------

Data collector name: ----------

Signature: ---------- Date: ----------

2. Participant Information Sheet (Amharic version)

ጥናቱ መረጃ ና ተሳታፊነት መግለጫ ቅጽ

የጥናቱ ዓላማ

ሄፓታ ይተስ ‘‘ቢ’’ እና ሄፓታይተስ ‘‘ሲ’’ቫይረሶች በአዕምሮ ህሙማን መካከል ያ ለዉን ስርጭት ለማጥናት የታቀደ ነዉ፡ ፡

በጥናቱ ስለ መሳ ተ ፍ

በዚህ ጥናት መሳተፍ በሙሉ ፈቃደኝነት ላይ የተመሰረተ ነዉ፡ ስለሆነም የሚንከባከቡት የአዕምሮ ህሙማን በጥናቱ እንዲሳተፉ ፈቃደኛነትዎን እንጠይቃለን ፡ለመሳተፍ ከፈቀዱ ፤5ሚሊሊትር የደም ናሙና ከክንዱ/ዷ ተወስዶ የላቦራቶሪ ምርመራ ይደረግለታል/ላታል ፡፡ የላቦራቶሪ ምርመራውም ሄ ፓታይተ ስ ‘‘ቢ ’’ እና ‘‘ሲ’’ ቫይረስን በደም ዉስጥ መኖርና አለመኖር ማረጋገጥ ይሆናል ፡ ፡ ደም ከመወሰዱ በፊት እና ከውጤት በሁዋላ በሰለጠነ ባለሙያ የምክር አገልግሎት ያገኛሉ፡ የደም ናሙናዉም የሚወሰደዉ ንጽህናዉ በተጠበቀ አዲስ እና በታሸገ መርፌ ና ስሪነጅ ነዉ፡ ፡

በጥናቱ ሊከሰቱ የሚችሉ ተያያዥ ችግሮች

5 ሚሊሊትር የደም ናሙናዉን ለመዉሰድ መርፌ ሲገባ ከሚፈጥረዉ የቅጽበትየ ህመም ስሜት በስተቀር የጎላ ችግር አያመጣም ነገር ግን ምቾት ካልተሰማው/ት ሀኪም እንዲያይልዎት ይደረጋል ፡ ፡

በጥናቱ በመሳተፍ የሚገኝ ጥቅም

የደም ናሙና የላብራቶሪ ዉጤት ምንም አይነት ችግር ካሳየ የመድሃኒት ትእዛዝና የባለ ሙያ ምክር ይሰጠዋል/ጣታል ፡ ፡

የጥናቱ መረጃዎች ሚስጥራዊነት

በጥናቱ ውስጥ የተሰበሰቡ ማናቸዉም ግላዊ መረጃዎች ሚስጥራዊነታቸዉ የተጠበቀ ይሆናል ፡፡

ከማንነትዎ ጋር በቀጥታ ተያያዥነት ያላቸዉ መረጃዎች በሙሉ በዋና ተመራማሪዉ ሚስጥራ ዊ በሆነ የመረጃ ጥንቅር ዘዴ ከተቀየሩ በኃላ ብቻ ለምርምር ሂደቱ የሚዉሉ ይሆናሉ ፡፡

የጥናቱን ዉጤት ስለ ማሳወቅ

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

ከጥናቱ ስለ መዉጣ ትና ስለ ማቋረጥ

ይህ ጥናት በፈቀደኝነት ላይ የተመሰረተ እንደ መሆኑ መጠን በማንኛዉም ወቅት በፈቃድዎ የሚንከባከቡትን ህመምተኛ ከጥናቱ ማስወጣት ይችላሉ ፡ ፡ ከጥናቱ ቢያስወጡትም እንኳን የተለመደዉን የህክምና እርዳታ በጤና ተቋ ሙዉስጥ በማንኛዉም ጊዜ የማግኘት መብት አለው/ላት

Annex II: Consent form

1. **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.

Guardians' name ----------

Relationship to participant: ----------

Signature---------- Date: ----------

Data collector name: ----------

Signature: ---------- Date: ----------

2. Consent form (Amharic version)

ስለ ስምምነቱ ማረጋገጫ ፊርማ

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

እኔ ---------- በጥናቱ ተሳታፊ /ተንከባካቢ መሆኔን በፊርማዬ እያረጋገጥሁ ይህንን ስወስን በጥናቱ ሳቢያ ሊከሰቱ የሚችሉ አደጋዎች በሚገባ የተረዳሁና ከጥናቱ በማንኛዉም ደረጃ የምንከባከበው ህመምተኛ ለመሰረዝ ብወስን ተገቢ የሆኑ ህክምናዎችና እገዛዎች ሁሉ እነደማይነፍጉብኝ በማመን ነዉ።። እነዚህ መረጃዎች ሁሉ በሚገባ በምረዳዉ ቋንቋ የተገለጸልኝ መሆኑን በፊርማዬ አረጋግጣለሁ ።።

የተነከባካቢው ሙሉ ስም---------- ፊርማ----------

የተሳታፊው ሙሉ ስም----------ፊርማ----------

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

የምስክር ሙሉ ስም ----------ፊርማ----------

ይህን ጥናት በተመለከተ ጥያቄ ቢኖርዎት ወይም ከዚህ ጋራ በተዛመደ መልኩ ስለሚያጋጥመዎት ድንገተኛ ችግር በሚከተለዉ አድራሻ ይጠቀሙ።።

ሞባይል+251912661669

የሕክምና ላብራቶሪ ሳይንስ ትምህርት ክፍል፤ የጤና ሳይንስ ኮሌጅ፤ አዲስ አበባ ዩኒቨርሲቲ

ኢ-ሜይል፤ endo.asnake@gmail.com

ለተጨማሪ መረጃ፡ አዲስ አበባ ዩኒቨርሲቲ ፤ የሕክምና ላብራቶሪ ሳይንስ ት/ክፍል ይጠይቁ።። ስልክ+251112755170

Annex III: Questionnaires

1. **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----------

1.4. Types of Psychiatric cases A. Depression B. Schizophrenia C.Bipolar

2. Back ground information

2.1. Residence A. Urban □ B. Rural □

2.2. Current occupational status

A.Self-employed □ B. Diver □ C. House Wife □ D. Student □ F. Other specify □

2.3. Religion

A.Chrsthian □ B. Muslim □ C. doesn't have □ D. others □ specify…….

2.4. Marital status

A. Maried □ B. single □ C divorced □ D. widowed □

2.4. Ethnicity

A. Amhara B. oromo □ C. Tgray □ D. Gurage □ E. Wolayta □
G. others □specify……..

2.5. 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 Multiple sexual pratener A. yes □ B. No □

3.3. Blood transfusion A. Yes□ B. No □

3.4. Abortion A. yes □ B. No □

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

3.6. Circumcision A. yes □ B. No □

3.7. Hospital admission A. yes □ B. No □

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

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

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

3.11. Ear piercing A. yes □ B. No □

3.12. Nose piercing A. yes □ B. No □

3.13. Uvuloctomy A. yes □ B. No □

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

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

3.16. Shaving A. yes □ B. No □

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

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

2. Questionnaires (Amharic version)

አዲስ አበባ ዩኒቨርስቲ

የህክምና ፋኩልቲ

የሚዲካል ላቦራቶትሪ ትምህርት ክፍል

ለመረጃ ስብሳቢዎች ፤ ጥያቄዉን ከጠየቃችሁ በኋላ መልሱን በተሰጠዉ ሳጥን ውስጥ ከተሰጡት አማራጮች አንዱን የከኤስ ምልክት ይጻፉ ፡፡

ጠ ቅ ላ ላ ጥ ያ ቄ

1.1. ኮድ----------

1.2. ፆታ ሀ. ወንድ □ ለ . ሴት □

1.3. እድሜ----------

1.4. መኖሪያ አካባቢ ሀ. ገጠር □ ለ . ከተማ □

1.5. ሥራ ሀ. የግል □ ለ . ሹፌር □ ሐ. የቤት እመቤ ት □

መ. ተማሪ □ ሠ. ገበሬ □ ረ. ሌላ □(ይገለጽ)---

1.6. ሃይማኖት ሀ. ክርስቲያን □ ለ. ሙስሊም □ ሐ.የለም□ መ.ሌላ□ (ይገለጽ)…….

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

1.8. ብሔር ሀ. ኦሮሞ □ ለ. አማራ □ ሐ.ትግሬ□ መ. ጉራጌ □ ሠ. ወላይታ ረ . ሌ ላ □

1.9 የአዕምሮ በሽታው አይነት ሀ. ስኪዝኦፍሪኒያ □ ለ. ድባቴ □ ሠ. ባይፖላር□

2.ሄፓታይተስ ‘‘ቢ ’’ እና ‘‘ሲ ’’ን በተመለከተ ጥያቄዎች

ከዚህ በታች ያሉትን በህይወት/ዋ አድርጎ /ጋ ያውቃል/ታውቃለች ?

2.1 ያባለዘር በሽታ ሀ. አዎ □ ለ.አይደልም □

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

2.3 ደም መቀበል ሀ. አዎ □ ለ. አይደልም □

2. 4 ማስወረድ ሀ. አዎ □ ለ. አይደልም □

2.5 ጤና ድርጅት ጥርስ ማስነቀል ሀ. አዎ □ ለ. አይደልም □

2.6 ግርዛት ሀ. አዎ □ ለ. አይደልም □

2.7 ሆስፒታል መተኛት ሀ. አዎ□ ለ. አይደልም □

2.8 ማንኛዉም አይነት ቀዶ ጥገና ሀ. አዎ□ ለ. አይደልም □

2.9 ሞኝ ባገኝ መበጣት ሀ. አዎ□ ለ. አይደልም □

2.10 በልምድ አዋላጅ መዉለ ድ ሀ. አዎ□ ለ. አይደልም □

2.11 ጆሮ መበሳት ሀ. አዎ□ ለ. አይደልም □

2.12. አፍንጫ መበሳ ት ሀ. አዎ□ ለ. አይደልም□

2.13. እንጥል ማስቆረጥ ሀ. አዎ □ ለ. አይደልም

2.14 ሰዉነት መነቀስ ሀ. አዎ□ ለ. አይደልም □

2.15. ድድ መነቀስ ሀ. አዎ □ ለ. አይደልም □

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

2.17ወፍ ከያዘዉ ሰዉ ጋር ንክኪ ሀ. አዎ □ ለ . አይደልም □

2.18. አዘውትሮ መጠጥ መጠጣት ሀ. አዎ □ ለ. አይደልም

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 [64].

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 [64].

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 [64].

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 [64].

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 [64].

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 [64].

WORKING WASH BUFFER

Dilute the 20X wash buffer concentrate with deionized or distilled water 1:20. For example, 5 ml of wash buffer concentrate should be diluted to a total volume of 100 mL with deionized or distilled water [64].

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 [64].

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 [64].

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): [64].

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 [64].

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 [65].

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 [65].

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 [65].

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 [65].

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 [65].

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 [65].

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 [65].

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 [65].

Annex IV: Annex 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: - Kassu Dasta, Assistant Professor

Signature: _____

Date: _____

Name: - Mintwab Hussen, Lecture

Signature: _____

Date: _____