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Assessment of Hepatitis B Sero prevalence , and associated factors among adult outpatients visiting selected Health facilities in Ethiopia.

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This is to certify that the thesis prepared by Abdurahman Shewmolo Bedru entitled " Assessment of Hepatitis B Sero prevalence , and associated factors among adult outpatients visiting selected Health facilities in Ethiopia" was submitted in partial fulfillment of the requirements for the Degree of Masters of Sciences in Clinical Laboratory Sciences (Diagnostic and Public Health Microbiology) complies with the regulations of the university and meets the accepted standards concerning originality and quality.

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Chair of Department of Graduate Program Coordinator

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Abbreviations

AAU	Addis Ababa University
CI	Confidence Interval
CMIA	ChemiluminuscentMicroparticle Immunoassay
DCs	Dendritic cells
DNA	Deoxyribonucleic acid
DRERC	Department of Research and Ethical Review Committee
EIA	Enzyme Immunoassay
ELISA	Enzyme-Linked Immunosorbent Assay
HBV	Hepatitis B Virus
HBsAg	Hepatitis B Surface Antigen
HBeAg	Hepatitis B Envelope Antigen
HBcAb	Hepatitis B Core Antibody
HBcAg	Hepatitis B Core Antigen
HCC	Hepatocellular Carcinoma
IFN	Interferon
ICT	Immunochromatography technique
IgM	Immunoglobulin M
MCH	Major Histocompatibility complex
NAT	Nucleic Acid Test
OD	Optical density
PCR	Polymerase Chain Reaction
RNA	Ribonucleic acid
SOP	Standard Operating Procedure
SPSS	Statical Package for Social Science
TLRs	Toll like receptors
TMB	Tetramethylbenzide
WHO	World health organization

Abstract

Background: Hepatitis B is a potentially life-threatening liver infection caused by the Hepatitis B virus and is the most serious type of viral hepatitis. Hepatitis B is a widespread infectious disease throughout the world. Hepatitis B virus is a DNA virus that causes acute and chronic hepatitis in humans. HBsAg is the main clinical marker indicating acute or chronic infection.

Objective: To assess the sero prevalence of Hepatitis B Virus, and associated factors among adult outpatients visiting selected Health facilities in Ethiopia.

Methods: Health facility-based cross-sectional study was conducted from May 2021 to October 2021 among Anitinatal care, Anti retrovirus therapy and adult outpatients visiting health facilities in selected towns of Ethiopia. A total of 3398 participants were selected for the prevalence study, and 1400 participants were selected for the risk factors assessment study from Addis Ababa, Ambo, Asela, and Batu towns. Blood samples were collected as part of routine medical services, and Leftover serum was tested for HBsAg using an enzyme-linked immunosorbent assay. All positive results within 10 % gray zone margins of OD were repeated for the same ELISA test and immunoassay. Data were analyzed using SPSS version 25 statistical package software.

Results: Of 3398 study participants 2406 (70.9%) were females. The mean and median age was 34.10 and 30 years respectively. The overall HBsAg prevalence was 7.42% (95% CI = 6.6 -8.3) (252/3398), without showing any significant difference in sex and age category. Of 1400 sub population which we assessed the risk factors for acquisition of HBV, 72.4 % (1013) were female and 80 .1 % (1121) live in urban area. Rural population were 2.25 times more likely to be infected by HBV than people from urban (OR = 2.248; 95% CI 1.37 -3.69, P = .001). More over family history of viral hepatitis were found to be the strongest predictors of HBV infection (OR = 3.84 (95% CI: 1.73-8.52), *P* =.001).

Conclusion: The sero prevalence of HBSAg in this study was moderately high. The prevalence was higher among rural residence and family history of HBV. Therefore, there should be organized health education emphasizing on rural population and screening of family member infected with HBV.

Keywords:-Hepatitis B virus, ELISA, Seroprevalence, Risk factor, and 0Ethiopia.

1. Introduction

1.1 Background

Viral hepatitis is a widespread infectious disease throughout the world [1]. Hepatitis B virus is the most serious type of DNA virus that causes acute and chronic hepatitis in humans which is a potentially life-threatening liver infection [2]. Chronic infection can cause liver cancer which becomes the second most common cause of death among cancer disease globally [3].The virus is highly infectious and can be transmitted from mother to child or via contaminated body fluid exposure such as unprotected sex, contaminated medical equipment, and blood donation [4]. It remains the leading cause of liver cirrhosis and hepatocellular carcinoma (HCC). Most the HBV-infected persons remain asymptomatic for long periods but are at risk of progressive liver disease and can transmit the virus to other susceptible individuals [5].

Hepatitis B virus (HBV) belongs to the *Orthohepadna* genus of the *Hepadnaviridae* family. It is primarily a concerted action of interferon-γ (IFN-γ) and cytolytic CD8+ T cells that target infected hepatocytes during acute phase of infection. Interferon's have an important role during acute HBV infection as the infected hepatocytes begin production of IFN-α/β that inhibits viral packaging. Innate sensing of viruses can occur through TLRs and cytosolic sensors that recognize viral DNA and RNA [6].

Immune responses involved in viral clearance comprise both humoral and cellular immunity. Major histocompatibility- complex (MHC) class II–restricted, CD4+ helper T cells contribute to generation of antibodies against viral envelope antigens that clear circulating virus particle. MHC class I–restricted, CD8+ cytotoxic T lymphocytes eliminate infected cells. HBV has the ability to stimulate the interferon pathway of the innate immune response during the early stage of infection. Cytokines have also been reported to control HBV replication in the transgenic mouse model, including interleukin 12 (IL-12) and IL-18, the effect being mediated by induction of IFN-γ and IFNα/β respectively [7].

The antigens on the surface of the virus are Hepatitis B surface antigen (HBsAg), Hepatitis B core antigen, and Hepatitis B envelope antigen consequently Antibodies which is immunological markers of the infection against HBV proteins are Anti Hepatitis B core antigen, and Hepatitis B envelope antibody is important markers of past or present HBV infection. In a typical Hepatitis B

infection, Hepatitis B surface antigen (HBsAg) will be detected within 2 to 5 weeks before symptoms or jaundice develop [8].

Different methods are used for the diagnosis of Hepatitis including Immunochromatography technique (ICT), Enzyme-Linked Immunosorbent Assay (ELISA), Enzyme Immunoassay (EIA), and Polymerase Chain Reaction (PCR) but ELISA, EIA, and PCR methods are expensive and are used in well-equipped laboratories and major tertiary care hospitals. Rapid diagnostic kits are a number one choice for developing areas because of less demand in expense, technical manpower, and infrastructure. The rapid card test is known to have less sensitivity and specificity than ELISA but some have sensitivity and specificity comparable to ELISA [9]. Among all HBsAg assays ELISA techniques were used for screening because of its effectiveness.

1.2 statement of the problem

In the world, the seventh-highest mortality cause was viral hepatitis in 2013. Hepatitis-related liver cancer and cirrhosis were responsible for 1.4 million estimated deaths per year. Hepatitis B virus was responsible for approximately 47% of these deaths. [3] In 2019 world health organization (WHO) estimates that 296 million people were living with chronic hepatitis B infection which resulted in an estimated 820 000 deaths, mostly from cirrhosis and hepatocellular carcinoma with 1.5 million new infections each year [10].

Worldwide viral hepatitis related mortality in absolute terms increased by 63% between 1990 and 2013, while the associated disability adjusted life years increased by 34% during this time. This global increase is largely the result of inadequate prevention measures combined with population growth in hepatitis endemic areas. An estimated 13 to 14 million people in the WHO European region are chronically infected with hepatitis B and about 36,000 people die every year as a consequence. In Europe, chronic HBV infection is a major cause of liver cirrhosis and 10–15% of hepatocellular carcinoma (HCC), cases is attributed to chronic hepatitis B (CHB).[11]

In African Region, hepatitis B is highly endemic and probably affects an estimated 5–8% of the population, mainly in West and Central Africa. [12] Viral hepatitis is also a growing cause of mortality among people living with HIV. About 2.6 million people living with HIV are co-infected with the hepatitis B virus. In Sub-Saharan Africa over 60 million people live with chronic hepatitis B, and 4.8 million of them are under-five children. The African prevalence of hepatitis B infection is estimated at 6.1%.[8]According to one systematic review and meta-analysis which do on articles between 2005 and 2020 the east African hepatitis B burden range from 1.05% (95% CI = 0.845 to 1.278%) to 20.896% (95% CI = 16.188 to 26.260%) with an overall pooled prevalence of 6.025% (95% CI = 5.414 to 6.667%) [13].

In Ethiopia, different studies came up with diverse prevalence rates. A recent systematic review and meta-analysis showed a ranged prevalence from 1% to 36% with an overall pooled prevalence of 6% (95% CI: 5 to 6%) [4]. Previous studies included a small sample size and one group of patients which may not tell us the real burden of HBV. To know the prevalence of the HBV we have included a diverse group of patients with relatively large sample size and the real burden could be estimated.

1.3 Significance of the study

To valuable information about hepatitis B infection in Ethiopia and, this study could have much importance for policy makers and health care workers to understand the current distribution of HBsAg among various group of patients. The findings could be useful to design intervention with regard to HBV infection. The data also represent different parts of Ethiopia and the result could be vital to estimate the current prevalence of HBV in Ethiopia.

2. Literature review

2.1. Seroprevalence of Hepatitis B virus

Viral hepatitis accounts for a significant global disease burden and a caused 1.1 million deaths from liver cancer and cirrhosis. According to a 2019 WHO report, 296 million people were living with chronic hepatitis B virus infection. New estimates show that about 1.5 million people newly acquire hepatitis B infection each year [14].

A Systematic review in 2016 on data published from 2005 to 2015 on the prevalence of hepatitis B and C in European (EU) and European Economic Association (EEA) countries on the general population and subpopulation like blood donors, pregnant women, injectable drugs user, migrants, prisoners, and homosexuals. After the full-text screening, a total of 125 articles were considered for inclusion: 48 on the general population, 32 on pregnant women, 32 on prisoners, and 13 on men who have sex with men (MSM). 211 prevalence data were identified, ranging from 0 to 33 estimates per country. 13 countries estimated HBV, general population, prevalence ranged from 0.1% to 4.4% in Ireland and Romania respectively. High-quality estimates for HBV prevalence in pregnant women were available for seven countries, ranging from 0.1% in Norway and Spain to 0.8% in France and Italy. For HBV, the prevalence in homosexuals ranged from 0.0% in Estonia and the United Kingdom to 1.4% in France. Prisoners HBV prevalence ranged from 0.3% to 25.2% in Ireland and Bulgaria respectively. Among blood donors, the prevalence of HBV ranged from 0.0% in Luxembourg and Finland to 3.2% in Bulgaria. Injectable drugs user National HBV prevalence estimates were available for seven countries and ranged from 0.5% in Ireland, Croatia, and Hungary to 6.3% in Portugal. The estimated prevalence of HBV for the general migrant population was available for five countries. Based on general population and blood donor estimates, the HBV prevalence in the EU/EEA as a whole is estimated to be 0.9% [15].

A cross-sectional study was conducted in Brazil from August to December 2016 in adults living in poverty. The study estimated the overall prevalence rate of HBV was 9.8% [16].

A cross-sectional study carried out in France in 2016 with the aim to estimate the seroprevalence of HBs antigen (HBsAg) and self-reported HBV vaccination history showed a 1•4% total prevalence. Variables associated with HBsAg positivity were being born in a moderate or high endemic zone or precarious housing. Their results suggest that drug use plays a small and

substantial role, respectively, in HBsAg carriage in people who use the drug (PWUD) born in high/moderate and low endemic zones [17].

In a systematic review and meta-analysis of 27 studies on Hepatitis B infection in the general population of China from 2013 to 2017 the pooled estimated prevalence of HBV infection was 6.89%. The overall prevalence of HBV infection in males (5.88%) was higher than that in females (5.05%), and rural areas (5.86%) which are higher prevalence than urban areas (3.29%). And also HBV infection in adults older than 20 years was approximately 7%, which was higher than that in children [18].

A cross-sectional prospective study conducted in Thailand and published between 1975 and 2015 was reviewed systematically. A high difference in prevalence estimates was observed. For the general population, the pooled prevalence estimate was 5.1% which would translate to an estimated number of individuals with CHB living in Thailand in 2015 as high as three million [19].

According to a Pakistan article review study in March 2011, there are an estimated 7-9 million carriers of hepatitis B virus (HBV) with a carrier rate of 3-5%. The prevalence of hepatitis B virus infection in the general population was 4.3%, healthy blood donors (3.9%), military recruits (4.3%), healthcare persons (3.3%), pregnant women (5.872%), prisoners (5.8%), surgical patients (7.4%), patients with cirrhosis (28.9%), patients with HCC (22%), patients with hepatitis (15.9%), patients with liver diseases (27.6%), multiply transfused patients (6.2%), ophthalmic patients (3.9%) and users of Injectable drugs (14.9%). Genotype D (63.7%) is the most prevalent genotype in the Pakistani population [20].

Another cross-sectional study conducted in India in 2018 shows the prevalence of HBsAg was 21.9% with 22.6% in females and 20.9% in males. This study has reported hyperendemic (21.9%) seroprevalence of HBsAg with an infectivity rate of 24.4%, more in the young and reproductive population [21].

A cross-sectional study conducted in October 2016 among the general population in Nigeria showed a total prevalence of 12.2%. , more than half of the participants, 527 (54.6%), had evidence of previous exposure to HBV. Only 76 (7.9%) showed serologic evidence of immunity to HBV through vaccination [22].

Another systematic review and Meta-analyses conducted in Nigeria from 2000 to 2013 on 46 studies showed a prevalence of 14.0% for blood donors, 14.1% for pregnant women attending antenatal clinics, 11.5% for children, 14.0% among adults, and 16.0% for studies evaluating adults and children with 13.6% pooled prevalence [23].

A systematic review and meta-analysis study from 2015 to 2019, in Ghana assessing the prevalence of HBV among the Ghanaian populace, estimated the prevalence of 8.36% for the adult population, 14.30% for the adolescent population, and 0.55% for children less than five years (pre-school). HBV infection prevalence in adults was the highest in the special occupation group (14.40%) and the lowest in blood donors (7.17). Across the country, the highest HBV infection prevalence rates were recorded in the age group of 20–40 years [24].

Epidemiological surveys with the cluster sampling method conducted in Urban and Rural Populations in Northern Gabon, Central Africa in 2009 showed a significantly lesser prevalence of hepatitis B virus surface antigen in rural than in urban with 7.6% and 12.9% respectively [25].

A cross-sectional study, which assessed the prevalence of HBV infection, from 2015 to 2018 at CHR-Sokodé and USP of Ogaro, in Togo, came up with a 20.33% (610/3000) overall prevalence of HBV. The highest prevalence (17.50%) was shown in an urban zone [26].

East Africa's systematic review and meta-analysis of hepatitis B virus (HBV) infection in articles from 2005 to 2020, 2021 revealed a widely varied prevalence ranging from 1.05% to 20.896%. The pooled prevalence among the population was 6.025% (95% CI = 5.414 to 6.667%) with a heterogeneity of 97.55% ($P > 0.0001$) [13].

According to an Ethiopian systematic review and meta-analysis conducted in 2019. There was a wide variation in the estimate of HBV prevalence ranging from 1% in the Amhara region to 36% in Addis Ababa city. Based on the random-effects model, the overall pooled prevalence among 106,125 was 6% (95% CI: 5 to 6%) with heterogeneity index (I2) of 97.77% ($p < 0.001$) [4].

Another retrospective study was conducted from January to February 2019, on laboratory logbook registered results, from January 2016 to December 2018 for three years period in Bahir Dar Ethiopia. The overall seroprevalence of HBsAg was 78/2010 (3.9%) [27].

2.2. Factors associated with hepatitis B infection

A survey of 30-cluster sampling method in the southeastern region of Turkey among Rural and Urban populations in 2005, showed a statistically significant difference between urban and rural regions in terms of positivity. The prevalence of HBsAg was 8.2% and 6.2% in rural and urban areas respectively.(OR: 0.74; 95% CI: 0.55-0.98). Family history jaundice was a statistically significant risk factor for positive HBsAg in rural areas (adjusted OR: 2.15; 95% CI: 1.30-3.56) but not for urban (adjusted OR: 1.48; 95% CI: 0.96-2.27) [28].

A cross-sectional study among People Living with HIV (PLWHIV) Attending CTC Mawenzi Regional Hospital Kilimanjaro, Northern Tanzania from March to April 2017 revealed a total prevalence of 8% with a significant association between the living area and positivity. People from rural areas had a high prevalence compared to urban areas (OR 8.71, 95% CI: 1.029 - 73.66) [29].

A case-control study conducted on Risk factors of hepatitis B virus surface antigen carriage and serological profile of HBsAg carriers in Lomé Togo, from October 2016 to March 2017 showed that Carriage of HBsAg was associated with an ethnic group, age group, marital status, mode of contamination knowledge, and HBV serological status knowledge. While there was no statistically significant association between HBsAg positivity and previous hospitalization, surgery, dental care, tattooing, and a neighbor suffering from Hepatitis B [30].

A cross-sectional study was conducted in Rwanda, in 2019 using data from a nationwide HBV screening campaign organized by the Rwanda Biomedical Centre from March to October 2018 on 327,360 populations. The total prevalence was 3.9% (12,865/327,360) (3.9%). The highest positivity of 4.2% was found in the age group 35–44 years, but there was no significant difference odds ratio [OR = 1.057, 95% CI (0.904–1.235)]. Male [OR = 1.348, 95% CI (1.30 - 1.40)], unmarried [OR = 1.092, 95% CI (1.10–1.16)], previous positive TB screening test [OR = 2.352, 95% CI (1.63–3.39)], history of surgical operation [OR = 1.082, 95% CI (1.00 -1.17)], history of traditional operational practices or scarification [OR = 1.187, 95% CI (1.13 -1.24)], and viral hepatitis family history[OR = 1.367, 95% CI (1.21 - 1.53)] were associated in statistically significant manner with HBV infection [31].

A cross-sectional study conducted on seroprevalence and associated risk factors for Hepatitis B Virus infections among apparently healthy pregnant mothers attending ANC in Rubkona primary

health care center in Rubkona County, Unity State, South Sudan, from March 1 to July 29, 2020, resulted in the overall HBV infection Sero-prevalence of 16 (6.8%), 95% CI; 3.8-10.3) and Having history jaundice AOR= 10.91:95%CI (2.6-45.2)], multiple sexual partner history [(AOR= 9.5:95%CI (2.3-39.7)] and history abortion, [AOR= 5.5: 95%CI (1.5-23.5)] to be associated factors of seroprevalence of HBV infection [32].

Cross-sectional observational study Mulago National Referral Hospital, Uganda, antenatal clinic. randomly selected 340 pregnant women attending their first antenatal visit at Mulago Hospital antenatal clinic. We recruited 340 participants. The prevalence of hepatitis B virus infection among pregnant women attending the antenatal clinic in Mulago Hospital, in our study, was 2.9% [33].

Another Institution-based cross-sectional study was conducted on magnitude and risk factors with HBV Infection among expectant mothers Presented to Antenatal Care Clinics in North Ethiopia Adigrat from February 1 to March 30, 2019; as a result, the overall prevalence of hepatitis B virus infection among the study participants were 9.2%. 46.7% of HBV-infected study subjects were from the age group 25-34 years. The study established that marrieds were more infected by hepatitis. Abortion history [OR = 0.12 (95% CI: 0.03, 0.47), P < 0.01] and tattoo history [OR = 0.21 (95% CI: 0.07, 0.62), P < 0.01] were found to be statistically significantly associated with the magnitude of HBV infection among expectant mothers [34].

Institution-based cross-sectional study was conducted from January to April 2019 in the South Ethiopia region Gedeo Zone among 479 pregnant women who visited health facilities. The study estimated 9.2% HBV prevalence moreover blood transfusion history [AOR = 5.2, 95% CI: 2.1, 12.5], hospital admission history [AOR = 3, 95% CI: 1.4, 6.6], abortion history [AOR = 4.1, 95 % CI: 1.5, 11.7], age [AOR = 5.1, 95 % CI: 1.5, 18.0], and HIV status [AOR = 8.1, 95 % CI: 1.9, 36.0] had a statistically significant association with HBsAgsero-positivity[35].

Another facility-based systematic sampling technique cross-sectional study was conducted from March 4 to April 4, 2019, among 589 pregnant women in the public health facilities of Jigjiga town, Eastern Ethiopia. Overall, 8.5% (95% CI: 6.5–10.7) of the study subjects were positive for HBsAg. HBV family history [AOR = 4.96, 95% CI (2.11–10.60)], sharp materials sharing history [AOR = 2.78, 95% CI (1.13–6.83)], surgical history [AOR = 3.41, 95% CI (1.26–9.24)],

and having multiple sexual partners [AOR = 6.12, 95% CI (2.12–17.64)] were significant predictors of HBV infection [36].

A community-based cross-sectional study was conducted in Benchi Maji Zone, Southwest Ethiopia, from November 1, 2017-January 30, 2018 indicated that seroprevalence of HBsAg among adults was 55/612 (9.0%) moreover gum tattoo (AOR=23.9, 95% CI (2.2-26.3)), body tattoo (AOR=6.8, 95% CI (1.1-43.1)), jaundiced person contact (AOR=20.7, 95% CI (6.7-63.8)) were significantly associated with seroprevalence of HBsAg[37].

Community-based cross-sectional study conducted on mothers in Gondar, Ethiopia, from March to November 2016 came up with 3.8% sero-prevalence of HBsAg and Statistically significant association result of HBV infection with age (AOR= 6.960, 96% CI, 2.047-23.659, P= 0.002), hospital admission history (AOR= 3.279, 95% CI, 1.054-10.195, P= 0.04), circumcision history (AOR= 4.394, 95% CI, 1.463-13.198, P= 0.008), jaundiced family history (AOR= 3.877, 95% CI, 1.274-11.795, P= 0.017) and abortion history (AOR= 4.867, 95% CI, 1.438-16.473, P= 0.011)[38].

Another cross-sectional study was conducted among 363 pregnant women during routine antenatal clinic visits in West Hararghe public hospitals, Ethiopia from April to May 2017. The overall prevalence of HBsAg was 6.1% (95% CI 3.9-8.5). Abortion history (aOR=4.3, 95% CI 1.3-15.0), tonsillectomy (traditional) (aOR=4.4, 95% CI 1.1-17.8), admission (aOR=4.4, 95% CI 1.2-16.9), multi sex partners (aOR=6.3, 95% CI 1.7-23.4) and familial liver disease (aOR=8.2, 95% CI 2.1-32.8) were showed Statistically significant association with HBV infection. [39]

A cross-sectional study was conducted from February to May 2020 among pregnant women attending ante-natal care (ANC) at Wolaita Sodo Teaching and Referral Hospital. A total number of 252 pregnant women were included in the proposed study. Respondents were asked to complete the questionnaires and offered testing for Hepatitis B surface antigen (HBsAg) detection. Chi-square and odds ratios were used to determine the association between dependent and independent variables. Among all studied pregnant women the prevalence of HBV infection was 7.5% [40].

Another cross sectional study was conducted Mekele Tigray regional state Northern Ethiopia, from August to October 2014 in HIV/AIDS positive adult individuals. Socio-demographic data and other explanatory variables were collected from 508 study participants using pre-tested and

structured questionnaire-based interviews. Serum hepatitis B surface antigen (HBsAg) was detected using commercially available rapid test and third generation enzyme-linked immunosorbent assay (ELISA). A total of 508 study participants, 305 females and 203 males were included. The sero-prevalence of HBsAg was 5.9 % [44].

An institution-based cross-sectional study was conducted in Wolayta Sodo Teaching Referral Hospital from October 15 to December 10, 2017 in ART patients using a systematic random sampling technique. Hepatitis B surface antigen (HBsAg) was detected from serum using an advanced quality one-step rapid test kit. A total of 442 study participants, 187 males and 255 females, were included in this study. Overall prevalence of HBsAg was 37 (8.4%) [42].

Another study conduct on Dessie Referral Hospital and Kemise General Hospital from September 2020 to February 2021 were included in the study include 1283 adults were admitted out of which, 1080 adults have completed measurements and had been taken into consideration for this examination, and others had been excluded from the examination because of exclusion criteria. The overall prevalence of HBV among adults was 27.4% [43].

Hospital based cross-sectional study was conducted from May 2 to July 2, 2014 in Hawassa University Referral Hospital. Samples were taken consecutively to get the calculated sample size of 348 adults living with HIV. Pretested interviewer administered structured questionnaire was implemented. The prevalence was 6.9% [44].

Institution based cross-sectional study was conducted among health professionals at University of Gondar Hospital from January to February, 2015. Self-administered questionnaire was used to collect sociodemographic variables and blood sample was also taken to determine hepatitis B virus sero status. The prevalence of hepatitis B in health professionals at UOG hospital was found to be 4.52% [45].

A cross-sectional facility-based study was conducted among 361 systematically selected pregnant women who received antenatal care in Ambo central Ethiopia, from March 25 and May 10, 2019. Data were gathered through face-to-face interviews and blood samples were taken. Prevalence of hepatitis B virus infection was 4.99% [46].

3. Objective

3.1 General Objective

Assessment of Hepatitis B Sero prevalence , and associated factors among adult outpatients visiting selected Health facilities in Ethiopia.

3.2 Specific Objectives

- To estimate the sero-prevalence of hepatitis B virus among HIV, ANC, and adult outpatients in selected towns of Ethiopia using ELISA, and Immunoassay methods.
- To determine the risk factors of hepatitis B virus infections among HIV, ANC, and adult outpatients in selected towns of Ethiopia.

4. Hypothesis

Ho: there is no difference in the magnitude of HBSAg among age and sex.

Ho: there is no significant association among hepatitis B virus and risk factors.

5. Material and Method

5.1 Study area

The study was conducted in health facilities of several towns found in different regions of Ethiopia. Ethiopia has eleven administrative regions and two self-administrative cities with estimated population were 114,963,588 in 2020 according to UN [46].

Towns in which sample and prevalence data were collected Northern Ethiopia, Debark,and Gonder, North Eastern Ethiopia, Dessie, and kombolcha, South Eastern Ethiopia, Dubti, and Jigjiga, Western Ethiopia, Gambela, South Western &Southern Ethiopia, Jimma, Arbaminch and Jinka, Central Ethiopia, Addis Ababa (Four primary and secondary health facilities), (Adisketema health center, Dagmawi Minilik referral Hospital, Meshualkya health center, and St. Peter specialized hospital), South, and Eastern central Ethiopia, Asela, Batu, and Ambo.

Risk factor assessment data were collected from the following towns. Asela, Batu, Ambo, and Addis Ababa.

5.2 Study design and period

Health Facility-based cross-sectional study was conducted to assess the prevalence of HBV and associated factors for the participant in the prevention of HBV infection in selected centers. The study was conducted from May 2021 to October 202.

5.3 Population

5.3.1 Source of population

All patients attended selected health facilities as part of routine clinical services.

5.3.2 Study population

All patients attended HIV, ANC, and OPD clinics as part of routine clinical services at selected health facilities which fulfilled the inclusion criteria during the study period.

5.4 Eligible Criteria

5.4.1 Inclusion Criteria

(a) All the patients who attend for ANC, and ART, and/or other serological tests

(b) All the patients who have attended adult OPD clinic and sent to the laboratory with LFT tests

(c) Patients with complete socio-demographic and basic clinical data pertinent for their tests.

(d) Patient age above age of 18 years.

5.4.2 Exclusion criteria

Patient not willing to participate in the study

5.5 Study Variable

5.5.1 Dependent variable

Seroprevalence of HBSAg

5.5.2 Independent Variable

Age, sex, occupational and education status, residence, patient type (HIV, ANC and OPD visitors).

5.6 Sample size calculation and sampling method

5.6.1 Sample size calculation

Based on the recent systemic meta-analysis the overall prevalence of hepatitis B virus in Ethiopia is 6% [4]. A 95% level of confidence (z=1.96) and margin of error (d) will be used.

Formula $samplesizen = \frac{z^2 * p(1-p)}{d^2}$

Where n = Sample size α = level of significance z = at 95% confidence interval Z value (a = 0.05) =>Z α/2=1.96 p =Proportion of occurrence of the event estimated 0.2 d = Margin of error at (5%) (0.0012)

$n = \frac{1.96^2 * 0.06(1-0.06)}{0.012^2}$

$= \frac{3.84*0.06(1-0.06)}{0.000144}$

$= \frac{3.8416*0.06*0.94}{0.000144}$

= 1504.6 = 1505 +10% non response rate 1505+151=1656

Calculated sample size were 1656, but to get more representative sample size in the various groups of pateints, 3400 data samples were used for the prevalence and 1400 data for the associated risk facror study were used .

5.6.2 Sampling Method

A consecutive non-probability sampling technique was used to collect the data. All patients visiting the health facility during the sample collection period were included as far as they fulfill the inclusion criteria.

5.7 Measurement and data collection

5.7.1 Data collection

Sex and age data for prevalence were collected from participants record but for risk assessment study each participant was questioned for age, sex, educational status, residence, employment status, and all the risk assessment questions. The questionnaire was prepared first in English, and then translated to Amharic, and other local language, Informed consent was obtained from each subject before recruitment. A face-to-face interview was conducted by the data collector or principal investigator, to fill a questionnaire designated for the study.

5.7.2 Laboratory analysis

5.7.2.1 Sample collection

The sample collection was mainly done for the routine clinical services. Only leftover samples were aliquated into Nunc tubes and stored at -20^{O}C until it transported to Addis Ababa, EPHI laboratory for ELISA and immunoassay. All the samples were collected using an SST tube and let the tube stand at room temperature for 30 minutes, then centrifuged for 15 minutes at 1100-1300 rpm. After the requested tests are completed, left-over (1-2ml) serum samples are stored using Nunc tubes in duplicate (0.5ml to 1ml per Nunc tube). All laboratory tests were done at the Ethiopian Public Health Institute laboratory.

5.7.2.2 Laboratory method

5.7.2.2.1 ELISA Method

Serum samples were tested for hepatitis B surface antigen (HBsAg) at Ethiopian Public Health Institute by using an enzyme-linked Immunosorbentassay (Wantai Hepatitis B Virus Diagnostics kit) according to the manufacturer's instructions with a principle of chromogen solutions containing TMB and urea peroxidase are added to the wells in presence of antibody-antigen-antibody HRP sandwich immune-complex, the colorless chromogens are hydrolyzed by the bound HRP conjugate to a blue-colored product. The blue color turns yellow after stopping the reaction using stop solution. The color intensity can be measured and it is proportional to the amount of antigen captured in the wells and to its amount in the sample respectively. Wells containing samples negative for HBsAg remain colorless.

The results are calculated by relating each specimen absorbance (A) value to the Cut-off value (C.O.) of the plate.

Calculation of the Cut-off value (C.O.) = Nc + 0.06 (Nc = the mean absorbance value for three negative controls).

The test results are valid if the Quality control criteria are fulfilled.

-The A value of the Blank well, which contains only Chromogen and Stop solution is < 0.080 at 450nm.

- The A values of the Positive control must be $\geq$ 0.800 at 450/600-650nm
- The A values of the Negative control must be $\leq$ 0.100 at *450/600-650nm*

If one of the Negative controls absorbance (A) values does not meet the Quality Control criteria, it should be discarded and the mean value calculated again using the remaining two values. If more than one negative control A values do not meet the Quality Control Range specifications, the test is invalid and must be repeated. Based on this assay a result with a value A / C.O. $\geq$ 1, A / C.O. < 1, and *A*/ C.O. = 0.9-1.1 is considered as positive, Negative Results, and border line respectively but all positive and result within 10% gray zone margins OD repeated for the same ELISA test and all 10 % gray zone and positive result from the repeated sample sent to immunoassay.

5.7.2.2.2 Immunoassay method

For all repeated gray zone and positive ELISA results another hepatitis B surface antigen-antibody test was conducted by automated ElectroChemiLuminescence (ECL) technology for immunoassay (Elecsys® hepatitis B surface antigen assay, Roche Diagnostics) according to the Sandwich principle. at first incubation 50 µL of sample, two biotinylated monoclonal anti-HBsAg Antibodies, and a mixture of monoclonal anti-HBsAg antibody and polyclonal anti-HBsAg antibodies labeled with a ruthenium complexa) form a sandwich complex and on the second incubation After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin then the reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier. Results are determined automatically by the software by comparing the electrochemiluminescence signal obtained from the reaction product of the sample with the signal of the cutoff value previously obtained by calibration COI $\geq$ 1 was a positive result for the technique. This study used all the test results from this assay as a final result to determine the prevalence of HBsAg.

5.8 Quality Assurance

The reliability of the study findings was kept guarantee by implementing quality control measures throughout the whole process of research activity. To minimize error, data were collected by trained laboratory personnel and principal investigators entered into a structured Excel sheet daily. At the end of data collection, the principal investigator rechecked all the data entered for completeness, accuracy, and clarity and prepared for SPSS analysis. All laboratory analyses were carried out using standard operating procedures.

5.9 Data analysis and interpretation

Data were entered, cleaned, and analyzed using SPSS version 25 statistical package software (SPSS Inc., Chicago, IL) and analyzed using descriptive statistics, chi-square and binary logistic regression for the assessment of age, sex, and risk factor relationship with HBSAg positivity.

5.10 Operational Definitions

Seropositive: - Refer the person who is positive for hepatitis B virus in both ELISA and Immunoassay test.

Eligible participant: – A person who fulfills the requirement to respond question and give blood sample.

5.11 Ethical clearance

Before starting the research work, ethical clearance were obtained with protocol number DRERC/638/21/MLS from the Departmental Research and Ethics Review Committee (DRERC) of department of Medical Laboratory Sciences, College of Health Sciences, Addis Ababa University, and Regional Health Bureau Public Health Emergency Management and Health Research Directorate with reference number A/A/H 13148/227. A formal letter of cooperation was prepared for all study sites. All eligible risk factor study participants were informed as their participation was voluntary and information obtained at any course of the study will be kept confidential.

5.12 Result Dissemination

This study on completion could serve as reference material to physicians or any health professionals, researchers, experts, and policymakers for intervention. To reach these bodies the finalized paper will be submitted to Addis Ababa University, College of Health Sciences, and Department medical of Laboratory Sciences. So it can serve as a reference in the library. In addition, a copy of this material will be given to sampling collected hospitals. Additional effort will also be made to present at conferences to reach the medical/scientific community and publish the article in reputable journals after the final reports.

6. Results

6.1 HBV Prevalence study

6.2 Socio-demographic characteristics

A total of 3400 participants were approached and 3398 (99.9%) participants were available for analysis. The majority of participants were female 2406 (70.9%) the mean and median age range was 34.10 and 30 years respectively with the age range is 18-88. Of 3398 were 1857 (54.6%) between 18 to 30 years of age, followed by 885 (26%) between 31 and 45 years of age, 471(13.9%) 45-60 years age, and 185 (5.4%) above 61 years of age. (Table 1)

Table 1

Demographic variables of HBV prevalence study among adult outpatients attendant of selected health facilities in different towns of Ethiopia, 2021. N = 3398

Demographic Variables	Frequency	Percent
Age		
18-30	1857	54.6
31-45	885	26.0
46-60	471	13.9
>61	185	5.4
Sex		
Male	992	29.2
Female	2406	70.8
Total	3398	100

6.3 HBV Prevalence

When the prevalence rate of HBsAg was analyzed, out of 3398 study participants tested, 7.4% (252/3398) were positive for HBsAg .The prevalence for men and women were 8.47% (82/992) and 6.98% (168/2406) respectively. Details of prevalence of HBV infection among different age groups 8.08% (150/1857) age 0f 18-30 years, 6.44% (57/885) age of 31-45 years, 8.07% (38/471) 46-60 years, and 3.785 (7/185) >61years age. There was no significant difference between HBsAg status and sex, and age category (Table 2).

Table2: Prevalence of HBV and its association with age and sex among adult outpatients attendant of selected health facilities in different towns of Ethiopia, 2021. N = 3398

Demographic Variable	Frequency (%)	Negative (%)	Positive (%)	p-value
Age				
18-30	1857(54.6)	1707(91.92)	150(8.08)	$X^2(3) = 6.256$, $P = .100$
31-45	885(26.0)	828(93.56)	57(6.44)	
46-60	471(13.9)	433(91.93)	38(8.07)	
>61	185(5.4)	178(96.22)	7(3.78)	
Total	3398	3146	252	
Sex				
Female	2406(70.8)	2238(93.02)	168(6.98)	$X^2(1) = 2.257$, $P = .133$
Male	992(29.2)	908(91.53)	84(8.47)	
Total	3398	3146	252	
Total	**3398**	**3146(92.58)**	**252(7.42)**	

6.4 Risk factors associated with HBV

6.5 Socio-demographic characteristics

Of 3398 total participants risk factors were analyzed for 1400 study participants and 72.4 % (1013) were female, 80.1 % (1121) live in urban area. Their age were ranging from 18- 85 years with a mean age of 35.90yrs and median age of 33yrs. Most variables do not showed significant association with HBsAg status. In risk factor assessment were positivity of HBV infection were 4.7% (21/444) ANC, 3.7% (15/402) ART, and 7.0% (39/554) was OPD patients. While there is significant difference between residence status and HBsAg ($X^2(1) = 10.787$, P = .001). The bivariate logistic regression analyses also proved this with (OR = 2.248; 95% CI 1.37 -3.69, P = .001) which showed people who came from rural area were 2.25 times more likely to be infected by HBV than people from urban (Table 3).

***Table 3**: Demographic variables of risk factor participants and its association with HBSAg among adult outpatients attendant of selected health facilities in Ethiopia, 2021. N = 1400*

Demographic Variable	Frequency (%)	Positive (%)	Negative (%)	P-value
Age				
18-30	633 (45.2)	35(5.5)	598(94.5)	$X^2(3) = .288$, $P = .962$
31-45	452 (32.3)	25(5.5)	427(94.5)	
46-60	250 (17.9)	12(4.8)	238(95.2)	
>61	65 (4.6)	3(4.6)	62(95.4)	
	1400	75	1,325	
Sex				
Male	387 (27.6)	26(6.7)	361(93.3)	$X^2(1) = 1.955$, $P = .162$
Female	1013(72.4)	49(4.8)	964(95.2)	
	1400	75	1,325	
Residence				
Urban	1121(80.1)	49(4.4)	1072(95.6)	$X^2(1) = 10.787$, $P = .001$/
Rural	279(19.9)	26(9.3)	253(90.7)	OR = 2.25 (95% CI: 1.37, 3.67), P =.001
	1400	75	1,325	
Occupation				
Employed	354(25.3)	22(6.2)	332(93.8)	$X^2(6) = 4.136$, $P = .658$
Unemployed	126(9.0)	6(4.8)	120(95.2)	
Student	99(7.1)	3(3.0)	96(97.0)	
Farmer	94(6.7)	7(7.4)	87(92.6)	
Housewife	439(31.4)	19(4.3)	420(95.7)	
Self-Employment	207(14.8)	12(5.8)	195(94.2)	
Merchant	81(5.8)	6(7.4)	75(92.6)	
	1400	75	1,325	
Educational Status				
Illiterate	157(11.2)	11(7.0)	146(93.0)	$X^2(4) = 1.835$, $P = .766$
Read and Write	172(12.3)	9(5.2)	163(94.8)	
Primary	459(32.8)	21(4.6)	438(95.4)	
Secondary	337(24.1)	17(5.0)	320(95.0)	
Higher Education	275(19.6)	17(6.2)	258(93.8)	
	1400	75	1,325	
Department				
ANC	444(31.7)	21(4.7)	423(95.3)	$X^2(2) = 5.534$, $P = .063$
ART	402(28.7)	15(3.7)	387(96.3)	
OPD	554(39.6)	39(7.0)	515(93.0)	
	1400	75	1,325	

6.6 Risk factors analysis

When associated risk factors for HBsAg seropositivity were analyzed, most of the expected risk factors like history of blood transfusion, multiple sexual partners, female genital mutilation/male circumcision, history of surgery, sharing of sharp materials, history of tooth extraction, body tattooing, family history of viral hepatitis infection, and abortion for female participants were assessed. On binary logistic regression analysis for risk factor prediction only family history of viral hepatitis were found to be the strongest predictors of HBV infection (OR = 3.84 (95% CI: 1.73, 8.52), *P* =.001) (Table 4)

Table 4*: Logistic regression and frequency of risk factors on HBV infection among adult outpatients attendant of selected health facilities in Ethiopia, 2021. N = 1400*

Predictive variable		Positive(%)	Negative(%)	COR(95%CI)	P-value
Do you have history of multi sexual partner in life?	Yes	34(5.6)	574(94.4)	1.09(.68, 1.73)	.732
	No	41(5.2)	751(94.8)		
Do you share sharp materials with others?	Yes	12(7.2)	155(92.8)	1.44(.76, 2.73)	.266
	No	63(5.1)	1170(94.9)		
Do you have history of tattooing?	Yes	16(8.0)	184(92.0)	1.68(.95, 2.99)	.076
	No	59(4.9)	1141(95.1)		
Is there family history of viral hepatitis infection?	Yes	8(16.7)	40(83.3)	3.84(1.73, 8.52)	.001*
	No	67(5.0)	1285(95.0)		
Do you have history of tooth extraction?	Yes	15(4.2)	339(95.8)	.73(.41, 1.3)	.281
	No	60(5.7)	986(94.3)		
Do you have a history of blood transfusion?	Yes	7(7.3)	89(82.7)	1.43(.64, 3.21)	.385
	No	68(5.2)	1236(94.8)		
Do you have a history of surgery?	Yes	12(5.8)	195(94.2)	1.10(.58, 2.08)	.761
	No	63(5.3)	1130(94.7)		
Do you have FGM/ Circumcision?	Yes	54(5.8)	876(94.2)	1.32(.79, 2.21)	.295
	No	21(4.5)	449(95.5)		
Do you have a history of abortion? For female participants	Yes	12(4.7)	245(95.3)	.84(.45, 1.58)	.588
	No	37(4.9)	719 (95.1)		

7. Discussion

The study aimed to establish HBV prevalence and associated predicting risk factors in order to fill the data gap for the infection as a result the estimated total prevalence was 7.4% for Hepatitis B virus infection without showing any association with sex and age category. On sub-analysis for risk factor assessment residence showed an association with HBsAg and family history of viral hepatitis was found to be the strongest predictor of HBV infection.

In our study, most of the expected risk factors like blood transfusion, genital mutilation, multiple sexual partners, history of surgery, body tattooing, history of abortion for female participants, tooth extraction, and sharing of sharp materials were found to be statistically insignificant for seropositivity for HBsAg in this study. On the contrary, a study from Jigjiga town, Ethiopia reported that risk factors, including, use sharing of sharp materials, family history, multiple sexual partner, and surgical history associated with HBV infection (36). Additionally some in Mawenzi Regional Hospital Kilimanjaro, Northern Tanzania other factors such as living area people from rural areas had a high prevalence compared to urban areas (29). Another study reported in southern Ethiopia Gedeo Zone history of blood transfusion, and abortion history as factors associated with acquisition of HBV infection (35).

Our hepatitis B surface antigen sero-prevalence study was lower compared to a study report from India (21.9%) (21), Nigeria (13.6%) (23) and (12.2%) (22), Brazil (9.8%) (16), and Ethiopia (9.2%) (34) and (9.0%) (37), the difference might be due to the sample size, sampling and screening method, socio cultural and behavioral practice and other factors.

While it was higher than France (1.4%) (17), Thailand (5.1%) (19) and Ethiopia (3.9%) (27) and (3.8%) (38), this variation could also be a result of difference in the study participants, sample size, sampling and screening method, knowledge, attitude, and practice between the study population and the influence of predictive factors.

However, it is relatively comparable with study done in China (6.89) (18), West Hararghe, Ethiopia (6.1%) (39), and with a systematic review of East Africa (6.025%) (13) and Ethiopia (6%) (4). this could be due to the similarity in knowledge, attitude, and practice between the study participants and other factors.

The chi-squire analysis of demographic characteristics on 1400 subpopulations for risk factors assessment revealed there was a statistically significant association between HBsAg positive results and residence. People who came from rural areas were 2.25 times more likely to be infected by HBV than people from urban (OR = 2.248; 95% CI 1.37 -3.69, P = .001). The study was in agreement with the study conducted in Turkey (OR: 0.74; 95% CI: 0.55-0.98) (28) and Tanzania (OR 8.71, 95% CI: 1.029 - 73.66) (26) though it was contrary to study from Togo (17.5%) (30) and Gabon (12.9%) (25). This variation might be due to differences in life style, economic back ground, and knowledge and practice towards HBV infection risk factors.

Family history of viral hepatitis infection was significantly associated with HBsAg positivity (OR = 3.84 (95% CI: 1.73, 8.52), P =.001), this result was in agreement with study done in Rwanda ([OR = 1.367, 95% CI (1.21, 1.53)]) (31) and Ethiopia ([AOR = 4.96, 95% CI (2.11–10.60)]) (36). This could be as result of lack of knowledge in the transmission of HBV infection and other factors.

8. Strength and Limitation

8.1. Strength

As compared to the previous study conducted the study uses enhanced laboratory methods like ELISA and immunoassay to determine the result for the assessment of the magnitude of hepatitis B virus with somewhat huge number of participant.

8.2. Limitation

Since there was resource and time constraint, data on risk factors were not collected for all study participants.

9. Conclusion

Of the total of 3398 study participant the estimated HBV prevalence was 7.42%. In this study, there was no significant association between HBV with sex and age However, residence and family history of HBV showed significant statistical association with seroprevalence of HBV.

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10. Recommendation

Thus, we recommend provision of routine screening and vaccination service together with accurate information on risk factors such as family history for the transmission of HBV. In addition to this I recommended that the federal ministry of health need to incorporate routine HBV screening in rural populations. Further study is conducted for mostly rural populations. To health care provider who work at HBV counseling and teaching session the people that they should aware of HBV.

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Annex I Consent form or Information sheet for study subjects (English version)

Principal Investigator: Abdurahman Shewmolo. Addis Ababa University College of Health Sciences

Purpose: The purpose of this study is the overall seroprevalence of the Hepatitis B virus in Ethiopia.

Procedures to be carried on: you are invited to participate in the study after giving your consent by giving samples

Risks associated with the study: There is no risk and serious invasive procedure at the beginning as well as at the end of the study and there is no additional time required from you to stay during the study.

Benefits of the study: There will be no financial benefit to you. But the result of this study will benefit the participant by investigating hepatitis B seroprevalence. There will be no compensation for using the sample.

Confidentiality of your information: The results of the laboratory findings will be kept confidential and could only be accessed by the researcher and the responsible physician. There will be no personal information to be attached to your data.

Termination of the study: We will respect your decision if you, later on, change your mind.

Your withdrawal of consent will not affect your right to receive medication.

Based on the above information I agree to participate in the research

Signature: _____ Date: _____

Name of Data collector _____ Signature _____

If you have any question you can ask the principal investigator

Abdurahman Shewmolo Addis Ababa University College of Health Sciences, Department of Medical Laboratory Sciences

Cell phone: +251-913057372 E-mail:www.abdukt25@gmail.com

Annex II: Informed consent form (Amharic version)

የተሳታፊዎች መረጃ ቅጽ

ጥናቱን የሚያጠናው ፤ አብዱራህማን ሸዉሞሎ

በአዲስ አበባ ዩኒቨርሲቲ ጤና ሳይንስ ኮሌጅ የህክምና ላቦራቶሪ ሳይንስ ዲፓርትመንት

የጥናቱ አላማ

የጥናቱ አላማ በኢትዮጵያ ዉስጥ ያለዉ የጉበት በሽታ አምጪ ቫይረስ ስርጭት ምን ያህል እንደሆነ ማጥናት ነዉ። ጥናቱ በአዲስ አበባ ከተማና በሌሎች የክልል ከተሞች ሆስፒታሎችና ጤናጣቢያዎች ዉስጥ ይሆናል። በጥናቱ ወቅት ከእርስዎ የሚጠበቀው በጥናቱ ለመሳተፍ ፈቃደኛ ከሆኑ ለምርመራዉ የሚሆን የደም ናሙና መስጠት ነዉ።

ለጥናቱ ተሳታፊዎች ያለው ልዩ ጥቅም

በጥናቱ ለሚሳተፉ ፍቃደኛ ተሳታፊዎች ምንም አይነት የገንዘብ ክፍያ የለውም ነገርግን ከጥናቱ የሚገኘው ውጤት ለርስዎ ህክምና ተጨማሪ መረጃ ለማግኘትና በዉጤቱም ህክምና ለማግኘት ይጠቅማል::

በጥናቱ ተሳታፊዎች ላይ ያለው ጉዳት

በጥናቱ መጀመሪያም ይሁን መጨረሻ በዚህ ጥናት ላይ በመሳተፍዎ ሊደርስብዎ የሚችል አንድም ጉዳት አይኖርም። በጥናቱ ምክንያት የሚያባክኑት ተጨማሪ ጊዜም አይኖርም።

የመረጃ ሚስጥራዊ አጠባበቅ

የሚሰጡት መረጃ በጥናቱ ወቅትም ሆነ ከዚያ በኋላ ባሉት ጊዜያት ሙሉበሙሉ ሚስጥራዊነቱ የሚጠበቅና መረጃውም የሚያዘዉ በስም ሳይሆን በመለያ ቁጥር ይሆናል። በጥናቱ ላይ ያለ መሳተፍ መብት አለዎት።

ይህ መረጃ በጥንቃቄ የሚያዝ ይሆናል። በመጨረሻም የጥናቱ ውጤት ለሚመለከተዉ አካል ለጥናቱ አላማና ለህክምና ባለሙያዎች ብቻ የሚገለፅ ይሆናል።

ያስታውሱ ፤ስለዚህ ጥናት ማንኛውም ጥያቄ ካለዎት በማንኛውም ጊዜ ከዚህ በታች በተጠቀሱት አድራሻዎች መጠየቅ ይችላሉ።

እኔም የጥናቱ ተሳታፊ ይህንን በመገንዘብ ጥናቱ ላይ ለመሳተፍ ተስማምቼያለሁ።

ፊርማ ----------

መረጃውን የሰበሰበው ግለሰብ ስም----------

ፊርማ ----------

የዋና ተመራማሪው አድራሻ፤

አብዱራህማን ሸዉሞሎ፤ የሕክምና ላቦራቶሪ ቴክኖሎጂ ዲፓርትመንት፣ የጤና ሳይንስ ኮሌጅ፣ አዲስ አበባ ዩኒቨርሲቲ- አዲስአበባ፣ ኢትዮጵያ

ኢ-ሜይል፣www.abdukt25@gmail.com

ስልክ+251-913057372

Annex III: English version Questionnaires

Part one: socio-demographic characteristic

I. Participant code _____

1. Sex 1. Male 2. Female

2. Age in years: _____

3. Residence 1. Urban 2. Rural

4. Occupation 1. Employed 2. Unemployed 3. Student 4. Farmer

5. House wife 6. Merchant 7. Others Specify_____

5. Education Status

1. Illiterate 2. Read and write only 3. Primary (grade1-8) 4. Secondary (grade9-10) 5. Preparatory (grade11-12) 6. Higher level

Part Two: Associated risk factors of hepatitis B viruses infection

1	Do you have history of multi sexual partner in life?	1. Yes.	2.No.
2	Do you share sharp materials with others?	1. Yes.	2.No.
3	Do you have history of tattooing?	1. Yes.	2.No.
4	Is there family history of viral hepatitis infection?	1. Yes.	2.No.
5	Do you have history of tooth extraction?	1. Yes.	2.No.
6	Do you have a history of blood transfusion?	1. Yes	2. No
7	Do you have a history of surgery?	1. Yes	2. No
8	Do you have FGM/ Circumcision?	1.yes	2.No
9	Do you have a history of abortion? For female participants	1. Yes	2. No

D. Health facility information

1. Health facility Name _____

2. The clinic the participant came from

1. ANC, 2. OPD, 3. ART

Annex IV: Amharic Version Questionnaire

<u>ክፍል አንድ ማህበራዊና ስነ ሕዝባዊ መረጃ</u>

I. የተሳታፊ ኮድ _____

1. ጾታ 1. ወንድ 2. ሴት

2. ዕድሜ በዓመት: _____

3. ነዋሪ 1. የከተማ 2. ገጠር

4. ሥራ 1. ተቀጣሪ 2. ሥራ አጥ 3. ተማሪ 4. ገበሬ

5. የቤት እመቤት 5. ነጋዴ 6. የግልስራ

7. ሌሎች ይግለጹ _____

5. የትምህርት ሁኔታ

1. ያልተማሩ 2. ማንበብ እና መጻፍ ብቻ 3. የመጀመሪያ ደረጃ (ክፍል 1 - 8)

4.ሁለተኛ ደረጃ (ክፍል 9-12) 5. ከፍተኛ ትምህርት

ክፍል ሁለት- ለጉበት በሽታ ሊያጋልጡ የሚችሉ ሁኔታዎች (ምክኒያቶች)

1	ከአንድ ሰዉ በላይ የግብረ ስጋ ግንኙነት አድረገው ያውቃሉ?	1. አዎ 2. የለም
2	ስለታማ ነገሮችን ከሌሎች ሰዎች ጋር ተጋርተ ውያውቃሉ?	1. አዎ 2. የለም
3	ንቅሳት ተነቅሰው ያውቃሉ?	1. አዎ 2. የለም
4	በቤተሰብዎ ዉስጥ የጉበት በሽታ ያለበት ሰዉ አለን?	1. አዎ 2. የለም
5	ከዚህ ቀደም ጥርስዎን ተነቅለው ያውቃሉ?	1. አዎ 2. የለም
6	ከዚህ ቀደም ደም ተለግሰው/ ተሰጥዎት ያውቃሉ?	1. አዎ 2. የለም
7	ከዚህ ቀደም ቀዶ ጥገና ተደርጎልዎት ያውቃል?	1. አዎ 2. የለም
8	ተገርዘዋል?	1. አዎ 2. የለም
9	ከዚህ ቀደም ውርጃ ገጥምዎት ያውቃል? ለሴቶች	1. አዎ 2. የለም

የጤና ተቋሙ መረጃ

የጤና ተቋሙ ስም_____

ወደ ላብራቶሪ የተላኩበት የምርመራ ክፍል

1. ኤ.ን.ሲ 2. ኦፒዲ 3. ኤ.አር.ቲ

Annex VI: Oromiffa version of Questionnaire

Kutaa Tokko Oddeeffaannoo Hawaasummaa Fi Ummataa

- Kodii Hirmataa: _____
  1. Saala A.Dhiraa B. Dhalaa
  2. Umurii Waggaan _____
  3. Jireenyaa A. kan magaalaa B. kan Baadiyyaa
  4. Hojii A. Qacaramaa B. hojii dhaba C. Barata D. qotee bulaa E. Hadhaa warraa F. Daldala G. hojii dhuunfaa H. kan biro ibsaa _____
  5. Haala Barnoota A. kan hin barannee B. dubbisuu fi barreessuu qofa C. sadarkaa jalqaba (kutaa 1-8) D. sadarkaa 2ffaa (kutaa 9-12) E. dhabbataa barnoota olaanaa

Kutaa Lama Haaloota (Sababoota) Dhukkubaa Tiruutiif Saaxiluu Danda'an

1	Nama Tokko ol waaliin walqunnamtii saalaa gootaanii beektuu?	1. Eyyee B. Lakkii
2	Meeshaalee qaraa qabuun namoota biro waaliin fayyadamtaanii beektuu?	1. Eyyee B. Lakkii
3	Tumaatii goodhattanii beektuu?	1. Eyyee B. Lakkii
4	Maatii keessaan keessaa dhukkuba tiruu namni qabu jira?	1. Eyyee B. Lakkii
5	Kanan duraa ilkaan keessaan tumaattanii beektuu?	A.Eyyee B. Lakkii
6	Kanan duraa dhignii sinif ergifame/sinif kenname beektuu?	A.Eyyee B. Lakkii
7	Kanan duraa baqasaanii hoodhuun isinif godhame beektuu?	A.Eyyee B. Lakkii
8	Dhaqna qabatanittuu?	A.Eyyee B. Lakkii
9	Kanan duraa ulfi isin irraa bahee beektuu? dubartootaaf	A.Eyyee B. Lakkii

Odeeffannoo dhaabbata fayyaa

Maqaa dhaabbata fayyaa_____

Kutaa qorannoo gara laaboraatoorii ittiin ergame

1. Eensii B. OPD C. ART

Annex IV: Standard operation procedures (SOP)

Materials and Equipment

Supplies and reagents

- Latex gloves
- K2 EDTA test tube
- Disposable syringe
- Cotton
- Automated ELISA analyzer
- Automated ELISA reader
- Fully automated immunoassay analyzer
- ELISA kit
- Micropipette different type
- Micro tips
- Nunc tube

SOP for ELISA

Principle:

The test is an enzyme-immunoassay based on a sandwich principle. Polystyrene microtiter strip wells have been coated with monoclonal anti-HBs(antibody to HBsAg). A patient serum sample is added to microwells. During incubation, the specific immune-complex formed in case of the presence of HBsAg in the sample is captured on the solid phase. After washing to remove serum proteins, a second antibody conjugated to the enzyme HRP and directed against a different epitope of HBsAg is added to the wells. During the second incubation step, these HRP conjugated antibodies will be bound to any anti-HBs-HBsAg complexes previously formed during the first incubation, and the unbound HRB conjugate is then removed by washing. After washing to remove unbound HRP conjugate, chromogen solutions containing TMB and urea peroxidase are added to the wells in presence of antibody-antigen-antibody HRP sandwich immune-complex, the colorless chromogens are hydrolyzed by the bound HRP conjugate to a blue-colored product. The blue color turns yellow after stopping the reaction using stop solution. The color intensity can be measured and it is proportional to the amount of antigen captured in the wells and to its amount in the sample respectively. Wells containing samples negative for HBsAg remain colorless.

ASSAY PROCEDURE

Step1 Reagents preparation: Allow the reagents and samples to reach room temperature (18-30°**C**) for at least 15-30minutes. Check the Wash buffer concentrate for the presence of salt crystals. If crystals have formed in the solution, resolubilize by warming at **37°C** until crystals dissolve. Dilute the stock wash Buffer **1 to 20** with distilled or deionized water. Use only clean vessels to dilute the buffer.

Step2 Numbering Wells: Set the strips needed in strip-holder and number sufficient number of wells including six calibration curve standards wells **(e.g. B1-G1; H1-E2)** and one Blank (**e.g. A1**, neither samples nor HRP Conjugate should be added into the Blank well). If the results will be determined by using a dual-wavelength plate reader, the requirement for use of a Blank well could be omitted. Use the only number of strips required for the test. Run the standards in duplicates.

Step3 Adding Sample: Add **50μl** of Calibration curve standards and **50μl** specimens into their respective wells. **Note: Use a separate disposal pipette tip for each specimen to avoid cross-contamination.**

Step4 Adding HRP-Conjugate: Add **50μl** of HRP-Conjugate Reagent into each well except into the Blank and mix gently. **Never add HRP Conjugateto the Blank well.**

Step5 Incubating: Cover the plate with the plate cover and incubate for **60minutes at 37°C**. It is recommended to use a thermostat-controlled water tank to assure temperature stability and humidity during the incubation. If a dry incubator is used, do not open the door frequently.

Step6 Washing: At the end of the incubation, remove and discard the plate sealer. Wash each well **5times** with diluted Wash buffer. Each time, allow the microwells to soak for 30-60 seconds. After the final washing cycle, turn the plate onto blotting paper or a clean towel and tap it to remove any remainders.

Step7 Coloring: Dispense **50μl** of Chromogen A and **50μl** Chromogen B solution into each well including the **Blank** and mix by tapping the plate gently. Alternatively, Soln A and B can be mixed (1:1 ratio) and then add 100 ul in a single step. Incubate the plate at **37°Cfor 15minutesavoiding light**. The enzymatic reaction between the Chromogen A/B solutions and

the HRP-Conjugate produces blue color in Calibration curve standards wells (except for 0mIU/ml) and in anti-HBs positive sample wells.

Step8 Stopping Reaction: Using a multichannel pipette or manually add **50μl** Stop Solution into each well and mix gently. The blue color will turn yellow after stopping the reaction.

Step9 Measuring the Absorbance: Calibrate the plate reader with the Blank well and read the absorbance at **450nm**. If a dual filter instrument is used, set the reference wavelength at **630nm**. Calculate the results (**Note:** read the absorbance within **10minutes** after stopping the reaction).

Quality control range

The A value of blank well which contains only chromogen and stops solution should be less than 0.080 at 450nm. The A value of positive control must be more than or equal to 0.800 at 450nm The A value of negative control must be less than 0.100 at 450 nm.

Interpretation of the results

Negative Results

Sample giving A value less than cut-off value is negative for this assay which indicates that no HBsAg antibodies have been detected with this HBsAg ELISA kits therefore the patient is probably not infected with hepatitis B virus.

Positive Results:

Samples giving A value greater than or equal to the cut-off value are considered initially reactive which indicates that HBV surface antigen has probably been detected with this HBsAg ELISA kit.

Borderline

Sample with A value to the cut-off ratio between 0.9 and 1.00 are considered borderline samples and retest is recommended. A repeatedly positive sample can be considered positive for HBsAg.

Declaration

I undersigned declares that this research thesis is my original work in partial fulfillment of the requirements for the masters of degree in Diagnostic and Public Health Microbiology, I also declare that it has never been presented in this or any other university and that all resource and material in the proposal have duly acknowledged.

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