Addis Ababa University

College of Health Science

Department of Medical Laboratory Sciences

MAGNITUDE OF HEPATITIS B VIRUS, HEPATITIS C VIRUS AND HIV AMONG FEBRILE PATIENTS ATTENDED HEALTH INSTITUTION AT AWRA AND GULINA DISTRICT, AFAR REGION, ETHIOPIA

By: Rago Edao (BSc)

Advisor: Kasu Desta (MSc, PhD Fellow, Assistant Professor)

Biruk Zerfu (MSc, PhD Fellow)

A Research thesis submitted to the Department of medical laboratory sciences, and college of health sciences, Addis Ababa University, in partial fulfilment of Masters of Science degree in clinical laboratory sciences (diagnostic and public health microbiology)

July, 2021

Addis Ababa, Ethiopia

Addis Ababa University

School of Graduate Studies

This is to certify that the thesis prepared by Rago Edao, entitled, magnitude of hepatitis B virus, hepatitis C virus and HIV among febrile patients attending health institute at Aura district, Afar Region, Ethiopia and submitted in partial fulfillment of the requirements for Master of Science degree in Clinical Laboratory Sciences (diagnostic and public health microbiology) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

Signed by the Examining Committee:

External Examiner_____ Signature _____Date _____

Internal Examiner_____ Signature _____ Date _____

Advisor: Mr. Kassu Desta (MSc, PhD Fellow, Assistant Professor) Signature _____Date_____

Mr. Biruk Zerfu (MSc, PhD Fellow) Signature _____ Date _____

Acknowledgement

First, I would like to thank the almighty God for allowing me to perform this activity. Secondly, I would like to thank Addis Ababa University College of health science, department of medical laboratory sciences for providing the opportunity to develop this research thesis. Thirdly, I express my deepest gratitude to my advisor Mr. Kassu Desta (MSc, Ph.D. fellow, Assistant professor) and Mr.Biruk Zerfu for their invaluable commitment, support, and guidance during the entire development of the thesis.

My special thanks also go to St. Peter specialized Hospital, Laboratory department and all laboratory staff who support my paperwork by affording laboratory kits and assist me during analysis. My deepest gratitude also goes to Mettu blood bank head Mr. Diriba Gemechu and all laboratory staff for their good cooperation and assistance in performing ELISA tests for positive samples.

Abbreviation

AAU	Addis Ababa University
AIDS	Acquired Immunodeficiency Syndrome
ART	Antiretroviral Therapy
CDC	Center for Disease Control
DNA	Deoxyribonucleic Acid
DRERC	Departmental Research and Ethics Review Committee
ELISA	Enzyme Linked Immunosorbent Assay
HBcAg	Hepatitis B core Antigen
HBsAg	Hepatitis B surface Antigen
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HIV	Human Immunodeficiency virus
HRP	Horseraddish Peroxidase
MBBC	Mettu Blood Bank Center
PI	Principal Investigator
PLHIV	People live with Human Immunodeficiency Virus
RNA	Ribonucleic Acid
SOP	Standard Operating Procedure
VMMC	Voluntary medical male circumcision
WHO	World Health Organization

Table of Contents

List of Tables

List of figures

Abstract

Background: Hepatitis B, Hepatitis C, and HIV are among the major public health concerns globally and are highly infectious diseases. During the first six months, most of the infections with viral hepatitis are often asymptomatic; therefore, detection of the virus at the early stage is difficult. The burden of viral hepatitis B and hepatitis C infections and co-infection with HIV still underreported in Ethiopia due to the lack of a coordinated health system and data management inability at the central level. In spite of increasing studies on viral hepatitis and their co-morbidity at different parts of the country, few data exists in Awra and Gulina district of Afar region.

Objective: To assess the burden of hepatitis B & C viruses and HIV among patients attending health facilities and association of viruses with socio-demographic factors and each other virus.

Method: A cross-sectional study was performed among 400 sera that were collected from Kelewani primary hospital and Derayitu health centre of Awra and Gulina district of Afar Region, north-eastern part of Ethiopia from February to May 2019. A pilot study were performed to assess the positive rate of both HCV, HBV and HIV to proceed with the research and continuous monitoring of the refrigerator was checked during the last couple of months to assure sample storage and stability before analysis. The two hepatitis viruses were screened by Anti-HCV and HBsAg rapid test kits, the seropositive sera were subjected to ELISA. HIV was screened sequentially by following HIV rapid test algorithm of Ethiopia; positive sera were confirmed by ELISA. Data were entered, coded and analyzed using SPSS statistical software version 25. A p-value < than 0.05 was considered statistically significant,

Result: A total of 400 sera (59.8% of female) of age ranging from 4-80 years (mean= 25.9, SD = ±11.6) were used for this study. Of all samples 13% (52/400), were sero-positive for one or two viruses, specifically 9.8 %(39/400), 1% (4/400) and 2.3% (9/400) for HBV, HCV and HIV respectively. 0.5% (2/400) was corresponded to the co-infections of HCV-HBV and HBV-HIV. The rates of positivity among both sexes were relatively similar; from the group in female participant sera, 61.5% (24/39), 50% (2/4) and 55.6% (5/9) were accounted by HBV, HCV and HIV respectively. HIV was more common in age category of 30 years and above (COR=7.536, 95% CI=1.543-36.803, p-value = 0.013).

Conclusion: The burden of HBV and HIV at this study area were found high among febrile patients. Similarly, HIV was significantly distributed among elderly febrile patients that may be an indication for these contagious viruses to be circulated in the community.

Introduction

1.1 Background

Hepatitis is an emerging global health problem, which is an infectious and non-infectious agents, such as viruses, alcohol, medications, autoimmune disorders, and metabolic conditions, induce liver inflammation commonly caused by a viral infection. Of these, hepatitis B and hepatitis C infections account for a substantial proportion of liver diseases worldwide (1).

Both HBV and HCV are blood borne pathogens that are spreading predominantly by percutaneous and mucosal contact with blood and other bodily fluids, such as saliva, menstrual, seminal, and vaginal fluids, which considered as a vehicle of transmission. Sexual transmission of hepatitis B among unvaccinated men who have sex with men and heterosexual people with multiple partners of unprotected sex, injection drug use with used needles that have been previously contaminated and transfusion of unscreened blood (2, 3).

According to 2016 WHO reports, 27 million people (10.5% of all people estimated to be living with hepatitis B) are aware of their status and 80-90% of infants infected during the first year of life develop chronic diseases, 30-50% of children infected before 6 years develop chronic infections and nearly 5% of adults otherwise health workers also develop chronic diseases. Around 30% of people infected with hepatitis C spontaneously clear the virus within 6 months of infection without treatment, while the remaining 70% develop chronic hepatitis C virus (4).

However, different studies around the globe suggest that there has been a decrease in the prevalence of HCV since the last half of the 20th century; because most countries have an age-specific prevalence of past and present infection, country surveillance shows a decline in the disease and improved injection safety. Still, an estimated 1.75 million new cases of HCV occur due to unsafe health care practice (including unsafe injection) and unsafe injection drug use (5).

Human immunodeficiency virus is a virus that attacks cells that help the body fight infection, making a person more vulnerable to other infections and diseases. The virus transmitted through contact with infected blood and bodily fluids; such as contact through unprotected sex, through sharing of needles or other drug injection equipment, through mother-to-child

transmission during pregnancy or breast-feeding, and through receipt of infected blood transfusions and plasma products during medical care in some parts of the world (6).

As HIV shares similar transmission routes with HBV and HCV, PLHIV exposed to or have an ongoing exposure to HBV and HCV. Furthermore, the transmission efficiency of HCV increased in the presence of HIV. The spontaneous clearance of both HBV and HCV is less likely in PLHIV, with higher viral loads and rapid and severe disease progression (7).

In Sub-Saharan Africa, around 8%of HIV-positive people infected with HBV. As a result, understanding HBV is critical for guiding appropriate antiretroviral treatment (ART) selection and monitoring/prevention of liver-related problems. Chronic HCV and HIV co-infection hastens the progression of end-stage liver disease, with HCV infection being the primary cause of non-AIDS-related liver disease (8).

Approximately 8% of HIV infected individuals are co infected with HBV in sub-Saharan Africa. Therefore, knowledge of HBV is important to guide optimal selection of antiretroviral therapy (ART) and monitor/prevent liver related complication. Chronic HCV and HIV co-infection also results in an accelerated progression to end-stage liver disease, with HCV infection being a leading cause of non–AIDS-related deaths among HIV+ individuals (6, 8).

Diagnosis of HCV infection was performed by direct and indirect methods. In indirect methods, antibodies such as Anti-HCV IgM for recent infection and Anti-HCV IgG for old infection, in which secretions against Hepatitis viruses were measured. In the direct method, virus antigens were purified and detected by nucleic acid. Rapid immunoassay tests were used for screening and recombinant immunoblot tests in order to confirm HCV infection (12).

HIV infection accelerates the progression of HBV related liver disease via direct interaction of HIV and HBV in target cells such as the hepatocyte and HIV of multiple cells in the liver and increased microbial translocation and elevated lipopolysaccharide (LPS) in the portal and systemic circulations activating Kupffer cell and hepatic stellate cell (HSC) activation and exhaustion of HBV-specific T-cells (9).

HIV diagnosed by detection of antibodies in patient’s serum or plasma representing the presence of viral nucleic acid either by PCR, p24 antigen, or rising viruses in cell culture. Most commonly antibody test is used for the detection of HIV infection. Rapid tests are quick and easy assay devoid of any complex equipment and confirmed by western blot assay. Capillary blood in addition to serum and plasma can be as a sample (10).

The introduction of combination therapy involving the administration of three drugs has the potential to reduce the mortality and morbidity related to HIV-1 infection which plays significant role in revision of immune system, by repressing viral replication and reducing the viral load below the level of detection (50 RNA copies/ml) and measured by elevated level of CD4+ T-lymphocyte (10). Treatment options are available for both viruses to suppress replication and prevent progresssion of liver diseases for HBV and cure infection and sustained virologic response for HCV (11,12). Vaccine is available for HBV to effectively reduce the virus whereas no vaccine developed for HCV (13, 14).

1.2 Statement of the Problem

Viral hepatitis is a serious disease that kills an increasing number of people every year. The illness burden remains considerable because the majority of affected persons are ignorant of their status (15).Because of its widespread prevalence; particularly in rural areas, and the high cost of prevention, management and treatment, hepatitis B virus (HBV) infection is a major burden in most developing nations (16).

Globally, 257 million people estimated to have chronic HBV infections in 2015, with 71 million having chronic HCV infections (2). Infection with the chronic hepatitis B virus ranges from high (8% in Africa, Asia, and the Western Pacific) to medium (2% to 7% in Southern and Eastern Europe) to low (2 % in Western Europe, North America, and Australia)(17). Studies conducted on the prevalence of HCV estimates range from 2.9% in Africa to 1.3% in America (18,19).

There are 37.9 million HIV-positive people in the globe, with 1.5 million new infections per year and 36.2 million adults. By the end of the year, 21% of people will be unaware of their legal standing (20).

Nearly 70 million Africans suffer from chronic viral hepatitis, with just one in ten having access to testing and treatment. As a result, the condition frequently advances to severe liver disease, resulting in the death of the patient (21).

Studies done on viral hepatitis in sub-Saharan Africa show that thesero-prevalence of hepatitis B surface antigen remains high, which is also similar to study conducted in Burkina Faso and Malawi and anti-HCV of the later range from 0.17% to 18% (22,23,24).

Lack of adequate epidemiological information limits planning and monitoring of prevention and treatment programmes tailored to the epidemics with new infections with viral hepatitis do not develop acute symptoms, thus do not come to health care system and therefore underestimate the true number of new infection (18,25).

In Ethiopia, different studies from various groups of populations show the prevalence of the hepatitis virus ranging from low to high, which expresses how much of an impact viral hepatitis have on liver diseases (26,27,28). Despite the work on the diagnosis, treatment and prevention of viral hepatitis and HIV is encouraging as a country, still the burden of the disease is the major challenge for both clinical and community as the whole; therefore, this study aims to address burden of the disease at Aura district of Afar region.

1.3 Significance of the Study

The rational of studying burdens of viral hepatitis and HIV will have practical vital value for patients, health care providers, researchers and policy-makers in the Ministry of Health. This study will help both the community and individual at large on the early detection, diagnosis and treatment.

This study can also influence the country to incorporate hepatitis as one of the emerging causes of public health problems that damage the society in large if they do not give due attention early. The study also provides the public health department to allocate resources that is sufficient enough to combat the diseases and to plan effective preventive program.

2. Literature Review

A retrospective study conducted to determine the prevalence of HBV in Shenzhen People's Hospital, China, from 2015 to 2018 shows 9.69% of positive cases of HBsAg. The male to female ratio is 1.16:1. The infections were almost entirely in the 20–49 age group and 50+ age group, accounting for 73.19% and 25.47%, respectively. The prevalence among the age group of 6-19 is low and less than five years is rare. From which they recommends that all peoples free from the virus should obtain the vaccine (29).

The sero-prevalence of HBV, HCV, and co-infection among patients in an eastern India health institute was investigated 316 (2.92%) of the 10802 samples sent for analysis were HBsAg positive, 115 (1.07%) were anti-HCV antibody positive, and 7 (0.07%) were positive for both HBV and HCV. Anti-HCV antibodies were more frequent in children under the age of ten (30).

The study conducted in the Tertiary Care Hospital of Kolkata, India on serum samples separated and analyzed for anti-HCV antibody 3rd generation ELISA showed eight out of 7897 (0.1%) were confirmed positive for anti-HCV antibody, of which 62.5% were males and 37.5% were females. The highest prevalence was found in the age group of 40-60years (37.5%). One of the patients suffered from both HCV and HBV. They conclude from the study that adequate public awareness by improving the prevailing health care practices and counselling of those affected results in a reduction in the transmission of the virus (31).

According to a study conducted to determine the prevalence of Hepatitis B Surface Antigen, Antibodies to Hepatitis C Virus, and Human Immunodeficiency Virus in a hospital-based population, the prevalence of HBsAg was 0.89%, anti-HCV was 0.28% and anti-HIV antibody was 0.35%. From this study, they conclude that the study throws light on the magnitude of viral transmission in the community in the state of Rajasthan and could be used as a reference in the future (32).

A serological screening conducted to estimate the prevalence of hepatitis B and hepatitis C in patients attending a tertiary hospital in Jalingo, Taraba State, Nigeria showed 70 (13.6%), 85 (16.6%) and 5 (1.0%) were positive for HBsAg, anti-HCV and co-infections, respectively. About 14.7% and 20.1% were the highest gender-based prevalence of HBsAg and anti-HCV respectively, and they were recorded for the male gender. The age group of 21-40 had a high prevalence of 19.2% of HBsAg, whereas the age group of 61 and above had a high

prevalence of 31.3%. From their results, they conclude the augmentation of the existing intervention toward the prevention, diagnosis and treatment of diseases (33).

A cross sectional study conducted to determine the prevalence of HIV, HBV, HCV and syphilis among individuals attending anonymous testing for HIV in Luanda, Angola showed 8.8% (38) were seropositive for HIV-1 and/or HIV-2, 9.3% (40/431) were HBsAg-positive, while 8.1% (35/431) had antibodies to HCV. The rates of co-infections were 2.3% (10/431) for HBV/HIV, 0.9 % (4/431) for HCV/HIV and 0.9% (4/431) for HCV/HB. Three individuals were seropositive for HIV, HBV and HCV (34).

A cross-sectional study conducted to detect HBsAg from randomly collected sera of 200 participants from three public hospitals in Ankpa,kogi state, Nigeria showed 17 (8.5%) were positive for HBsAg with males having a higher prevalence (10.89%) than females (6.06%) and the age group with the highest rate of infection was 21-44years. Patients' occupation and marital status were significantly higher in relation to HBsAgsero-positivity. They recommend the study screening of patients who are coming for routine hospital care (35).

A prospective study conducted in critically ill emergency medical department patients in Istanbul, Turkey, to estimate the sero-prevalence of HBV, HCV and HIV shows a prevalence of 5%, 1.8% and 0.2% for HBV,HCV and HBV-HCV co-infections, with no HIV detected during the study period, which is also consistent with the very low number of reported cases in Turkey (36).

Studies conducted in the general population of Burkina Faso reveal a prevalence of HBV and HCV of 14.7% and 1% respectively. The burden of HBV was more in men (18.58%) than in women (11.06%), from which they conclude that the high prevalence of HBV and low prevalence of HCV warrant enhancing the campaign to reduce the spread of the virus (37).

Study conducted to determine the prevalence of HCV on febrile patient from public and private health institute of Lagos, Nigeria. From a total of 89 blood samples screened for anti HCV ELISA technique, 5(5.61%) were positive for anti-HCV antibody with no significance ($P>0.5$) of sex and other demographic factors, and conclude that public awareness against HCV infection and prevention should be intensified to eradicate future outbreak of cases (38).

A study conducted on the sero-prevalence of HCV and HIV co-infection in sub-Saharan Africa involved 33 African countries revealed an overall pooled sero-prevalence of 2.98% of HCV with a burden of difference in different groups of the population. The pooled prevalence was 11.87% across all high risk groups and 5.73% among the HIV-infected population (39).

A systematic study conducted on the epidemiology of HCV in Djibouti, Somalia,Sudan and Yemen which shows a pooled prevalence of 0.9%,1% and 1.9% in Somalia, Sudan and Yemen respectively with 0.3% of the only study done in general population of Djibouti in blood donors; with different prevalence among the risk groups (40).

A study conducted on the prevalence of HBV and HCV infections among military personnel at the Bahir Dar Armed Forces, reveals that 4.2% and 0.2% prevalence respectively, with no co-infection detected. High prevalence of HBsAg was diagnosed in the age group of over 40 (COR=7.6, 95%CI=2-29, p-value=0.0003). From this, they conclude that strengthening HBV screening strategies among military personnel to reduce the disease is the best way to go (41).

A study conducted to determine the prevalence of HCV and HIV infection among voluntary counseling and testing attending private health facilities in Bahir Dar showed a 2.1% and 3.7% prevalence respectively. The age group 41-50 was significantly associated with HCV (AOR = 65.65; 95% CI 4.57–943) and married participants revealed a significant association with HIV infection (AOR = 7.92, 95% CI 1.32–47.31) from which they conclude that large scale research is required to elaborate on potential factors associated with the viruses (42).

A study conducted to determine the prevalence of HBV and HCV showed that, from 765 participants, 2.6% and 16.2% were found respectively. High frequency was found (17.8%) in males followed by females (14.1%). Similarly, HBV and HCV were found to be more common among married individuals (17.1%) compared to unmarried (13.5%). The superior frequency of HBV and HCV was recorded in patients in the age group of greater than 60 years (40%) followed by the age group of 21-40 (15.2%). Significance associations were observed among the age group. They recommended that public health authorities should instruct the general public concerning prevention (43).

A cross sectional study conducted to determine HIV and malaria infection and risk factors in North West Ethiopia shows a 13.8% prevalence of HIV and concludes that health professionals should strengthen provider initiative counselling and testing programs for HIV prevention, control strategy and approach febrile illness patients for the diagnosis of HIV (44).

A study conducted to determine the prevalence of HIV among elderly people and associated factors in northeast Ethiopia shows 6.2% of HIV. The authors conclude that HIV prevalence in Habruworeda was higher than the national level and effort should be considered in designing new HIV intervention programs targeting older people (45).

The systematic study on viral hepatitis in Ethiopia reveals the overall pooled prevalence of HBV at 7.4% with variation among different groups of the population: 5.2% in HIV-infected individuals, 8.0% in community based studies, and 8.4% in blood donors. The study also shows an overall pooled prevalence of HCV of 3.1%, which shows high anti-HCV in HIV-infected individuals unlike in other subgroups (5.5%, 95%CI: 3.8–7.8%, p = 0.01). From this study, they conclude that diagnostic and treatment algorithms for viral hepatitis in the health care system and implementation of prevention and control strategies need urgent attention (46).

A community-based study conducted in the South Omo zone shows the prevalence of HBV at 8.0% and that of HCV at 1.9%. The two among study participants showed sero-positive for HBV and HCV 0.3%, with high HBV and anti-HCV in the male participants than females (COR=1.63,95% CI=0.87-3.04, p-value=0.12) and (COR=1.35,95% CI=0.42-4.29, p-value=0.61), from which they concluded that provision of mass screening, health education, and treatment are important in the reduction of the virus (47).

The first evidence of an HIV epidemic in Ethiopia was detected in 1984. The national HIV prevalence is 0.9%; the urban prevalence is 2.9% and the rural prevalence is 0.4%. The report also shows that the prevalence of the virus varies according to the region, ranging from less than 0.1% in Somalia to 4.8% in the Gambela region. The percentage of people with knowledge of HIV prevention is 41.7% and 18.8% for women in urban and rural areas, and that of men is 47.7% and 37% for urban and rural populations respectively. From these studies, the Afar Region accounts for 14.1% of women and 32.6% of men, which is relatively low in comparison to the region (48).

3. Objective of the study

3.1 General Objective

To determine the burden of hepatitis B virus, hepatitis C viruses and HIV among febrile patients attending health facilities and associated factors.

3.2 Specific Objective

To determine the burden of hepatitis B, hepatitis C and HIV among febrile patients attending health facilities.

To assess the association of each virus with different risk factors and each other's.

4. Materials and Methods

4.1 Study Area

The Afar region is one of the nine regions of Ethiopia and is geographically located in the northeast of the country. The region shares a common international border with Eritrea from the north and northeast and with Djibouti from the east, with Tigray in the northwest and Amhara in the southwest. It shares a border in the south with the state of Oromia and the Somali region in the southeast of Ethiopia. The region has a total population of 1.5 million and is divided into five administrative areas. Kelewina is one of the five regions in the Afar region. It borders Administrative Area 1 to the southeast, the Amhara region to the southwest, Tigray to the northwest, and Administrative Area 2 to the north. The administrative centre of the Awra and Gulina district is Kelewina. The total population of the Aura region is 246,822, with 140,741 men and 106,081 women.

4.2 Study design and period

A cross sectional study was conducted to determine the magnitude of hepatitis B, hepatitis C and HIV from stored serum sample collected from febrile patients attended health institutions in the Afar region from February 2019 to May 2019. Serum samples in the previous studies were properly stored at -20℃ and daily monitoring of the refrigerator maintained. Socio-demographic factors were collected from previously assessed data on sero-prevalence of human brucellosis and risk factors among febrile patients in the study area and hence retrospective.

4.3 Population

4.3.1 Source population

All patients who attended Drayitu health center and Kelewina primary hospital in Awra and Gulina district, Afar region, Ethiopia, for study of human brucellosis.

4.3.2 Study population

The study population was febrile patients that attended Drayitu Health Center and Kelewina Primary Hospital in Aura district, Afar Region, Ethiopia, during the study period.

4.4 Inclusion and Exclusion criteria

4.4.1 Inclusion Criteria

All sera with clear identification number and samples with sufficient volume were included in the study.

4.4.2 Exclusion criteria

The insufficient samples were excluded since requires a large volume of serum for analysis of each virus by ELISA and rapid tests. Samples with invisible identification number were also excluded to reduce clerical error.

4.5 Study Variables

4.5.1 Dependent Variable

The burden of hepatitis B virus, hepatitis C virus and HIV among the study participants.

4.5.2 Independent Variable

Socio-demographic factors

- ✓ Age
- ✓ Sex
- ✓ Marital status
- ✓ Educational status
- ✓ Occupation
- ✓ Residency

4.6 Sample size determination and sampling technique

4.6.1 Sample Size Determination

The estimated sample size is about 384 participants assuming the proportion of 50%. Sample Size derived using the following formula

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

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

P = proportion of 50% q=1-p

d= Margin of error (5%) = 0.05

n= required sample size

n= $(1.96)^2$ (0.5) (0.5)/ $(0.05)^2$ =384

For the calculation, 95% confidence interval and 5% margin of error are used. To reduce errors arising from complaints due to insufficiency of sample, 5% of sample size added giving a sample of 400.

4.6.2 Sampling Technique

All 444 sera samples analyzed for the previous study were stored at -20℃. Due to the large volume of samples required for various investigations, insufficient samples from the stored sera were excluded from the investigation. Hepatitis B, hepatitis C, and HIV were analyzed using samples with an acceptable volume of sera and clear identification number, collected for the investigation of brucellosis and malaria. A convenient sampling technique was used to select sample from the stored sera.

4.7 Measurement and data collection

4.7.1 Data Collection and laboratory tests

From previously prepared and stored at-20◦c serum samples, detection of HIV, HCV and HBV was undertaken. All selected sera were screened for HCV and HBV viruses using anti-HCV antibody and HBsAg, which is a rapid test that follows an immune-chromatographic technique. From the screened samples, positive results from the rapid tests were further

analyzed by ELISA tests to confirm the result. Similarly, all selected sera were analyzed for HIV using the current national algorithm for HIV/AIDS diagnosis, which is Stat Pak, Abon, and SD Bioline. Positive results for HIV/AIDS checked using ELISA testing for confirmation. The respondents' age, marital status, and occupation were collected from a questionnaire.

4.7.1.1 Hepatitis B test

HBsAg rapid test principle

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

HBsAg ELISA test principle

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

4.7.1.2 Hepatitis C test

Anti-HCV rapid test principle

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

Anti-HCV ELISA test principle

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

4.7.1.3 HIV test

Two types of serological tests employed as screening and confirmatory tests for HIV diagnosis in the serum samples. HIV rapid tests, based on the current national algorithm that performed at St. Peter specialized hospital and ELISA test for all positive samples analyzed at Metu Blood Bank.

HIV STAT-PAK®test principle

The Chembio HIV 1/2 STAT-PAK™ assay employs a unique combination of a specific antibody binding protein, which is conjugated to colloidal gold dye particles, and HIV 1/2 antigens, which are bound to the membrane solid phase. The sample applied to the sample well followed by the addition of running buffer. The buffer facilitates the lateral flow of the released products and promotes the binding of antibodies to the antigens. If present, the antibodies bind to the gold conjugated antibody binding protein. In a reactive sample, the dye conjugated-immune complex migrates on the nitrocellulose membrane and captured by the antigens immobilized in the test (T) area producing a pink/purple line. In the absence of HIV antibodies, there is no pink/purple line in the TEST (T) area. The sample continues to migrate along the membrane and produces a pink/purple line in the CONTROL (C) area containing immunoglobulin G antigens. This procedural control serves to demonstrate that specimen and reagents properly applied and migrated through the device.

Interpretation of results

Nonreactive result: one pink/purple line in the control area, with no line in the test (T) area indicates a nonreactive test result. A nonreactive test result at 15 minutes indicates that HIV-1 and HIV-2antibodies.

Reactive results: Two pink/purple lines, one in the test area and one in the control area indicate a reactive test result. The line in test area may look different from the line in control area. Test result with visible lines in both test and control areas, regardless of intensity, is considered reactive. Reactive result means that HIV-1 and/or HIV-2 antibodies detected in the specimen.

HIV Abon test principle

The HIV 1/2/O Tri-line Human Immunodeficiency Virus Rapid Test Device test strip is precoated with HIV-1 and subtype O antigens on T1 test line and HIV-2 antigen on T2 test line. Firstly, specimen and then buffer are adding to the specimen well, thus starting the migration of the specimen/buffer. The specimen/buffer passes the conjugate pad, which

contains a mixture of HIV-1 envelope and capsid antigens and HIV-2 envelope antigen. These detection antigens are conjugated to latex particles. If present, the HIV-1 of HIV-2 antibodies reacts and binds to the detection antigen-conjugate. The antibody/antigen-conjugate mixture then migrates further and binds to antigens present on the test lines. If the specimen contains antibodies to HIV-1, the specimen will bind to the T1 test line and produce a line, if specimen contains antibodies to HIV-2, the specimen will bind to the T2 test line. As liquid continues to migrate down the test strip,the control line will appear, If the control line is present, in addition to either or both test lines, then the test is reactive for HIV1/2 antibodies. If the specimen does not contain HIV-1 or HIV-2 antibodies, no coloured lines will appear for either of the test lines region indicating a non-reactive result. Please note that the appearance of coloured lines at T1 and T2 is highly unlikely to be indicative of co-infection with HIV-1 and HIV-2 but rather is a result of cross-reactivity between antigens. A coloured line will appear in the control line region if the migration of liquid has been successful and must be present for the test to be valid. Its presence does not confirm sufficient specimen addition.

Interpretation of the result

Reactive: two or three distinct coloured line appears. One line should always appear in the control line region (C), and another one or two apparent coloured line should appear in the test line region(s) (T1 and /or T2).

The intensity of the colour in the test line region (T1 and /or T2 will vary but any shade of the colour in test line region (T1 and/or T2) should be considered reactive.

Dual infection of HIV-1 and HIV-2 is quite rare. Dual reactivity observed in AbonHIV1/2/0 Tri-line HIV rapid test device, i.e. HIV-1 line and HIV-2 line both reactive, is more likely to be caused by antibody cross reactivity. Any specimen with dual reactivity shouldrefer for specific HIV-2 confirmatory testing, if a discretionary result is required.

Non-reactive: one coloured line appears in the control region (C).No apparent coloured lines appear in the test line regions (T1 and/or T2).

HIV SD Bioline test principle

The SD Bioline HIV-1/23.0 kit is a rapid qualitative test for the detection of antibodies to all isotypes (IgG, IgM, IgA) specific to HIV-1 including subtype-0 and =HIV-2simultaneously in human serum, plasma or whole blood. The SD Bioline HIV1/2 3.0 test contains a membrane strip, which is pre-coated with recombinant HIV-1 capture antigen (gp^{41}, p^{24}) on test band 1 region and with recombinant HIV-2 capture antigen (gp36) on test band 2 region,

respectively. The recombinant HIV-1/2 antigen (gp41, p24 and gp36)- colloid gold conjugate and the specimen sample move along the membrane chromatographically to the test region(T) and form a visible line as the antigen-antibody-antigen gold particle complex forms with high degree of sensitivity and specificity. This test device has a letter of 1 and 2 and C as test line1 (HIV-1), Test line 2 (HIV-2) and control line on the surface of the device. Both the Test lines and control line in result window are not visible before applying any sample. The control line uses for procedural control. Control line should always appear if the test procedure, were performed properly and the test reagents of control line are working.

Interpretation of the test

Negative result: The presence of only control line (C) within the result window indicates a negative result.

Positive result

The presence of two line as control line(C) and test line 1 (1) within a result window indicates a positive result for HIV-1.

The presence of two line as a control(C) and test line 2 (2) within attest window indicates a positive for HIV-2.

The presence of three line as control line (C), test line 1 (1) and test line 2 (2) within the result window indicates a positive result for HIV-1 and/or HIV-2.

If the colour intensity of the test line 1 is darker than one of the test lines 2 in the result window, the result interpreted as HIV-1 positive.

If the colour intensity of the test line 2 is darker than one of the test lines1in the result window, the result can be interpreted as HIV-2 positive.

4.8 Data Quality Assurance

Quality assurance and quality control standard operating procedures were following, and internal quality control materials were included according to the manufacturers' instruction of the test kits. A brief orientation provided for laboratory professionals processing the samples on the proper management of handling the results. In addition, there was a continuous follow-up by the principal investigator and supervisor during each step of the process. A known positive and negative sample was receiving from the Adera clinic to check the rapid test kit for HCV and HBV. The result was entering into statistical software to summarize and analyze the data.

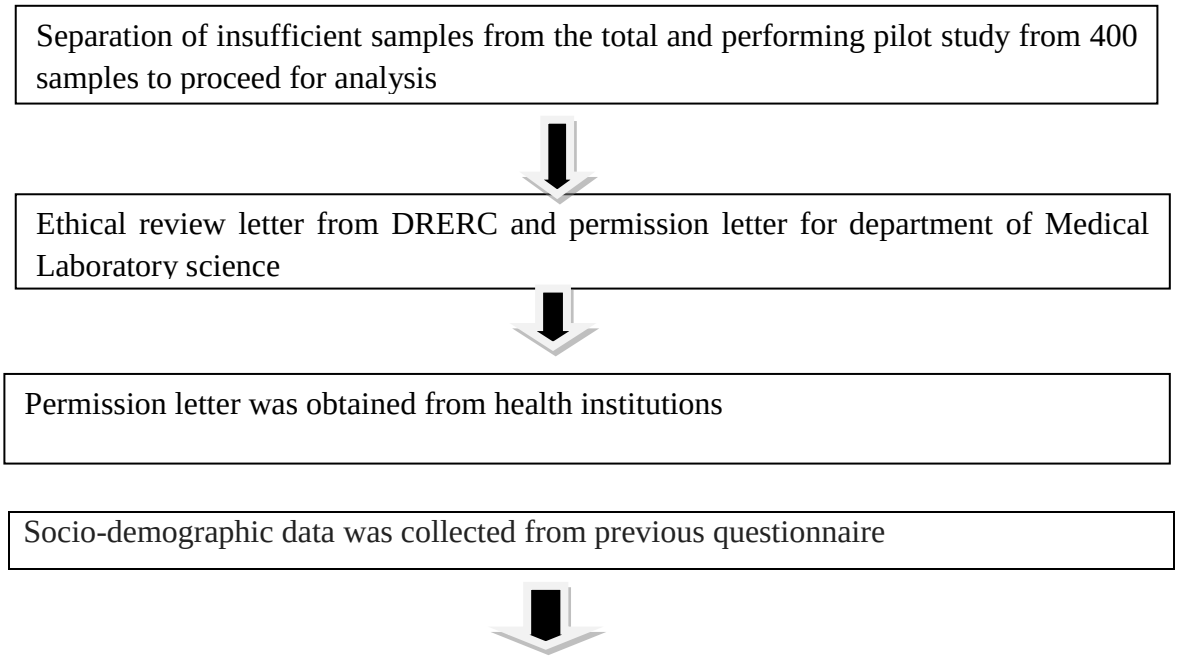
4.9 Data Processing and analysis

Collected data entered and analyzed was by SPSS version 25 statistical software. Data were clean and edited before analysis. Categorical variables were summarizing using proportion and percentages. Continuous data were described in terms of central tendency (mean and median) and dispersion (standard deviation).95%confidence interval and p-value were used to measure the strength of association and identify statistically significant results. P-value ≤ 0.05was considered statistically significant. Bivariate logistic regression analysis was used to determine the risk factors associated with viral diseases.

4.10 Ethical Consideration

An ethical clearance was obtained from the institutional review board of the department of medical laboratory science, College of health science, Addis Ababa University (DRERC/555/20/MLS).HIV test kit was obtained from St. Peter specialized hospital by model 22/health serial №. 171906. For those positive values of the three tests ELISA were performed for confirmation at Mettu Blood Bank (MBBC//152/13). Permission letter was obtained from health institutions. Those individuals infected with the viruses were contacted via the health institutions to receive the necessary counselling and treatment. To keeps confidentiality, all processed laboratory results were coded and locked separately before entered into the computer. After entered into the computer, the results locked by password and disclosed to anybody other than the principal investigator. A prior investigation into human Brucellosis among febrile patients at the health facility compiled the consent and assent. These consent and assent had selected for this research purpose since there were no variables that exposed confidentiality of the participants.

4.10 Overall workflow Diagram



HIV test was performed based current algorithm	HBsAg was performed	Anti-HCV was performed	ELISA test were done for all positive samples

5. Operational Definition

Febrile patients: patients with fever and axial body temperature of ≥ 37.5℃.

HBV positive: Serum positive for HBsAg by rapid test and ELISA method.

HBsAg: low (<2%), intermediate (2-8%) and high (>8%) according to WHO (17).

HCV positive: Serum positive for anti-HCV by rapid test and ELISA method.

HIV positive: serum positive for HIV antibody for rapid test of HIV-1/2.

6. Results

6.1Socio-demographic Characteristics

Out of 400 samples were selected for analysis of the viruses. The remaining 44 samples excluded due to insufficient sample volume and vague identification number. From the total 400 participants, 239 (59.8%) were females and 161(40.2%) were males. The mean age was 25.9(SD±11.6) with age ranged from 4-80 years. Majority of the age group were 15-29, 53.8% (217/400). Regarding to marital, educational status, residency and occupation, 70.5% (282/400) were married 46.5% (185/400) were illiterate 77% (308/400) were rural residences and 56.25% (225/400) were pastoralist respectively (Table 1).

Table 1:- Socio-demographic characteristics viral hepatitis and HIV Burden among febrile patients attending health institute at Aura district, Afar Region, Ethiopia (from February to May 2019).

Variable	category	Frequency(n=400)	Percentage (%)
Age category	4-14	52	13
	15-29	217	54.3
	30-44	102	25.5
	>44	29	7.2
Sex	Male	161	40.2
	Female	239	59.8
Marital Status	Single	118	29.5
	Married	282	70.5
Educational status	Illiterate	185	46.25
	Grade 1-8	128	32
	High School and above	87	21.75
Residency	Urban	92	23
	Rural	308	77
occupation	Pastoralist/agro-pastoralist	225	56.25
	Others	175	43.75

n=total number of study participants
Others= student, government worker and daily labour
Married= divorced, widowed and married

6.2 Burden of HBV, HCV and HIV infection

Among all samples screened for HBsAg, anti-HCV antibody and HIV rapid test,10% (40/400) by HBsAg,1.25% (5/400) by anti-HCV anti-body and 2.5% (10/400) by HIV rapid tests were found positive for HBV, HCV and HIV respectively. From positive results detected by rapid test and further analyzed for confirmation by ELISA method, 9.8% (39/400), 1% (4/400) and 2.3% (9/400) were positive for hepatitis B virus, hepatitis C virus and HIV respectively. From the participants included in the study 9.3% (15/161), 1.24% (2/161) and 3.5% (4/161) males were infected with hepatitis B virus, hepatitis C virus and human immunodeficiency virus respectively. High burden of HIV infections were found in the male participants 3.5% (4/161) than females 2.1%(5/239). With respect to the age category of individuals participated in the study 12%(26/217), 1.4%(3/217) and 0.9%(2/217) were detected for hepatitis B virus, hepatitis C virus and HIV as confirmed by ELISA tests from previously diagnosed samples with HBsAg, anti-HCV and HIV test algorithm orderly were within 15-29years. Regarding to the marital status of the study participants that were screened for hepatitis B virus, hepatitis C virus and HIV 9.6%(27/282), 1.42%(4/282) and 2.5%(7/282) tested positive in the above order were married. Two patients had concurrent infection for HCV-HBV and HBV-HIV. Concerning with participants occupation in the study 9.8% (22/225), 1.3 % (3/225) and 1.8%(4/225) who were tested positive for hepatitis B, hepatitis C virus and HIV accordingly were nomadic/semi nomadic. 6.5 % (6/92) of the urban dwellers were screened positive for hepatitis B virus that was less prevalent when compared to the rural community included in the study. The HIV burden was higher in the urban community at Aura district of Afar region that detected from febrile patients during the study period (**Table 2**).

Table 2:- Prevalence Of HBV, HCV and HIV among febrile patients at health institutions of Afar region, Ethiopia.

Variables		Total test	HBV	HCV	HIV
			Pos (%)	Pos (%)	Pos (%)
Sex	Male	161	15(9.3)	2(1.25)	4(3.5)
	Female	239	24(10)	2(0.84)	5(2.1)
Age category	4-14	52	3(5.8)	0(0)	0(0)
	15-29	217	26(12)	3(1.4)	2(0.9)
	30-44	102	9(8.8)	1(1)	7(6.9)
	>44	29	1(3.4)	0(0)	0(0)
Educa. status	Illiterate	185	16(8.6)	1(0.54)	7(3.8)
	Grade 1-8	128	16(12.5)	1(0.8)	1(0.8)
	High school and above	87	7(8)	2(2.29)	1(1.15)
Marital status	Single	118	12(10.2)	1(1)	2(1.7)
	Married	282	27(9.6)	4(1.4)	7(2.5)
Occupation	Pastoralist/agro-pastoralist	225	22(9.8)	3(1.3)	4(1.8)
	Others	175	17(9.7)	2(1.1)	4(1.8)
Residency	Rural	308	33(10.7)	5(1.6)	5(1.6)
	Urban	92	6(6.5)	0(0)	4(4.3)

Others= government worker, student and daily labor

Married= divorced, widowed and married

6.3 Risk factors associated with HBV based on bivariate analysis

With male participants 9.3% (15/161) were positive for HBV and 10% (24/239) of females, the differences were not statistically significant (COR=0.92, 95% CI=0.467-1.814,P-value=0.811). Among the educational status 12.5% (n=16) from the elementary school were positive for HBV and there is no statistical difference between educational status and HBV (COR=0.765, 95% CI=0.27-2.171, P-value=0.615). Relating to with the age of participants 8.8% (9/102) of 30-44 were positive for HBV with no statistical significance between age category and HBV positivity (COR=2.710, 95% CI=0.329-22.322and P-value=0.354). The

burden of HBV with the pastoralist /agro-pastoralist communities were 9.8% (22) and was statistical no significance between occupation of the participants and of HBV prevalence (COR=0.985, 95% CI=0.506-1.917 and P-value=0.964). The participants from the urban community 6.5% (6/92) were positive for HBV and no statistical significance between places of residency and HBV (COR=0.581, 95% CI=0.236-1.434 and P-value=0.239) (**Table 3**).

Table 3:- Socio-demographic risk factors associated with burden of HBV

Variables		Total tests	HBV			
			Pos (%)	COR	CI (95%)	P-value
Sex	Male	161	15(9.3)	1		
	Female	239	24(10)	0.92	0.467-0.814	0.811
Age category	4-14	52	3(5.8)	1.714	0.170-17.276	0.647
	15-29	217	26(12)	3.812	0.497-29.205	0.198
	30-44	102	9(8.8)	2.710	0.329-22.322	0.354
	>44	29	1(3.4)	1		
Educational status	Illiterate	185	16(8.6)	1.082	0.428-2.735	0.868
	Grade 1-8	128	16(12.5)	1.633	0.642-4.152	0.303
	High school and above	87	7(8)	1		
Marital status	Single	118	12(10.2)	1.069	0.522-2.189	0.855
	Married	282	27(9.6)	1		
Occupation	Pastoralist/agro-pastoralist	225	22(9.8)	0.985	0.506-1.917	0.964
	Others	175	17(9.7)	1		
Residency	Rural	308	33(10.7)	0.581	0.236-1.434	0.239
	Urban	92	6(6.5)	1		

Others= government worker, student and daily labour

Married= divorced, widowed and married

6.4 Risk factors associated with HCV based on bivariate analysis

The burden of HCV among the female participants taking part in the studies were 0.84%(n=2/239) and male were1.24%(n=2/161); however, both sex groups had no statistical significance with the sero-prevalence of HCV(COR=1.491, 95% CI=0.208-10.69, p-value=0.69). Relating to educational status of the partakers, the illiterate community accounts 0.54(1/185), elementary school 0.8%(1/128) and high school and above measure 2.3%(2/87) but, had no statistical significance with HCV burden(COR=4.329, 95% CI=0.387-48.4, p-value=0.234). Concerning to the marital status and occupation of the participants the distribution of the virus was similar; however the burden of the virus was high among the rural residence than that of urban (Table 4).

Table 4:- risk factors associated with HCV based on bivariate analysis

Variable		Total tests	HCV Pos(%)	COR	CI (%)	p-value
Sex	Male	161	2(1.24)	1.491	0.208-10.69	0.69
	Female	239	2(0.84)	1		
Age	4-29	269	3(1)	1.46	0.2-14.2	0.7
	30-80	131	1(0.76)	1		
Edu. status	Illiterate	185	1(0.54)	1		
	1-8	128	1(0.8)	1.449	0.09-23.377	0.794
	High school and above	87	2(2.3)	4.329	0.387-48.4	0.234
Marital status	Single	118	1(0.84)	1		
	Married	282	3(1.06)	1.258	0.130-12.219	0.843
Occupation	Pastoralist/agro-pastoralist	225	3(1.3)	1.316	0.183-9.435	0.785
	Others	175	2(1.1)	1		
Residence	Rural	308	4(1.6)	0.000	0.000	0.997
	Urban	92	0(0)	1		

Others= government worker, student and daily labour

Married =divorced, widowed and married (the first two is only 7 that is why I include them under married)

6.5 Risk factors associated with HIV based on bivariate analysis

The burden of HIV among female participants in the studies were 2.1% (5/239) and of 3.5% (4/161) males but, was not statistical significance with the burden of HIV (COR=1.192, 95% CI =0.315-4.5 and p-value=0.795). The occurrence of HIV among age category of 4-29 were 0.7% (n=2/269) and age group of 30-80 years were 6.9% (7/131) that was statistically significant with HIV (COR=7.536,95% CI=1.543-36.803,p-value=0.013). Regarding to educational status, the burden of HIV was high in the illiterate community 3.8% (7/185) though, it was not statistically significant with HIV (COR=4.994, 95%=0.607-41.097, p-value=0.135). The burden of HIV for those who had not married were 1.7% (2/118) and of marriage history were 2.5% (7/282) that was relatively high but no statistical significance (COR=0.935, 95% CI =0.457-1.915,p-value=0.855). The majority of HIV positive among the participants were urban dwellers of 4.3% (4/92) and peoples of other than pastoralists with disease proportion of 2.9% (5/175) despite, both of them have no statistical significances with the burden of HIV (**Table 5**).

Table 5:- risk factors associated with prevalence of HIV

Variable		Total tests	HIV Pos (%)	COR	CI (%)	P-value
Sex	Male	161	4(3.5)	1.192	0.315-4.5	0.795
	Female	239	5(2.1)	1		
Age	4-29	269	2(0.7)	1		
	30-80	131	7(6.9)	7.536	1.543-36.803	0.013
Educa. Status	Illiterate	185	7(3.8)	4.994	0.607-41.097	0.135
	High school and above	87	1(1.4)	1.477	0.091-23.93	0.784
	Grade 1-8	128	1(0.8)	1		
Marital status	Single	118	2(1.7)	1		
	Married	282	7(2.5)	0.935	0.457-1.915	0.855
occupation	Others	175	5(2.9)	1.625	0.430-6.144	.474
	Pastoralist/agro-pastoralist	225	4(1.8)	1		
Residence	Rural	308	5(1.6)	1		
	Urban	92	4(4.3)	2.755	0.724-10.478	0.137

7 Discussion

The objective of the study was to assess/determine the burden of hepatitis B, hepatitis C and Human immunodeficiency virus among febrile patients from Afar health institution of Aura districts. Out of the total participants 59.75% were female and 41.25 were male which had consistent proportion with studies conducted in Gonder (5).

The study revealed that burden of hepatitis B virus disease in this area was 10% by rapid HBsAg and 9.8% by ELISA, which is generally categorized as high endemic according to world health organization classification(17) and higher than Ethiopian national pooled prevalence 7.4%(46). The prevalence of HBV among febrile patients attending the facility during the study period was foundhigh compared to the studies conducted in different areas: Bahir Dar 4.2%(41),in Pakistan 2.6% (43),in Nigeria 8.5%(35), in Turkey 5%(36) and in India 2.92%(30). Nearly similar burden to studies done in China 9.69% (29).The result of study was lower than studies done in different areas such as Nigeria 13.6%(33) and Burkina Faso 14.4%(37). This might be because of variation of the geographical location and community awareness in the study area.

The burden of hepatitis C virus infection was found 1% which was almost alike to studies done in Somalia 0.9%,Sudan 1% and Yemen 1.9%(40). The result was lower than studies conducted in Bahir Dar 2.1%(42) and national overall pooled prevalence of Ethiopia 3.1%(46), in Pakistan 16.2%(43), Lagos Nigeria 5.61% (38) and an overall pooled prevalence of Africa2.98%(38). The result was higher than studies done in India 0.1% (31) this difference and alike of the result might be because of the uniformity of the social behaviour life style and disparity of the study area, period and population characteristics difference accordingly.

The burden of HIV among febrile patients attending the health institution of the of Aura districts through the study period was 2.3% which was lower than studies conducted on the Northwest Ethiopia 13.8%(44)and northeast Ethiopia 6.2% of HIV(45).high than studies performed in Turkey(36), India of Rajasthan states(32) and Ethiopia(48) with occurrence rate of 0%, 0.35% and 0.9% respectively. The difference might be due to the life style character of the community, difference in the sample size, area of the research and study period.

Among male participants 9.3% (15/161) were positive for HBV and 10% (24/239) of females, the differences were not statistically significant (COR=0.92, 95% CI=0.467-1.814, P-value=0.811). The burden of anti-HCV from the female participants taking part in the studies were 0.84%(2/239) and that of male were1.24%(2/161) positive for anti-HCV; however, had no statistical significance with the sero-prevalence of anti-HCV(COR=1.491, 95% CI=0.208-

10.69, p-value=0.69) this finding was unmatched with studies conducted in South Omo from a total of 304 female participants 5.6% (17/304) were positive for HBsAg and from a total of 318 male participants 8.8% (28/318) were positive for HBsAg. While 1.6%(5/304) females were positive for anti-HCV and 2.2% (7/318) males were positive(47). The difference might be due study area, sex ratio and sample size.

The burden of HIV among female participants in the studies were 2.1% (5/239). HBV positivity among males were 3.5% (4/161) but, was not statistical significance with the burden of HIV (COR=1.192, 95% CI =0.315-4.5 and p-value=0.795).This finding was comparable with the results obtained in male participants 3.8%(8/211) however, not equivalent with female participants 3.5%(6/171) (42). This might be because of volunteers had probably know their status prior to testing.

From the study participants two of them had concurrent infection. One between anti-HCV and HBV that accounts 0.25% and the other between HIV and HBV which contribute 0.25% respectively that was lower than studies conducted in Angola 0.9%(4/431) and 2.3%(10/431) respectively(34). The difference might be due study period specificity of the population.

With regard to marital status the burden of HBV was slightly higher in unmarried participants 10.2% (12/118) than those who had previous exposure of marriage 9.57% (27/239), but the difference was not statistically significant (p-value>0.05). High burden of hepatitis B virus infection was detected amongst the age group of 15-29 with 12%(26/217) however had no statistical significance association(COR=3.812, 95% CI=0.492-29.205, p-value=0.198).the result was incomparable with community based study of south Omo by age category of 18-29 and 6.9% prevalence of the viral infection. The burden of the virus in the age category of 30-44 was 8.8%(COR=2.710, 95% CI=0.329-22.322) similar finding of community based study of south Omo 8.8% but no significant association exist(47). In relation to educational status 8.6% were illiterate (COR=1.307 95% CI=0.461-3.706 p-value=0.615).

The burden of anti-HCV with respect to marital status was higher in married participants 1.06% (3/282) than single 0.85% (1/118) (COR=1.258, CI=0.130-12.219, p-value=0.843) which was no statistically significant with marital status. this finding lower than study conducted in Bahir Dar 1.4% in unmarried groups and 1.7% among the married participants(42).

8 Strength and Limitation of the study

8.1 Strength of the study

The study was conducted on stored sera without evaluating any clinical approach associated with the disease. Therefore, research is capable of providing evidence of the burden of virus distribution in the study area.

8.2 Limitation of the study

Lack of collecting history of the participants regarding the awareness on the transmission of the viruses, severity and symptoms relating to the diseases and social impacts exerted on their daily life by questionnaire development. Inability to assess life style of the population since most of the communities in arid areas are nomadic and move from place to place to search grasses for the cattle.

9 Conclusion and Recommendations

9.1 Conclusions

The study reveals high burden of HBV and HIV among the study participants that were selected from febrile patient without clinical sign and symptoms relating to the viruses, and that of HCV was relatively low. There were statistically significant association shown between HIV and age category of 30-80 years. As a result, the regional health department and health worker in the study area should consider serious health education and community awareness, as well as serological disease screening for individuals who require medical treatment and perform provider initiating counselling and testing services for the early detection of the diseases.

9.2 Recommendation

- Establish a comprehensive national surveillance studies to assess the burden of HCV, HBV and HIV is important in the arid areas of the country
- Awareness creation for health worker is crucial to reach the large community in the area
- Formulation of the national guidelines on the diagnosis of hepatitis is essential to understand the burden of the virus at the national level.
- Increasing HBVvaccination coverage for the whole population to decrease co-morbidity with other viruses
- Increasing access to treatment to reduce the viral load and improve the quality of life
- Early screening of the viruses are important since most of the infections are usually asymptomatic to reduce occurrence of complication of the diseases
- Setting preventive measure to reduce infections that accelerates diseases progression to chronic hepatitis

10 Reference

1. Shaw-Stiffel TA, Mandell G, Bennett J. Chronic hepatitis. Principles and Practice of Infectious Diseases New York, NY: Churchill. 2000:1297-331.
2. Organization WH. Guidelines for the prevention care and treatment of persons with chronic hepatitis B infection: Mar-15: World Health Organization; 2015.
3. Ryerson AB, Schillie S, Barker LK, Kupronis BA, Wester C. Vital Signs: Newly Reported Acute and Chronic Hepatitis C Cases—United States, 2009–2018. Morbidity and Mortality Weekly Report. 2020;69(14):399-404.
4. World health organization on hepatitis B and hepatitis C report on 2019 July 9 and 18. https://www.who.int/news-room/fact-sheets/detail/hepatitis.
5. Organization WH. Global hepatitis report 2017: World Health Organization; 2017.
6. Research reports: HIV/AIDS. National institute of drug abuse 2012. Available at http://www.drugabuse.gov/publications/research-reports/hivaids/letter director.
7. Mason LM, Duffell E, Veldhuijzen IK, Petriti U, Bunge EM, Tavoschi L. Hepatitis B and C prevalence and incidence in key population groups with multiple risk factors in the EU/EEA: A systematic review. Eurosurveillance. 2019;24(30):1800614.
8. Coffie PA, Egger M, Vinikoor MJ, Zannou M, Diero L, Patassi A, et al. Trends in hepatitis B virus testing practices and management in HIV clinics across sub-Saharan Africa. BMC infectious diseases. 2017;17(1):141-8.
9. Singh KP, Crane M, Audsley J, Lewin SR. HIV-Hepatitis B virus co-infection: epidemiology, pathogenesis and treatment. AIDS (London, England). 2017;31(15):2035-52.
10. Zulfiqar HF, Javed A, Afroze B, Ali Q, Akbar K, Nadeem T, et al. HIV diagnosis and treatment through advanced technologies. Frontiers in public health 2017; 5:1-16.
11. Aryal S. Hepatitis B Virus- Structure, Epidemiology, Symptoms, Pathogenesis, Diagnosis, Treatment and Vaccines, 2019 [cited 2020, July 16], available from: https://microbiologyinfo.com/hepatitis-b-virus-structure-epidemiology-symptoms-pathogenesis-diagnosis-treatment-and-vaccines/.
12. Moosavy SH, Davoodian P, Nazarnezhad MA, Nejatizaheh A, Eftekhar E, Mahboobi H. Epidemiology, transmission, diagnosis, and outcome of Hepatitis C virus infection. Electronic physician. 2017;9(10):5646-56.
13. Kwon SY, Lee CH. Epidemiology and prevention of hepatitis B virus infection. The Korean journal of hepatology. 2011; 17:87-95.

14. Lee DH, Cho Y, Seo JY, Kwon JH, Cho EJ, Jang ES, et al. Polymorphisms near interleukin 28B gene are not associated with hepatitis B virus clearance, hepatitis B e antigen clearance and hepatocellular carcinoma occurrence. Intervirology. 2013;57(1):167-85.
15. Jefferies M, Rauff B, Rashid H, Lam T, Rafiq S. Update on global epidemiology of viral hepatitis and preventive strategies. World journal of clinical cases. 2018;6(13):589-99.
16. Zampino R, Boemio A, Sagnelli C, Alessio L, Adinolfi LE, Sagnelli E, et al. Hepatitis B virus burden in developing countries. World journal of gastroenterology. 2015;21(42):11941-53.
17. Schweitzer A, Horn J, Mikolajczyk RT, Krause G, Ott JJ. Estimations of worldwide prevalence of chronic hepatitis B virus infection: a systematic review of data published between 1965 and 2013. The Lancet. 2015;386(10003):1546-55.
18. Petruzziello A, Marigliano S, Loquercio G, Cozzolino A, Cacciapuoti C. Global epidemiology of hepatitis C virus infection: An up-date of the distribution and circulation of hepatitis C virus genotypes. World journal of gastroenterology. 2016;22(34):7824-40.
19. Gower E, Estes C, Blach S, Razavi-Shearer K, Razavi H. Global epidemiology and genotype distribution of the hepatitis C virus infection. Journal of hepatology. 2014;61(1):S45-S57.
20. Global HIV/AIDS fact sheet: https://www.unaids.org/en/resources/fact-sheet
21. WHO Africa. [Cited 2019,June10], available from:https://www.afro.who.int/health-topics/hepatitis.
22. Spearman CW, Afihene M, Ally R, Apica B, Awuku Y, Cunha L, et al. Hepatitis B in sub-Saharan Africa: strategies to achieve the 2030 elimination targets. The lancet gastroenterology & hepatology. 2017;2(12):900-9.
23. Stockdale AJ, Mitambo C, Everett D, Geretti AM, Gordon MA. Epidemiology of hepatitis B, C and D in Malawi: systematic review. BMC infectious diseases. 2018;18(1):516.
24. Lingani M, Akita T, Ouoba S, Sanou AM, Sugiyama A, Tarnagda Z, et al. High prevalence of hepatitis B infections in Burkina Faso (1996–2017): a systematic review with meta-analysis of epidemiological studies. BMC public health. 2018;18(1):551.
25. Mohd Hanafiah K, Groeger J, Flaxman AD, Wiersma ST. Global epidemiology of hepatitis C virus infection: new estimates of age-specific antibody to HCV seroprevalence. Hepatology. 2013;57(4):1333-42.

26. Yazie TD, Tebeje MG. an updated systematic review and meta-analysis of the prevalence of hepatitis B virus in Ethiopia. BMC infectious diseases 2019; 19(1):917. https://doi.org/10.1186/s12879-019-4486-1.
27. Abate M, Wolde T. Seroprevalence of human immunodeficiency virus, hepatitis B virus, hepatitis C virus, and syphilis among blood donors at jigjiga blood bank, eastern Ethiopia. Ethiopian journal of health sciences2016; 26(2):155-162.
28. Taye S, Abdulkerim A, Hussen M. Prevalence of hepatitis B and C virus infections among patients with chronic hepatitis at Bereka Medical Center, Southeast Ethiopia: a retrospective study. BMC research notes. 2014;7(1):1-4.
29. Tao J, Zhang W, Yue H, Zhu G, Wu W, Gong W, et al. Prevalence of Hepatitis B Virus Infection in Shenzhen. Scientific reports. 2019;9(1):1-9, China, 2015–2018.
30. Rahaman J, Sengupta M, Barik G, Sarkar S, Sarkar R, Sengupta M. Seroprevalence and Co-infection of Hepatitis B and Hepatitis C Among Patients in a Tertiary Care Hospital in Eastern India. The Journal of the Association of Physicians of India. 2019;67(9):27-9.
31. Chakraborty A, Pramanik SB, Roy DS, Sarkar S, Chakraborty M, Nandi A. A retrospective study on the sero-prevalence of Hepatitis C infection in a Tertiary Care Hospital in Kolkata, India. Int J Curr Microbiol App Sci. 2015;4(3):115-23.
32. Sood S, Malvankar S. Seroprevalence of Hepatitis B surface antigen, antibodies to the Hepatitis C virus, and human immunodeficiency virus in a hospital-based population in Jaipur, Rajasthan. Indian journal of community medicine: official publication of Indian Association of Preventive & Social Medicine. 2010;35(1):165-9.
33. Omote V, Kashibu E, Ojumah I, Adda D, Etaghene J, Ukwamedua H. Serological screening of hepatitis B virus and hepatitis C virus among patients attending a tertiary hospital in Jalingo, Taraba state, Nigeria. Saudi Journal for Health Sciences. 2018;7(3):167-71.
34. Guimarães Nebenzahl H, Lopes A, Castro R, Pereira F. Prevalence of human immunodeficiency virus, hepatitis C virus, hepatitis B virus and syphilis among individuals attending anonymous testing for HIV in Luanda, Angola. SAMJ: South African Medical Journal. 2013;103(3):186-8.
35. Omatola CA, Onoja BA, Agama J. Detection of hepatitis B surface antigen among febrile patients in Ankpa, Kogi State, Nigeria. Journal of Tropical Medicine. 2020;2020.
36. Ozturk TC, Guneysel O, Tali A, Yildirim SE, Onur OE, Yaylaci S. Hepatitis B, Hepatitis C and HIV seroprevalence in critically ill emergency medicine department patients in a

tertiary inner city hospital in Istanbul, Turkey. Pakistan journal of medical sciences. 2014;30(4):703.

37. Tao I, Compaoré TR, Diarra B, Djigma F, Zohoncon TM, Assih M, et al. Seroepidemiology of hepatitis B and C viruses in the general population of Burkina Faso. Hepatitis research and treatment. 2014;2014.
38. Ogunware AE, Onakomaiya AO, Adekoya AA. Seroprevalence of Hepatitis C Virus Infection amongst Febrile Patients Attending Selected Public and Private Hospitals in Lagos State, Nigeria. Asian Journal of Immunology. 2020:9-15.
39. Rao VB, Johari N, du Cros P, Messina J, Ford N, Cooke GS. Hepatitis C seroprevalence and HIV co-infection in sub-Saharan Africa: a systematic review and meta-analysis. The Lancet infectious diseases.2015;15(7):819-24.
40. Chaabna K, Kouyoumjian SP, Abu-Raddad LJ. Hepatitis C virus epidemiology in Djibouti, Somalia, Sudan, and Yemen: systematic review and meta-analysis. PloS one. 2016;11(2):e0149966.
41. Birku T, Gelaw B, Moges F, Assefa A. Prevalence of hepatitis B and C viruses infection among military personnel at Bahir Dar Armed Forces General Hospital, Ethiopia. BMC research notes. 2015;8(1):1-6.
42. Ameha Z, Tadesse S, Assefa A, Tessema B. Prevalence and associated factors of Hepatitis C virus and human immunodeficiency virus infections among voluntary counseling and testing clients attending private health facilities in Bahir Dar city, North West Ethiopia 2014. BMC research notes. 2019;12(1):1-6.
43. Daud M, Hassan A, Ahmad A, Ali F, Khan MA, Rehman MU. Prevalence of Hepatitis B and C Infection in District Dir, KhyberPaktunkhwa, Pakistan. World J Zoolog. 2015;10(2):142-6.
44. Wondimeneh Y, Gebrecherkos T, Muluye D, Damtie D, Ferede G. HIV and Malaria Infections and Associated Risk Factors Among Febrile Illness Patients in Northwest Ethiopia. Turkiye Parazitol Derg. 2018;42(3):180-6.
45. Fentaye S, Yibeltal D, Tessema Z. Prevalence of HIV/AIDS Among Elderly People and Associated Factors in Habru Woreda, Amhara Region, Northeast Ethiopia. HIV/AIDS (Auckland, NZ). 2020;12:411-23.
46. Belyhun Y, Maier M, Mulu A, Diro E, Liebert UG. Hepatitis viruses in Ethiopia: a systematic review and meta-analysis. BMC infectious diseases. 2016;16(1):1-14.

47. Woldegiorgis AE, Erku W, Medhin G, Berhe N, Legesse M. Community-based sero-prevalence of hepatitis B and C infections in South Omo Zone, Southern Ethiopia. Plos one. 2019;14(12):e0226890.
48. CSA I. Central Statistical Agency (CSA)[Ethiopia] and ICF. Ethiopia Demographic and Health Survey 2016. Addis Ababa, Ethiopia, and Rockville, Maryland, USA: CSA and ICF;2016.

ANNEX-

Annex- Ⅰ : Principle and procedure of test

A) HBsAg rapid test principle

The principle of the test is immune-c°Chromatography, which has two unique site immune-assays on a membrane. The test sample flows through the membrane assembly of the cassette, the colour monoclonal anti-HBsAg, colloidal gold conjugate complexes with HBsAg in the sample. This complex moves further on the membrane to the test region where immobilized by another monoclonal anti-HBsAg antiserum coated on the membrane. Pink-purple colour band formation confirms a positive test result and absence of the colour band in the test region indicates a negative test result. Unreached conjugate and unbound complex, if any moves further on the membrane and are subsequently immobilize by the anti-rabbit antibodies coated on the membrane at the control region, forming a pink/purple colour band. This control band serves to validate the test result (Test kit insert sheet).

Test procedure

1. Open the sealed pouch by tearing along the notch. Remove the test from the pouch
2. Immerse the strip into the container with arrow end toward the container do not immerse the maximum line. Take the strip out after 8-10 seconds and lay the strip flat on a dry, non-absorbent surface.
3. Wait 10-20 minutes and read result. Do not read result after 30 minutes.

Result interpretation

Positive result: Distinct colour bands appear on the control and regions test both test line and control line indicate that the specimen contains detectable amount of HBsAg.

Negative result: Only one-colour band appears on the control region. No apparent band on the test region. This indicates that there is no detectable HBsAg in the serum.

Invalid: No visible band at all or only one coloured band appears at test region**.**

B) HBsAg ELISA test principle

This is a Sandwich Enzyme linked Immune-sorbent assay method in which polystyrene micro well stripis pre-coated with monoclonal antibodies specific to HBsAg. A Participant serum or plasma sample is added to the micro well together with a secondary antibody conjugated with horseradish peroxidase(HRP), and directed against a different epitope of HBsAg. During incubation, the specific immune-complex formed in the case of presence of HBsAg in the sample, captured on the solid phase. After washing to remove sample serum protein and

unbound HRP-conjugate, chromogen solution containing Tetra-methyl Benzedrine (TMB) and urea peroxidase added to the walls. In the presence of the antibody-antigen-antibody (HRP) sandwich immune-complex, the colourless chromogens hydrolyzed by the bound HPR conjugate a blue coloured product. The blue colour turns to yellow after stopping the reaction with sulphuric acid. The amount of colour measured is proportional to the amount of antigen in the sample (Test kit insert sheet).

Components: -Microwell Plate, Negative Control, Positive Control, HRP-Conjugate, Specimen Diluents, Wash buffer, Chromogen Solution A, Chromogen Solution B and Stop Solution.

Specimen collection, transportation and Storage.

Specimen collection:fresh serum or plasma specimen can be used.

Transportation and Storage: store specimen at 2- 8℃. Specimens not used for the assay within 1 weeks should be stored frozen (-20℃).

Procedure

Reagent preparation: allow reagents to reach room temperature (18-30℃).

1. Preparation: mark three wells as negative control and two wells as negative control and one blank.
2. Adding a Diluents: add 20µl of specimen diluents into each well except the blank
3. Adding specimen: add 100µl of positive control, negative control and specimen into their respective wells except blank.
4. Incubating: cover the plate with the plate cover and incubate at 37℃ for 60minutes
5. Adding HRP-conjugate: at the end of the incubation, remove and discard the plate cover. Add 50µl of HRP-conjugate into each well except blank, and mix by tapping the plate gently.
6. Incubating: cover the plate with plate cover and incubate at 37℃ for 30 minutes.
7. Washing: at the end of incubation, remove and discard the plate cover wash each well 5 times with diluted washing buffer. Each time allow the micro well to soak for 30-60 seconds. After the final washing cycle, turn down the plate onto blotting paper or clean towel and tap it to remove any remainders.
8. Colouring: add 50ul of chromogen solution A and then 50µl of chromogen solution B into each well including blank, mix gently incubate the plate at 37℃ for 30 minutes avoiding light. The enzymatic reaction between chromogen solutions and HRP-conjugate produces blue colour in positive control and HBsAg specimen wells.

9. Stopping reaction: Using a multichannel pipette or manual, add 50µl of stop solution into each well and mix gently. Intensive yellow colour develops in positive control and HBsAg specimen wells.
10. Measuring the absorbance: calibrate the plate reader with the blank well and read the absorbance at 450nm.

Quality control (Assay validation): the test results are valid if the quality control criteria fulfilled.

-The A value of the blank, well, which contain only chromogen and stop solution, is <0.80 at 450nm.

-The A value of the positive control must be ≥0.800 at 450/600-650nm or at 450 after blanking

-The A of the negative control must be ≤0.100 at 450/600-650nm or at 450nm after blanking. If one of the negative control 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 does not meet the quality control range specification, the test is invalid and repeated.

Interpretation of the results

Negative result (A/c.o.<1):Specimens giving absorbance less than the cut-off value are negative for this assay, which indicates that no hepatitis B virus surface antigen has been detected with theAID^{TM}HBsAg ELISA. Therefore, the patient is probably not infected with HBV and the blood unit does not contain hepatitis B virus surface antigen and could be transfused in case infectious diseases are absent.

Positive results (A/C.O.≥1): Specimens giving an absorbance equal to or greater than the cut-off are considered initially reactive, which indicates that hepatitis B virus surface antigen has probably been detected using AID^{TM} HBsAg ELISA. All initially reactive specimens should been retested in duplicate using AID^{TM} HBsAg ELISA before the final assay result interpretation. Repeatedly reactive specimens can be considered positive for hepatitis B virus surface antigen.

Borderline (A/C.O.=0.9-1.1):specimen with absorbance to cut-off ratio between 0.9 and 1.1 are considered borderline and retesting of these duplicate is required to confirm the initial result.

C) Anti-HCV rapid test principle

The HCV test strip (serum/plasma) detects antibodies to HCV through visual interpretation of colour development in the internal strip. Recombinant HCV antigen is immobilized on the test region of the membrane. During testing, the specimen reacts with recombinant HCV antigen conjugated to coloured particles and pre-coated onto the sample pad of the test. The mixture

then migrates through the membrane by capillary action and interacts with reagents on the membrane. If there are sufficient HCV antibodies in the specimen, a coloured band will form at the test region of the membrane. The presence of this coloured band indicates a positive result, while its absence indicates a negative result. The appearance of coloured band at the control region serves as a procedural control, indicating that the proper volume of specimen has been added and membrane wicking has occurred (Test kit insert sheet).

Test procedure

Allow the reagents to equilibrate temperature prior to testing

1. Remove the test from the sealed foil pouch use it as soon as possible. Best result obtained if the assay performed within one hour.
2. In test tube, transfer 2 drops (approximately 50µl) of serum of plasma to the sample pad of the strip, then add 1 drop of buffer and start the timer.
3. Wait for the lines to appear. The test should be read at 10 minutes. Do not interpret the after 20 minutes.

Result interpretation

Positive result: Two coloured lines appear. One line should always appear in the control region(C), and another line appears in the test region (T).

Negative: only one coloured line appears in the control region(C). No apparent coloured line appears in the test region (T).

Invalid: No line appears in the control region(C). Results from any test; which has not produced a control line at specified reading time, must be discarded.

D) Anti-HCV ELISA test principle

Polystyrene micro-well stripes are pre-coated with recombinant, highly immune-reactive antigens corresponding to the core and non-structural regions of HCV. During the first incubation step, anti-HCV specific antibodies, if present, will be bound to the phase pre-coated HCV antigens. The wells are washed to remove unbound serum proteins, and rabbit antihuman IgG antibodies (anti-IgG) conjugated to HRP is added. During the second incubation step, these HRP conjugated antibodies will be bound to any antigen- antibodies complexes previously formed and the unbound 18 HRP-conjugate is then removed by washing. Chromogen solutions containing Tetra-methyl Benzedrine (TMB) and urea peroxidase are added to the wells and in presence of the antigen-antibody-anti-IgG (HRP) immune-complex; the colourless chromogens are hydrolyzed by the bound HRP-conjugated to a blue coloured product. The blue colour turns to yellow after stopping the reaction with sulphuric acid. The amount of colour can be measured and is proportional to the amount of antibody in the sample (the inserted kit).

Components: microwell plate, negative control, positive control, HRP-Conjugate, Biotin Conjugate, wash Buffer, Chromogen solution A, Chromogen solution B, stop solution.

Storage and stability

The components of the kit will remain stable through the expiration date indicated on the label and package when stored between 2-8℃, do not freeze.

Reagent preparation**:** allow reagent to reach room temperature (18-30℃). Check the wash buffer concentrate for the presence of salt crystals.

Procedure

1. Preparation: mark three wells as negative control**,** two wells as positive controland one blank well. If the result will be determined by using dual wavelength plate reader, the requirement for use of blank well could be omitted. Use only number of strips required for the test.
2. Adding biotin conjugate reagent: Add 50µl of biotin conjugate reagent into each well except the blank.
3. Adding specimen: add 50µl of positive control, negative control and specimen into their respective wells except the blank, mix gently.
4. Incubating: cover the plate with the plate cover and incubate at 37℃ for 60 minutes
5. Washing: at the end of the incubation, remove and discard the plate cover, wash each well 5 times with diluted washing buffer. Each time allow the micro wells to soakfor 30-60 seconds. After the final washing cycle, turn down the plate onto blotting paper or clean towel and tap it to remove any remainders.
6. Adding HRP-Conjugate: Add 100µl of HRP-Conjugate into each well except the blank.
7. Incubating: cover the plate with the plate cover and incubate at 37℃ for 30 minutes.
8. Washing: at the end of the incubation, remove and discard the plate cover. Wash each well 5 times with diluted washing buffer. Each time allow the microwell to soak for 30-60 seconds. After the final washing cycle, turn down the plate onto blotting paper or clean towel and tap it to remove any remainders.
9. Colouring: add 50µl of chromogen solution A and then 50µl of chromogen solution B into each well including the blank, mix gently. Incubate the plate at 37℃ for 30 minutes avoiding light. The enzymatic reaction between chromogen solution and HRP-Conjugate produces blue colour in positive control and HCV antibody positive specimen wells.

10. Stopping reagent- using multichannel pipette or manually, add 50µl of stop solution into each well and mix gently. Intensive yellow colour develops in positive control and HCV antibody positive specimen wells.
11. Measuring the absorbance- Calibrate the plate reader with the blank well and read the absorbance at 450nm. If a dual the filter instrument is used, set reference wavelength at 600-650nm. Calculate the cut-off value and evaluate the result.

Quality control and calculation of the result

Each micro plate should be considered separately when calculating and interpreting the result of the assay regardless of the number of plates concurrently processed.

Calculation of the cut-off value (C.O.) = NC+0.12(NC= the mean absorbance value for the three-negative control).

Quality control (assay validation): 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 <0.80 at 450nm

- The A value of the positive control must be ≥10.80 at 450/600-650nm or at 450nm after blanking

-The A value of the negative control must be ≤0.100 at 450/600-650nm or at 450nm after blanking

Interpretation of the results

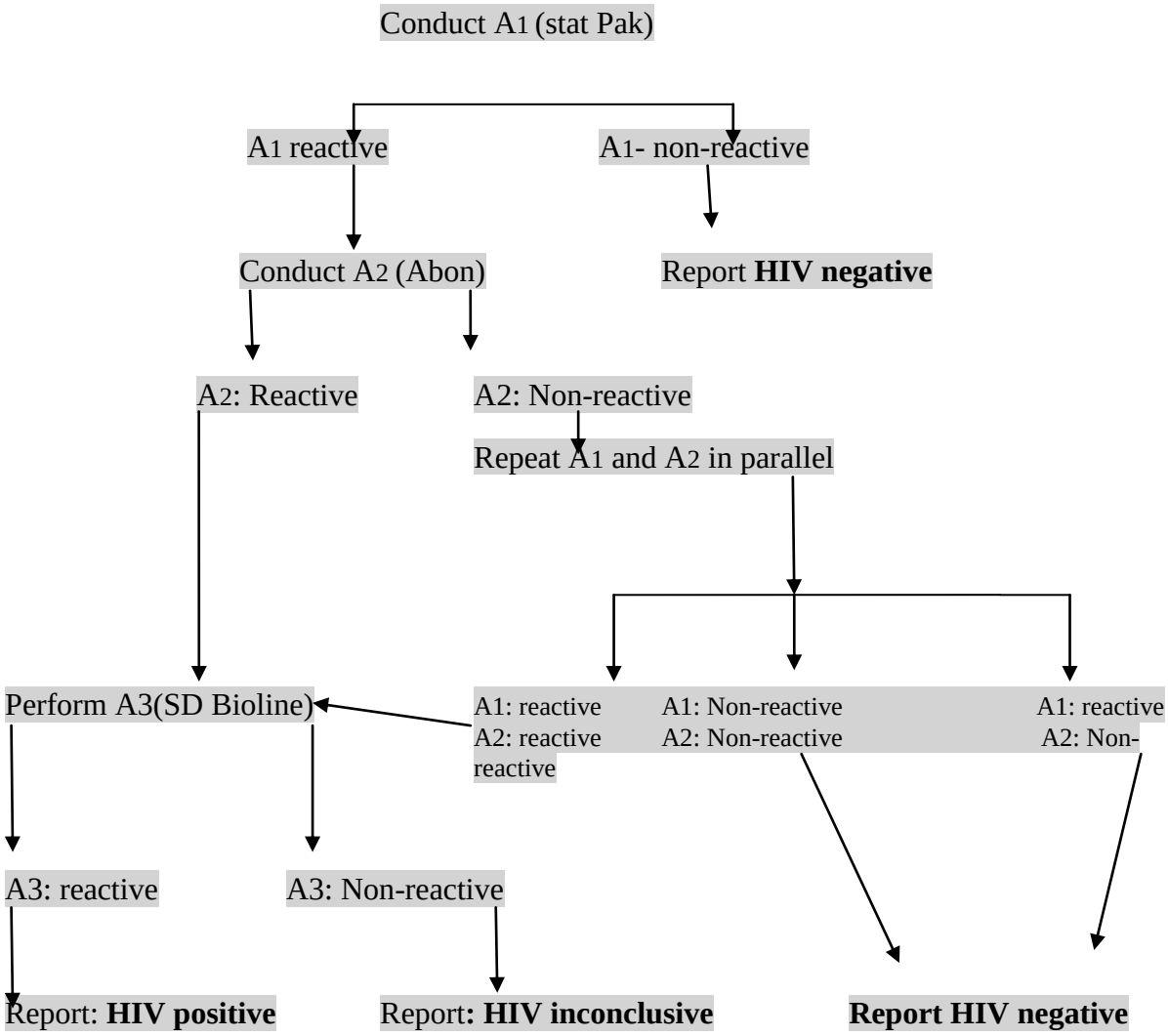
Negative result(A/C.O.<1): Specimens giving absorbance less than the cut-off value are negative for this assay, which indicate that no hepatitis C virus antibodies have been detected with AID™ anti-HCV ELISA. Therefore, the patient is probably not infected with HCV.

Positive Results (A/C.O.≥1): Specimens giving an absorbance equal to greater than the cut-off value is considered initially reactive, which indicates that hepatitis C virus antibodies have probably been detected using AID™ anti-HCV ELISA.

Borderline (A/C.O.=0.9-1.1): Specimens with absorbance to cut-off ratio between 0.9 and 1.1 are considered borderline and retesting of these specimens in duplicate is required to confirm the initial results.

HIV test

National HIV test algorithm for Ethiopia



E) HIV STAT-PAK® test principle

The Chembio HIV 1/2 STAT-PAK™ assay employs a unique combination of a specific antibody binding protein, which is conjugated to colloidal gold dye particles, and HIV 1/2 antigens, which are bound to the membrane solid phase. The sample is applied to the SAMPLE (S) well followed by the addition of running buffer. The buffer facilitates the lateral flow of the released products and promotes the binding of antibodies to the antigens. If present, the antibodies bind to the gold conjugated antibody binding protein. In a reactive sample, the dye conjugated-immune complex migrates on the nitrocellulose membrane and is captured by the antigens immobilized in the TEST (T) area producing a pink/purple line. In the absence of HIV antibodies, there is no pink/purple line in the TEST (T) area. The sample continues to migrate along the membrane and produces a pink/purple line in the CONTROL (C) area containing

immunoglobulin G antigens. This procedural control serves to demonstrate that specimen and reagents have been properly applied and have migrated through the device.

Test procedure

If the specimen to be tested is refrigerated, remove it from the refrigerator and allow it to come to a temperature of 18 to 30℃ prior to testing.

1. Remove the test device from its pouch and place it on a flat surface
2. Label the test device with patient name or identification number
3. Touch the 5µl sample loop provided to the specimen, allowing the opening of the loop to fill with the liquid
4. Holding the sample loop vertically, touch it to the sample pad in the centre of the sample well of the device to dispense~5µl of sample
5. Invert the running buffer bottle and hold it vertically over the sample well. Add 3 drops of buffer slowly, drop wise, into the sample well.
6. Read the test result 15 minutes after the addition of the running buffer. In some cases, a test line may appear in less than 15 minutes however, 15 minutes are needed to report a nonreactive. Read results in a well-lit area, do not read results after 20 minutes.

Interpretation of results

Nonreactive result: one pink/purple line in the control area, with no line in the test (T) area indicates a nonreactive test result. A nonreactive test result at 15 minutes indicates that HIV-1 and HIV-2antibodies.

Reactive results: Two pink/purple lines, one in the test area and one in the control area, indicate a reactive test result. The line in the test area may look different from the line in control area. Test result with visible lines in both test and control areas, regardless of intensity, is considered reactive. Reactive result means that HIV-1 and/or HIV-2antibodies have been detected in the specimen.

F) HIV Abon test principle

The HIV 1/2/O Tri-line Human Immunodeficiency Virus Rapid Test Device strip is pre-coated with HIV-1 and subtype O antigens on T1 test line and HIV-2 antigen on T2 test line. Firstly, specimen and then buffer are adding to the specimen well, thus starting the migration of the specimen/buffer. The specimen/buffer passes the conjugate pad, which contains a mixture of HIV-1 envelope and capsid antigens and HIV-2 envelope antigen. These detection antigens are conjugated to latex particles. If present, the HIV-1 of HIV-2 antibodies reacts and binds to the detection antigen-conjugate. The antibody/antigen-conjugate mixture then migrates further and binds to antigens present on the test lines. If the specimen contains antibodies to HIV-1, the

specimen will bind to the T1 test line and produce a line, if specimen contains antibodies to HIV-2, the specimen will bind to the T2 test line. As liquid continues to migrate down the test strip,the control line will appear, If the control line is present, in addition to either or both test lines, then the test is reactive for HIV1/2 antibodies. If the specimen does not contain HIV-1 or HIV-2 antibodies, no coloured lines will appear for either of the test lines region indicating a non-reactive result. Please note that the appearance of coloured lines at T1 and T2 is highly unlikely to be indicative of co-infection with HIV-1 and HIV-2 but rather is a result of cross-reactivity between antigens. A coloured line will appear in the control line region if the migration of liquid has been successful and must be present for the test to be valid. Its presence does not confirm sufficient specimen addition.

Test procedure

Allow the test device, buffer and specimen to reach room temperature (15-30℃) prior to testing.

1. Remove the test device from the foil pouch and use it as soon as possible
2. Place test device on a clean and level surface. Label with specimen ID. For serum/plasma specimens: hold the specimen dropper vertically and transfer one drop of serum/plasma to the specimen well of the test device, then add 1 drop of buffer and start timer.
3. Wait for the coloured red line to appear. Read results at 10 minutes. Do not read results after 20 minutes.

Interpretation of the result

Reactive: two or three distinct coloured line appears. One line should always appear in the control line region (C), and another one or two apparent coloured line should appear in the test line region(s) (T1 and /or T2).

The intensity of the colour in the test line region (T1 and /or T2 will vary but any shade of the colour in test line region (T1 and/or T2) should be considered reactive.

Dual infection of HIV-1 and HIV-2 is quite rare. Dual reactivity observed in Abon HIV1/2/0 Tri-line HIV rapid test device, i.e. HIV-1 line and HIV-2 line both reactive, is more likely to be caused by antibody cross reactivity. Any specimen with dual reactivity should be referred for specific HIV-2 confirmatory testing, if a discretionary result is required.

Non-reactive: one coloured line appears in the control region (C).No apparent coloured lines appear in the test line regions (T1 and/or T2).

G) SD Bioline test principle

The SD Bioline HIV-1/23.0 kit is a rapid qualitative test for the detection of antibodies to all isotypes (IgG, IgM, IgA) specific to HIV-1 including subtype-0 and =HIV-2simultaneously in human serum, plasma or whole blood. The SD Bioline HIV1/2 3.0 test contains a membrane strip, which is pre-coated with recombinant HIV-1 capture antigen (gp^{41}, p^{24}) on test band 1 region and with recombinant HIV-2 capture antigen (gp36) on test band 2 region, respectively. The recombinant HIV-1/2 antigen (gp41, p24 and gp36)- colloid gold conjugate and the specimen sample move along the membrane chromatographically to the test region(T) and form a visible line as the antigen-antibody-antigen gold particle complex forms with high degree of sensitivity and specificity. This test device has a letter of 1, 2 and C as test line1 (HIV-1), Test line 2 (HIV-2) and control line on the surface of the device. Both the Test lines and control line in result window are not visible before applying any sample. The control line is used for procedural control. Control line should always appear if the test procedure is performed properly and the test reagents of control line are working.

Procedure of the test

1. Remove the device from the foil pouch, place it on the flat, dry surface
2. Add 10µl of plasma or serum specimen into the sample well(s)
3. Add 4 drops of assay diluents vertically into sample well(s)
4. As the test begins to work, you will see purple colour move across the result window in the centre of the test device
5. Time to result is 10 to 20minutes. After adding, the diluents read the result after 10 minutes but not more than 20 minutes.

Interpretation of the test

Negative result: The presence of only control line (C) within the result window indicates a negative result.

Positive result

1. The presence of two line as control line(C) and test line 1 (1) within a result window indicates a positive result for HIV-1
2. The presence of two line as a control(C) and test line 2 (2) within attest window indicates a positive for HIV-2.
3. The presence of three line as control line (C), test line 1 (1) and test line 2 (2) within the result window indicates a positive result for HIV-1 and/or HIV-2.

If the colour intensity of the test line 1 is darker than one of the test lines 2 in the result window, the result can be interpreted as HIV-1 positive.

If the colour intensity of the test line 2 is darker than one of the test lines 1 in the result window, the result can be interpreted as HIV-2 positive.

Annex Ⅱ:- Thesis declaration:

I, the undersigned, have to accept all the scientific responsibilities and ethical conduct of the research projects. I have provided a timely progress report to my advisor and sought necessary advice and approval from my advisors in the course of the research. I have communicated timely with my advisors and all stakeholders regarding my project.

Name of the principal investigators: Rago Edao (BSc)

Signature_____ Date_____

Name of Advisors:

1. Kasu Desta (MSc, PhD Fellow, Assistant Professor) Signature_____ Date_____
2. Biruk Zerfu (MS, PhD Fellow) Signature_____ Date_____