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Prevalence of hepatitis B and C Viruses associated risk factors and knowledge, attitude and practice among refugees at Pugnido-I camp in Gambela, Ethiopia.

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This thesis prepared by Abiyu Ayele which is entitled Prevalence of Hepatitis B and C Viruses, Associated Risk Factors and Knowledge, Attitude and Practice among refugees at pugnido-I refugee camp in Gambela, Ethiopia**.** This is my original work and submitted for the partial fulfillment of the requirements for the degree of Master of Sciences in Clinical Laboratory Sciences (Diagnostic & Public Health Microbiology). It complies with the regulations of the University and meets the accepted standards with respect to originality and quality. All sources of material used for the thesis has been duly acknowledged.

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List of Abbreviations

CDC	Centers for Disease Control and Prevention
CHB	Chronic Hepatitis B
DNA	Deoxyribose Nucleic Acid
ELISA	Enzyme Linked Immunosorbent Assay
HAV	Hepatitis A Virus
HBcAg	Hepatitis B core Antigen
HBsAg	Hepatitis B surface Antigen
HBV	Hepatitis B Virus
HCC	Hepatocellular Carcinoma
HCV	Hepatitis C Virus
HDV	Hepatitis D Virus
HEV	Hepatitis E Virus
HIV	Human Immunodeficiency Virus
KAP	Knowledge, attitude and practice
MTCT	Mother to Child Transmission
RNA	Ribose Nucleic Acid
SNNPRs	Southern Nations Nationalities and Peoples' Regional States
SOPs	Standard Operating Procedures
UNHCR	United Nations High Commissioner of Refugees
WHO	World Health Organization

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Abstract

Background: In the last two decades Ethiopia has witnessed increasing immigration flows from South Sudan, Somalia and Eritrea. However, these immigrants are not screened for hepatitis B and C which could be additional burden for the local population.

Objective: To determine Prevalence of hepatitis B and C viruses, associated risk factors and knowledge, attitude and practice (KAP) among refugees at Pugnido-I refugee camp in Gambela, Ethiopia.

Methods: Cross-sectional study was conducted on 453 refugees at Pugnido-I refugee camp in Gambella region from January to May 2018.Socio-demographic, risk factors and KAP was assessed by using structured questionnaires. Five ml blood sample was collected with serum separator tube. HBsAg and anti-HCV rapid screening tests were performed and those positive samples were confirmed using ELISA method. Data was entered and analyzed using SPSS statistical software version 20. The odds ratio and 95% confidence interval was calculated to assess the strength of the association. P-value less than 0.05 were considered as statistically significant.

Results: The overall prevalence of HBV and HCV among refugees was 7.3 %(n=33/453) and 2.0 %(n=9/453) respectively. Of those 6.8% (n=25/370) and 1.4% (n=5/370) of females were positive for HBV and HCV respectively. And 9.6 % (n=8/83) and 4.8 % (n=4/83) of males were positive for HBV and HCV respectively There was no significant association between HBV and proposed risk factors (p>0.05), however, statistical significant association was observed between HCV and age group of 18-25years (AOR=0.045, CI 95%=0.005-0.378, P=0.004) and 26-35years (AOR=0.035, CI 95%0.004-0.301, P=0.002). From the total participants, 86.5%(n= 392/453) did not know how the disease is transmitted, 8.2%(n= 37/453) believed that hepatitis can be transmitted through food, 86.8%(n= 393/453) had no information about the availability of HBV vaccine and 98.5%(n=446/453) were not vaccinated.

Conclusion: The prevalence of hepatitis B and hepatitis C among refugees was intermediate which might be due to low knowledge and attitude towards the transmission and prevention of the disease. Therefore this indicates the need for creating awareness for refugees about the transmission and prevention mechanisms of hepatitis B and hepatitis C infection**.** Large scale study is recommended at national level.

Key Words: Hepatitis B virus, Hepatitis C virus, Pugnido Refugee camp, Gambella, Ethiopia.

1. Introduction

1.1. Background

Hepatitis B, C and other viruses can cause hepatitis. It is clinically silent infections for decades until developing cirrhosis, end-stage liver disease and hepato-cellular carcinoma. Hepatitis B and C viruses can cause both acute and chronic infection [1]. Infection with hepatitis B and C virus affects the liver and results in a broad spectrum of disease out come. An infection with HBV can spontaneously resolve and lead to protective immunity, result in a chronic infection and, in rare cases, cause acute liver failure with a high risk of dying [2].

HBV and HCV are transmitted by percutaneous or mucosal exposure to the blood or body fluids of an infected person, from an infected mother to her newborn during childbirth, through close contact within households, through exposures to unscreened blood transfusion or unsafe injections in health care settings, through injection-drug use, and from sexual contact with an infected person [1].

Refugee populations are more at risk of having sexually transmitted infections (STI), like HBV and HCV. The contributing factors include: origin from countries that are highly endemic for these infections, lack of information on hepatitis prevention directed to the migrant communities in the host country. No screening takes place for infectious diseases of sexual transmission, though this is recommended in the protocols of the Centers for Disease Control and Prevention [3]. Associated risk factors of hepatitis B and C viruses; Occupational exposure to blood and body fluids, history of sharp injury, blood donation family history of liver disease, tooth extraction, tattoo [4].

Diagnosis is based on clinical information and laboratory screening. HBV and HCV infection cannot be differentiated on the basis of clinical symptoms alone, and definitive diagnosis depends on the results of serologic testing such as rapid screening and ELISA [1].

About 350 to 400 million people infected with HBV due to this 1 million people deaths per year and also 130 to 170 million people are infected with HCV that cause of about 350,000 deaths per

year [5].The World Health Organization classifies countries according to the hepatitis B surface antigen (HBsAg) into low (<2%), intermediate (2–8%), and high (>8%) prevalence [6].

Today, many people are obligated to leave their country and seek asylum in foreign countries due to war and poverty, as well as to political, economic and social reasons in the last two decades Ethiopia has witnessed increasing immigration flows from the different neighbor countries like South Sudan, Somalia and Eritrea and others. Our public health policies are not focused on screening immigrants for an infectious disease and no systematic actions against blood borne diseases like hepatitis B and C are implemented [7].

1.2 Statement of the problem

Viral hepatitis is the tenth leading cause of death and the leading cause of liver cancer worldwide [6].About 85% of the world's population live in areas of endemic of HBV such as intermediate (2–7.9%) or high (> 8%).Some studies in specific populations indicated that prevalence of infection ranging from 0.1% to 8%.Most individuals with chronic hepatitis B or C are unaware of their infection status, because they can remain asymptomatic for year[8].About 350 and 80 million people are estimated to have been chronically infected by HBV and HCV respectively and three-to-four million new cases are detected each year [7].

About 3-4 million newly infected cases each year of HCV and a mean global sero-prevalence of 2.2-3.0%. Approximately 70% of those with acute infection develop chronic HCV infection and 20% of these individuals develop cirrhosis and 1-5% develops hepato-cellular carcinoma (HCC) during the two decades following initial infection. There is no effective vaccination to prevent against transmission of HCV. Chronic hepatitis C infection however, can easily detected through widely available screening blood tests and standard treatment regimens are moderately successfully (50% overall) in achieving sustained viral response (SVR) which in those with cirrhosis decreases disease progression to liver failure and HCC [9].

Studies from the Horn of Africa, particularly Ethiopia, report a HBsAg carriage rate between 5.4 and 15% and anti-hepatitis C virus positivity between 0.8 and 5.1% in the different groups Considered. Therefore, the arrival refugees may influence the prevalence of HBV and HCV among the Ethiopian population, especially if no appropriate preventive programmed are planned [10].

During the last two decades, refugees from countries with an increased prevalence of infectious diseases, such as viral hepatitis. The crowded living conditions and the avoidance of well-organized places of sheltering, due to the fear of repatriation or expulsion, characterize immigrant populations and facilitate the spread of these diseases [11]. In addition the increasing numbers of immigrants from high to intermediate or low endemic regions forms a new dynamic epidemiology of hepatitis transmission [12].

Increased migration volume and different hepatitis prevalence between immigration and emigration countries have changed the Hepatitis viruses epidemiology considerably[13].As we

know there are too many immigrants settled in Ethiopia from different neighbor countries mainly originated from Somali, Eritrea, South Sudan and other countries. These immigrants cross the border of the country without any health screening criteria and live at different refugee camps with cohesion of the local people. Therefore, different disease can be transmitted from refugees to local population unless extended health education and preventive mechanism planned in the country. Moreover, these immigrants living system is not limited to in the camp rather highly communicated to the local population no rule and regulation that restrict the movement of the refugees through the local society. There are insufficient information about the prevalence of hepatitis B and hepatitis C among refuges in Ethiopia. Therefore, this study was aimed to determine the prevalence of hepatitis B and C virus among South Sudan refugees at pugnido-I refugee camp Gambela, Ethiopia.

1.3 Significance of the study

This study provides the following information to the responsible bodies:

- The study generates information on the prevalence of hepatitis B virus and hepatitis C virus among refugees that helps health policy makers for designing appropriate intervention strategies.
- This study gives information to the local responsible bodies to be aware about the disease status among refugees to pay attention about the prevention of healthy population and treatment the exposed individuals.
- Moreover, the finding of the study uses to formulate preventive mechanisms to halt the spread of the disease to the local society as well as countrywide.
- Finally, the information obtained from knowledge, attitude and practice of participants about the disease can give clue to the responsible bodies for planning health education program based on the level of understanding of targeted group.

2. Literature review

A cross-sectional survey conducted by Quddus A.*et al*, in Pakistan to estimate Prevalence of hepatitis B among Afghan refugees living in Baluchistan. From a total of 903 Afghan refugees the investigators found that 75 study subjects were positive for HBsAg. From which, 37 husbands, 21 wives and 17 children has been positive result for HBsAg. The prevalence of HBsAg among Afghan refugees in Baluchistans Province was 8.3%, while the prevalence among husband, wife and children were 12.3%, 7.0% and 5.6%, respectively. From their result they concluded that vaccination is necessary for Afghanistan refugees [14].

Stevens K, *et al* conducted a cross-sectional study in India, to investigate hepatitis B prevalence and treatment needs among Tibetan refugees residing; The investigators selected 2,769 participants, 945 (34.1%) were from households , 1,153 (41.3%) were from the boarding school, and 671 (24.6%) were from the monastery. The assessment showed that from a total of 247 participants 8.9% were positive for HBsAg. Focusing just on the house hold sampling, 11.9% were positive for HBsAg. And they generalized that vaccination of HBV is important to stop the spread of infection [15].

Another study conducted by Hussein N R,*et al,* in Iraq to investigate Prevalence of HBV, HCV and HIV Infections Among Syrian Refugees in Kurdistan Region. From a total of 880 refugees, 34 cases (3.86%) were positive for HBsAg. All samples showed HCV negative. From the local people, 2,975 were volunteer to participate in the study of which, 30 (1.09%) were positive for HBsAg and only one was positive for HCV.The investigators conclude that the prevalence of HBV in Syrian refugees was nearly fourfold higher than that of indigenous people of Iraq[16].

The study conducted by Chironna M.*et al* in Kosovar 2001; Prevalence of Hepatitis Virus Infections in Kosovar Refugees;The investigator Among the 526 Kosovar refugees, Among the 526 refugees, the prevalence of total anti-HAV antibodies was 81%. The prevalence of anti-HEV antibodies was 25%. Fifteen subjects (2.9%) were positive for hepatitis B surface antigen (HBsAg), whereas 17.5% tested positive for anti-hepatitis B core antigen (anti-HBc).Whereas 25.9% were found to be positive for anti-HBc. None of the refugees tested positive for anti-HDV.The study group concludes that,an immunization policy against HBV infection, through vaccination of all newborns and children before adolescence, may be advisable [17].

A narrative systematic review conducted by Owiti J A, *et al*, in UK to assess Illness perceptions and explanatory models of viral hepatitis B & C among immigrants and refugees. By using `51 publications.The studies focused on hepatitis B and ethnic groups of South East Asian immigrants in USA, Canada, and Australia. Most immigrants lack of adequate knowledge of etiology, symptoms, transmission risk factors, prevention strategies, and treatment, of hepatitis HBV and HCV. The variation of knowledge becomes from Ethnicity, gender, better education, higher income, and English proficiency. Immigrants are high risk to HBV and HCV, because of lack of knowledge. The investigators conclude that, immigrants are vulnerable to HBV and HCV, because of poor knowledge [18].

A cross-sectional study in UK by Evlampidou I,*et al* in titled by Low hepatitis B testing among migrants. A total of 82 561 migrants who had informed to test HBV testing, 9627 (12%) were 'HBV tested. The test coverage indicate that Eastern Africa 20%,Western Africa 15%, South Eastern Asia 9%, Eastern Asia 5%.Of which born in UK 82 561 (17%)were identified with ≥2% HBV prevalence. The investigators concludes that, much greater support for primary care and awareness of national guidance are required[6].

The study conducted by Padovese V.*et al* in Malta with title of Prevalence of latent tuberculosis, syphilis, hepatitis B and C among asylum seekers in Malta. The investigator selected 500 migrants and screened for latent TB, hepatitis B, C and syphilis. Among them, 83.2% (n ¼ 416) were from Somalia, 8.2%, from Eritrea, 2.4%, from Ethiopia and 5.6%, from West African countries. 81.2%, were males and the mean age was 26.5 years. The study group suggests that, systematic screening for asymptomatic migrants recommended for HBV but not HCV [10].

In Italy the study conducted by Coppola N, *et al*, to estimate Hepatitis B virus infections in immigrant populations from different geographical regions such as South East Asia (0%-27.3%). The HBsAg Seroprevalence in sub-Saharan immigrants ranged from 7.4% to 13.9%.High prevalence were also observed in Albanian refugees two Greek studies, 11.7% and 15.3%, respectively. The investigators concluded that the immigrant's prevalence rose due to lack of health care management, funds and follow up. The investigators conclude that, HBV vaccination, good quality medical care and improved quality of life are the first steps [19].

Another study performed by Coppola N, *et al*, in 2017,to estimate Hepatitis B virus infection in undocumented immigrants and refugees in Southern Italy, show that of the 1,212 immigrants screened, 116 (9.6%) were HBsAg positive,490 were HBsAg negative/anti-HBc positive, and 606

were seronegative for both. The investigators suggests that, provide screening, HBV vaccination, treatment and education to immigrants [20].

The study conducted by Palumbo E, *et al,* in Italy, to determine prevalence of HBV-genotypes in immigrants affected by HBV-related chronic active hepatitis. From a total 556 men participants tested, 60 (10.7%) resulted HBsAg positive. From these 42 (70%) African 10 (16.6%) Asian and 9 (14.4%).In real-time PCR, Genotype distribution was as follow: genotype E, 16 (50%), genotype D, 9 (28.1%), genotype A, 7 (21.9%).The study group concludes that, prevalence of HBV-infection in immigrants, characterized by a different natural history and, a different response to antiviral treatment[21].

The systematic review and meta-analysis by Greenaway C.*et al*, 2015 in Germany, a total of 973 titles and abstracts were screened, 173 full text articles were reviewed, and 50 were included in this study. Immigrants account that 38635 which are around the world regions who arrived in Australia, Canada, Europe, Israel and the United States. The study resulted in anti-HCV prevalence of 1.9%.The investigators concluded, that adult migrants originating from Asia, Sub-Saharan Africa and Eastern Europe are at high risk for HCV and may benefit from targeted HCV screening. The investigators conclude that, migrants at increased risk for HCV have being benefit from targeted HCV screening [22].

The study conducted by Roussos A.*et al,* in Athens 2003;Prevalence of hepatitis B and C markers among refugees in Athens; The study group Twenty individuals (15.4 %) were HBsAg positive and 69 (53.1 %) were anti-HBc positive. The prevalence of HBsAg and anti-HBc was higher among refugees from Albania and Asia. The prevalence of these markers was found irrelevant to age or sex. Anti-HCV was detected in the serum of 3 individuals (2.3 %). No differences among age, sex or ethnicity regarding anti-HCV prevalence were found. The study group concluded that, HBV vaccination programs will be necessary [23].

A study conducted by Subramanian K, *et al,* in Australia to determine Hepatitis B status in refugees, increasing health burden in Western Australia. From a total of 478 with the majority migrated from Asia (57%) and Africa (35%). About 50% of CHB patients are at risk of cirrhosis and hepato- cellular carcinoma that do not get treatment. The study determines the prevalence of Asian and African immigrants 0.05%. The investigators conclude that, increasing economic burden of CHB patients and increasing direct costs of treatment [24].

Chloe J H, *et al*, conducted a cross-sectional study in USA to determine Hepatitis B and Liver Cancer Beliefs among Korean Immigrants in Western Washington. The investigators select 30 participant interviews and belief that, food is main cause of hepatitis B transmission. Some of them recognized by clinicians about HBV transition such as blood exposure, sexual contact, or maternal–child vertical transmission (no interviews).The majority believe that prevent by altering eating habits, avoiding contaminated meats. The investigators suggest that, vaccination will likely require careful attention for misconception of transmission of HBV [25].

Another study performed in USA by Rein B D, *et al*, to determine the Prevalence of Hepatitis B Surface antigen among refugees entering the United States Between 2006 and 2008.Refugees screened from 20 areas a total of 42,303 refugees. The study indicated that highest HBsAg prevalence from Africa (8.1%), Asia (4.8%) eastern Europe (2.6%), and South/Central America and the Caribbean (1.0%).In Africa, the highest prevalence was observed in refugees from Eritrea (15.5%); the lowest prevalence Burundi (3.0%).In Asia the highest prevalence from Myanmar (12.4%).Prevalence in European countries ranged from 0.08%in Russia to 5.9% in Moldova. Refugees from South American, Central American and Caribbean, prevalence below 2.0%, Haitian refugees, and prevalence were 2.6%.The assessor concludes that, refugee prevalence may differ from the prevalence among the general population [26].

In Northern California, a cross- sectional survey study was conducted by Levy V, *et al,* about 1,512 immigrant men aged 18 to 35 screened HBV prevalence and risk behaviors. Asian immigrants have had prior HBV infection (15.1%) and chronic infection (3.8%) compared to US born (prior 5.1%, chronic 0.6%) and Latino immigrant men (prior 2.0%, chronic 0.3%).The study group concludes that, HBV testing and vaccination of immigrants soon after US arrival should be encouraged [27].

The study conducted by Shire A M, *et al,* in Somalia, to know the status of Viral Hepatitis Among Somali Immigrants in Minnesota: Association of Hepatitis C With Hepato-cellular Carcinoma. The study group recruited 99 males and 66 females for anti-HCV from which 68 of 73 Somalis (93.2%) with positive anti-HCV test results had active HCV infection. Of 30 Somali patients with HCC, 22 (73.3%) tested anti-HCV positive viral hepatitis was diagnosed coincident with HCC. The investigators concluded that, screening immigrants for HCV infection may enhance the prevention, early detection, and optimal treatment of HCC [28].

A study performed by Cella E, *et al,* in 2016 to identify epidemiological analysis of Hepatitis B virus infection in migrants. The study group recruited 136 Malian participants screened, HBV positive 16 (11.7%),Hepatitis B serologic test results 13(81.25%) reactive for HBsAg and none for HBs-Abs. Additionally HBeAg was reactive 11(68.75%).HCV-Ab-positivity 18.75%, all HCV-RNA negative. The study group conclude that, give an important improvement in prevention campaigns and monitoring of the viral infection in migrants. [29].

The study conducted by Daw M.A.*et al,*in Libia.2016 to assess; Geographic integration of hepatitis C virus. A global threat prevalence of HCV in the world (14.7%-32%). It is alarming that > 20% of Egyptian blood donors are seropositive for HCV. Libya, considered an area of low endemicity for hepatitis C (1.2% average prevalence) In Iran, the prevalence of anti-HCV antibodies ranges from 0.2% to 6.25% . HCV prevalence was higher in Iraq (2.3%), Jordan (3.5%) and the Gaza strip (2.2%) Syria (1%).There is no national studies on the prevalence HCV in. The assessor concludes that, geographic integration of HCV is reflected in the prevention and treatment of this ongoing pandemic [30].

Short report presented by Franco-Paredes C, et al, in USA on untreated tropical infectious diseases among Sudanese refugees in the United States. The reporters during the study period from July 2005 to December 2006,medical evaluations of the 44 of 150 Atlanta area lost boys,
mean age 25 years were completed. Of the 31 patients in whom we were able to obtain hepatitis B diagnostic serologies, 10 (32%) showed evidence of HBsAg[31].

The study conducted by Chandrasekhar E, *et al,* in Chicago to identify of African-Born People With Chronic Hepatitis B Virus infection in the Chicago Metropolitan Area, 2012–2014.The study group selected 1,000 African-born people. Of which are Democratic Republic of the Congo (14%), Nigeria (13%), Ghana (11%), Somalia (11%), and Ethiopia (10%). Of which 35 (8%) HBsAg-positive people, 37% had evidence of past infection, and29% were immune. The study group concludes that, the large proportion of HBsAg-positive people reinforces the need for health promotion programs [32].

3. Objectives

3.1. General Objective

To determine the Prevalence of Hepatitis B and C Viruses, Associated Risk Factors and Knowledge, Attitude and Practice Among Refugees at Pugnido-I refugee camp in Gambela, Ethiopia.

3.2. Specific objective

- To determine prevalence of hepatitis B and C viruses among refugees
- To assess associated risk factors related to Hepatitis B and C Viral infections in refugees
- To assess knowledge, attitude and practice among refugees about Hepatitis B and C Viral infections

4. Hypothesis

Null hypothesis (H_O)

The Prevalence of HBV and HCV in this study group is not different from in the previous studies in Minnesota on Somalia migrants [28].

5. Materials and Methods

5.1. Study design and period

A cross-sectional study was conducted to determine the prevalence of HBV and HCV by taking blood samples and screened sera with rapid test and positive tests were further tested with ELISA, and associated risk factors as well as KAP was assessed using a semi-structured questionnaire. The study was conducted from January, 2018 to May, 2018 at Pugnido-I refugee camp in Gambella, Ethiopia.

5.2. Study area

Gambela is the region that situated in South-West part of Ethiopia and borders with Benishangul Gumuz and Oromiya regions to the North, the Southern Nations, Nationalities and Peoples' Regional State (SNNPRS) and the South-Sudan Republic to the South, Oromiya and SNNPRS to the east and the South-Sudan Republic to the west. Gambella is located to the southern direction and apart about 711.2 km from Addis Ababa. In the region there are six refugee camps which encompass the total of 285,846 refugees, namely Pugnido-II, 16, 820, Nguenyyiel 28,243, Kule 51,272, Jewi 56,989, Pugnido-I accounts about 62,925 and Tierkidi 69,597 refugees. Of which the study was conducted at Pugnido-I refugee camp.

5.3. Population

5.3.1. Source of population

All refugees who were living at Pugnido-I camp during the study period.

5.3.2. Study population

Refugees who have living at Pugnido-I camp and visiting the refugees camp health center during the study period and who were volunteer to participate in the study.

5.4. Inclusion and exclusion criteria

5.4.1. Inclusion criteria

Refugees aged ≥18 years.

5.4.2. Exclusion criteria

Refugees who knew their status of HBV and HCV.

5.5. Study variables

5.5.1 Dependent variables

- Prevalence of hepatitis B virus among refugees
- Prevalence of hepatitis C virus among refugees
- Knowledge, attitude practice (KAP) of refugees about HBV and HCV.

5.5.2. Independent variables

- Socio demographic (Sex, age, marital status, educational status)
- History of multi sexual partner in life
- Sharing of sharp materials
- History of tattooing
- Family history of liver disease
- History of tooth extraction
- HBV vaccination status
- Awareness of hepatitis/liver disease/
- Sexual transmission of hepatitis infection
- Body fluid transmission of hepatitis infection
- HBV have vaccine

5.6. Sample size and sampling procedure

5.6.1 Sample size determination

The estimated sample size was about 384 participants assuming a proportion of 50%.Sample size derived using the following formula:

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

Where: n= sample size; Zα/2 = standard normal distribution abscissa corresponding to 95% confidence interval (1.96); *P* = proportion of 50%, *Q = (1-P);* and *d* = desired level of precision (5%).

$$n = \frac{(1.96)^2 \times 0.5(1-0.5)}{(0.05)^2} = 384$$

For the calculation, a 95% confidence interval and a 5% margin of error is used. To minimize errors arising from the likelihood of non-compliance/non response/, ten percent (10%) of the sample size is added giving a final sample size of 422.However, we have collected 453 samples.

5.6.2. Sampling Technique

Purposive sampling technique was used to select refugee camps due to well organized laboratory, huge number of refugees in the camp and convenient sampling technique was used to select study participants who visited the clinic during the study period.

5.7. Data collection and laboratory Analysis

5.7.1. Data collection

Written consent was obtained from study groups and after that questionnaire was used to collect informa.tion about knowledge, attitude and practice of the disease, the exposure status of potential risk factors and to assess the socio demographic characteristics of the study participants was used.

The questionnaire was prepared in English language by principal investigator in simple understandable language. Information was given to the study participants about; the benefit of the study, individual's right, informed consent and before started the data collection

5.7.2. Laboratory analysis

After obtaining the participant written consent, 5ml of blood sample was collected from each refugee using gel and clot activator tubes. The tubes were label and process at the time of collection. The blood sample was centrifuge for 10 minutes at speed of 3000rpm and the serum was separated. After separation, all serum samples were tested for HBsAg with rapid screening method according to the manufacturer's instruction of the selected test kit then, all serum samples were transported using cold box to Ethiopian Airports Enterprise clinic laboratory and anti-HCV rapid screening was performed. All rapid positive tests were confirmed using ELIASA at Ethiopian Public Health Institute (EPHI). All serum samples which tested positive by rapid test for HBsAg and anti-HCV were further tested with ELISA for confirmation.

5.7.2.1. HBsAg rapid test principle

The principle of the test is immune-chromatography which has two unique site immune-assays on a membrane. The test sample flows through the membrane assembly of the cassette, the color monoclonal anti-HBsAg, colloidal gold conjugate complexes with HBsAg in the sample. This complex moves further on the membrane to the test region where it is immobilized by another monoclonal anti-HBsAg antiserum coated on the membrane. Pink-purple color band formation confirms a positive test result and absence of the color band in the test region indicates a negative test result. Un reacted 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 color band. This control band serves to validate the test result (Test kit insert sheet).

5.7.2.2. Anti-HCV rapid test principle

Rapid test which is followed the principle of immune-chromatography, unique two site immunoassay on a membrane. As the sample flows through the membrane of the test cassette, the test color HCV antibody colloidal gold conjugate complexes with anti-HCV in the sample. This complex moves further on the membrane to the test region where it is immobilized by HCV antigen coated on the membrane leading to formation of a pink-purple color band which confirms a positive test result. Absence of this color band in the test region indicates a negative test result. Un reacted conjugate and unbound complexes, if any moves further on the membrane and are subsequently immobilize by the anti-HCV coated on the membrane at the control region, forming a pink/purple color band. This control band serves to validate the test result (Test kit insert sheet).

5.7.2.3. HBsAg ELISA test principle

This is a Sandwich Enzyme linked Immune-sorbent assay method in which polystyrene micro-well strips are pre-coated with monoclonal antibodies specific to HBsAg. Participants serum or plasma sample is added to the micro-wells 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, is 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 are added to the walls. In the presence of the antibody-antigen-antibody (HRP) sandwich immune-complex, the colorless chromogens are hydrolyzed by the bound HPR conjugate a blue colored product. The blue color turns to yellow after stopping the reaction with sulfuric acid. The amount of color can be measured and is proportional to the amount of antigen in the sample (Test kit insert sheet).

5.7.2.4. 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

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 colorless chromogens are hydrolyzed by the bound HRP-conjugated to a blue colored product. The blue color turns to yellow after stopping the reaction with sulfuric acid. The amount of color can be measured and is proportional to the amount of antibody in the sample.

5.8. Data entry and analysis

The coded data was entered and analyzed by SPSS software version 20. In the analysis process, frequency distributions of variables were workout in order to describe in relation with the study population. Descriptive statistics like, percentage and ratio were calculated. The associations between dependent and independent variables were measured by means of odds ratio for which 95% confidence interval was calculated. Variable that show a statistically significant association ($p<0.05$) was analyzed at bivariate and multivariate level.

5.8.1. Quality assurance and quality control

The quality of data was controlled starting from the time of questionnaires preparations. The questionnaires were developed by reviewing relevant literatures on the subject to ensure reliability. Instruction was given for data collectors on the purpose of study and procedures of data collection before started the study. During data collection, the principal investigator was receiving questionnaires from data collectors and review for completeness, accuracy, and consistency. Standard operating procedure (SOPs) of tests was followed during laboratory analysis and a known positive and negative serum for HBV and HCV testing was used. The result was entering to statistical software to analyze and summarize the data.

5.9. Ethical considerations

An ethical review committee of the Department of Clinical Laboratory Sciences, School of Allied Health Sciences, College of Health Science, and Addis Ababa University was approved this study with an ethical letter. After written permission was obtain from the office of the Administration of Refugees and Returnees Affair and UNHCR. Names and any other sensitive personal information of individual study subject was not record during sample collection. Moreover the sample

collectors were laboratory professional working in the laboratory department of the refugee clinic and were being monitored daily by the principal investigator. Sample was collected after getting consent from the participant refugees. All positive results were communicated to the attending physician. The confidentiality of the test result of the study was kept by investigator.

5.10. The overall work flow diagram

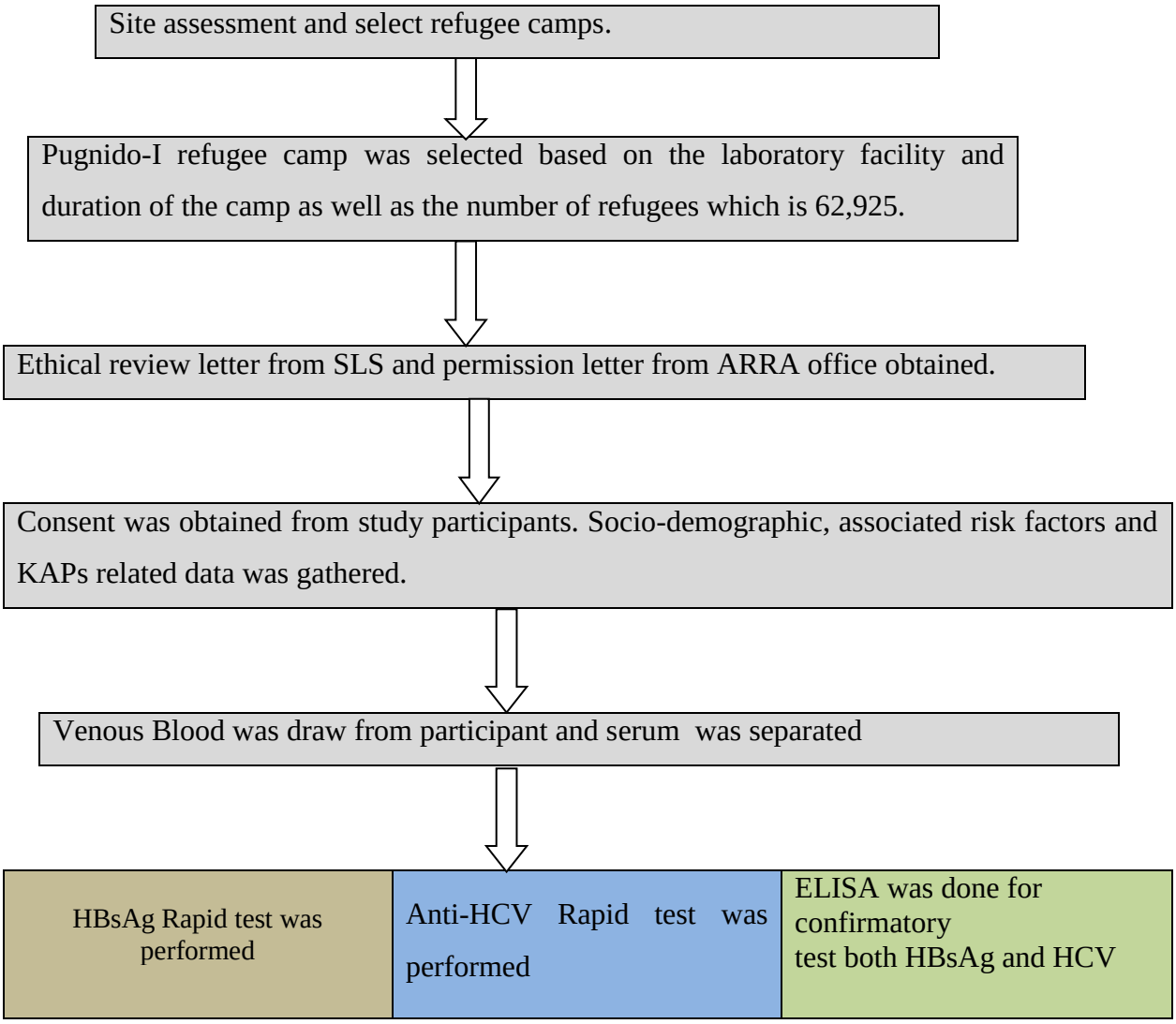

Fig-1 .Work flow diagram of the study

5.11. Operational definitions

Refugees: South Sudan immigrants live in pugnido-I camp in Gambella

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

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

HBsAg: low (<2%), intermediate (2–8%), and high (>8%) according to WHO [4]**.**

6. Results

6.1: Socio-Demographic Characteristics

During the study 473 volunteer clinic visitors have given their consent to be participating in the study and the questionnaire was filled, but 11 participants were excluded due to improper blood collection and 9 participants were rejected with incompleteness of the questionnaire with the total rejection of 4.2% (n=20/473). From the total of 453 participants 81.7 %(n=370/453) were female and 18.3%(n=83/453) were male and the age range of the participants 18-61years old and the mean age was 29.6±9.3SD. Majority of age group was 26-35, 43.3 %(n=196/453). Related to marital status and educational status, 83.7 %(n=379/453) of them were married and 36.6 %(n=166/453) participants were elementary level respectively as showed in (Table 6.1).

Table 6.1: Socio-Demographic Characteristics among refugees of Pugnido-I camp Gambela, Ethiopia, January to May 2018 (n=453), 2018.

Variables		**Frequency(n=453)**	**Percentage (%)**
Sex	Female	370	81.7
	Male	83	18.3
Age category	18-25	167	36.9
	26-35	196	43.3
	36-45	57	12.6
	46-55	24	5.3
	>55	9	2.0
Marital status	Single	25	5.5
	Married	379	83.7
	Divorced	22	4.9
	Widowed	27	6.0
Education	Illiterate	165	36.4
	Elementary	166	36.6
	High school	104	23.0
	Higher education	18	4.0

n=total sample size

6.2. Prevalence of HBV and HCV

All participants were screened with HBsAg and Anti-HCV rapid tests. From those 7.3 %(n=33/453) were positive for HBsAg and 2.2 %(n=10/453) were positive for Anti-HCV. ELISA confirmed prevalence of HBV and HCV among refugees was 7.3 %(n= 33/453) and2.0 %(n=9/453) respectively. Of those 6.8% (n=25/370) and 1.4% (n=5/370) of females were positive for HBV and HCV respectively. And 9.6 % (n=8/83) and 4.8 % (n=4/83) of males were positive for HBV and HCV respectively. In the case of Age category, 36-45years old 12.3 %(n= 7/57) were positive for HBV which is slightly higher than other age categories. Participants who were positive for HBV 7.7 %(n= 29/379) and 2.4 %(n=9/379) positive for HCV were married. In the case of educational status 12.0% (n=20/166) for HBV positive and 3.0% (n=5/166) for HCV positive refugees were educated at elementary level (Table 6.2).

Table 6.2: Prevalence of HBV and HCV among refugees of Pugnido-I camp Gambela Ethiopia January to May 2018 (n=453), 2018.

Variables		**HBsAg ELISA**		**Anti-HCVELISA**		**Total**
		Pos (%)	**Neg(%)**	**Pos (%)**	**Neg(%)**	
Sex	Female	25(6.8)	345(93.2)	5(1.4)	365(98.6)	370
	Male	8(9.6)	75(90.4)	4(4.8)	79(95.2)	83
Age category	18-25	7(4.2)	160(95.8)	2(1.2)	165(98.8)	167
	26-35	17(8.7)	179(91.3)	2(1.0)	194(99.0)	196
	36-45	7(12.3)	50(87.7)	2(3.5)	55(96.5)	57
	46-55	2(8.3)	22(91.7)	1(4.2)	23(95.8)	24
	>55	0(0)	9(100)	2(22.2)	7(77.8)	9
Marital status	Single	1(4.0)	24(96.0)	0(0)	25(100)	25
	Married	29(7.7)	350(92.3)	9(2.4)	370(97.6)	379
	Divorced	1(4.5)	21(95.5)	0(0)	22(100)	22
	Widowed	2(7.4)	25(92.6)	0(0)	27(100)	27
Educational status	Illiterate	7(4.2)	158(95.8)	4(2.4)	161(97.6)	165
	Elementary	20(12.0)	146(88.0)	5(3.0)	161(97.0)	166
	High school	5(4.8)	99(95.2)	0(0)	104(100)	104
	Higher education	1(5.6)	17(94.4)	0(0)	18(100)	18
Total		33(7.3)	420(92.7)	9(2.0)	444(98.0)	453

HBsAg-hepatitis B surface antigen,ELISA-Enzyme Linked Immunoassay,HCV-Hepatiti C virus.

6.3: Risk factors associated with prevalence of HBV

Among female participants 6.8% (n=25/370) were positive for HBV and 9.6% (n=8/83) of males, the difference was not statistically significant (COR=0.679, CI 95%=0.295-1.565, P=0.364).Among participants 7.7%(n=29/379) were married (COR=1.989, CI95%=0.26015.231, P=0.508), 1.4%(n=1/22) were divorced,7.4%(n=2/27) were widowed, and 4.0% (n=1/25) were single, there was no statistically significant difference between marital status and hepatitis virus infection. Regarding to educational status, those who were elementary level were 12.0 %(n= 20/166)positive for HBV but there was no statistically significant difference between educational status and HBV positivity(COR=2.329,CI95%=0.294-18.459,P=0.424).Participants who have exchange of sharp materials with other person have slightly higher positivity of HBV 6.3% (n=12/106), however there was no statistically significant difference between associated risk factors HBV reactivity(COR=0.828,CI 95%=0.281-2.438,P=0.731) as showed in (Table 6.3).

Table 6.3: Risk Factors associated with prevalence of HBV based on ELISA test among refugees of Pugnido-I camp Gambela, Ethiopia (n=453), 2018.

Variables		No(%)	HBsAg pos=no (%)	COR	CI (95%)	P- Value
Sex	Female	370(81.7)	25(6.8%)	0.679	0.295-1.565	0.364
	Male	83(18.3)	8(9.6%)	1		
Age category *	18-25	167(36.9)	7(4.2)			
	26-35	196(43.3)	17(8.7)			
	36-45	57(12.6)	7(12.3)			
	46-55	24(5.3)	2(8.3)			
	>55	9(2.0)	0(0)			
Marital status	Single	25(5.5)	1(4.0)	1		
	Married	379(83.7)	29(7.7)	1.989	0.260-15.231	0.508
	Divorced	22(4.9)	1(4.5)	1.143	0.067-19.424	0.926
	Widowed	27(6.0)	2(7.4)	1.920	0.163-22.584	0.604
Education status	Illiterate	165(36.4)	7(4.2)	0.753	0.087-6.493	0.796
	Elementary	166(36.6)	20(12.0)	2.329	0.294-18.459	0.424
	High school	104(23.0)	5(4.8)	0.859	0.094-7.810	0.892
	Higher education	18(4.0)	1(5.6)	1		
Multi sex	Yes	347(76.6)	21(6.1)	0.505	0.239-1.063	0.072
	No	106(23.4)	12(11.3)	1		
Sharps	Yes	64(14.1)	4(6.3)	0.828	0.281-2.438	0.731
	No	389(85.9)	29(7.5)	1		
Tattoo	Yes	18(4.0)	1(5.6)	0.741	0.095-5.747	0.774
	No	435(96.0)	32(3.4)	1		
Family liver*	Yes	8(1.8)	0(0.0)			
	No	445(98.2)	33(7.4)			
Tooth extract	Yes	51(11.3)	3(5.9)	0.775	0.228-2.636	0.683
	No	402(88.7)	30(7.5)	1		
Vaccine	Yes	9(2.0)	1(1.1)	1.609	0.195-13.271	0.658
	No	444(98.0)	32(7.2)	1		

Family liver history*, Age category ***,** not valid for association, COR-Crude odds ratio.

6.4. Risk Factors associated with HCV prevalence

Among female and male participants 1.4 % (n= 5/370) and 4.8% (n=4/83) were positive for HCV respectively, the difference was not statistically significant (COR= 0.271, CI 95%=0.071 1.030,P=0.055). Statistically significant association was showed on the age group of 18-25 and 26-35 years old with HCV positivity (COR=0.042, CI 95%=.0.005-0.374, P=0.003 and COR=0.036, CI 95%=0.004-0.295, P=0.002, respectively). Association was not performed for marital status and educational status. Among participants 1.6 %(1/64) have sharing of sharp materials; however there was no significant association between exchange of sharp materials and HCV positivity as demonstrated in (Table 6.4).

Table.6.4. Risk Factors associated with prevalence of HCV based on ELISA test among refugees at Pugnido-I camp Gambela Ethiopia January to May 2018 (n=453), 2018.

Variables		No (%)	Anti-HCV Pos	COR	CI (95%)	P- Value
Sex	Female	370(81.7)	5(1.4)	0.271	0.071-1.030	0.055
	Male	83(18.3)	4(4.8)	1		
Age category	18-25	167(36.9)	2(1.2)	0.042	0.005-0.374	0.003
	26-35	196(43.3)	2(1.0)	0.036	0.004-0.295	0.002
	36-45	57(12.6)	2(3.5)	0.127	0.015-1.052	0.056
	46-55	24(5.3)	1(4.2)	0.152	0.012-1.940	0.147
	>55	9(2.0)	2(22.2)	1		
Marital status*	Single	25(5.5)	0(0)			
	Married	379(83.7)	9(2.4)			
	Divorced	22(4.9)	0(0)			
	Widowed	27(6.0)	0(0)			
Education status*	Illiterate	165(36.4)	4(2.4)			
	Elementary	166(36.6)	5(3.0)			
	High school	104(23.0)	0(0)			
	Higher education	18(4.0)	0(0)			
Multi sex history	Yes	347(76.6)	7(2.0)	1.071	0.219-5.233	0.933
	No	106(23.4)	2(1.9)	1		
Sharps material	Yes	64(14.1)	1(1.6)	0.756	0.093-6.148	0.794
	No	389(85.9)	8(2.1)	1		
Tattoo history*	Yes	18(4.0)	0(0.0)			
	No	435(96.0)	9(2.1)			
Family liver*	Yes	8(1.8)	0(0.0)			
	No	445(98.2)	9(2.0)			
Tooth extract*	Yes	51(11.3)	0(0.0)			
	No	402(88.7)	9(2.2)			
HBV Vaccine *	Yes	9(2.0)	0(0.0)			
	No	444(98.0)	9(2.0)			

Variables marked with*: not valid for association, CI-Confident interval COR-Crude odds ratio,

6.5. Adjusted Odds Ratio of sex and age with prevalence of HCV

Adjusted odds ratio was performed between Sex and age with HCV and showed statistically significant association as follows: Sex (AOR=0.291,CI=O.072-1.177,P=0.083) age category of 18-25 years (AOR=0.045,CI=0.005-0.378,P=0.004) age category of 26-35 years (AOR=0.035,CI=0.004-0.301,P=0.002) and age category of 36-45 years (AOR=0.111,CI=0.013-0.974,P=0.047 (Table 6.5).These the above age categories were slightly less associated to HCV infection.

Table 6.5.Adjusted odds ratio for age and sex with HCV infection based on ELISA test among refugees at Pugnido-I camp Gambela Ethiopia January to May 2018 (n=453), 2018.

Variables		No (%)	Anti –HCV Positive	AOR	CI (95%)	P-Value
Sex	Female	370(81.7)	5(1.4)	0.291	0.072-1.177	0.083
	Male	83(18.3)	4(4.8)	1		
Age category	18-25	167(36.9)	2(1.2)	0.045	0.005-0.378	0.004
	26-35	196(43.3)	2(1.0)	0.035	0.004-0.301	0.002
	36-45	57(12.6)	2(3.5)	0.111	0.013-0.974	0.047
	46-55	24(5.3)	1(4.2)	0.136	0.010-1.827	0.132
	>55	9(2.0)	2(22.2)	1		

AOR= Adjusted odds ratio

CI = Confidence interval

6.6. Knowledge, Attitude and Practices (KAP) assessment on HBV and HCV

6.6.1. Knowledge of participants

Majority of participants 79.2% (n=359/453) were never heard about HBV and HCV and 86.5% (n=392/453) and 91.4% (414/453) were never know the transmission of HBV and HCV. About 89.0 % (n=403/453) participants have no ideas about liver cancer with the causative agent of HBV and HCV. In case of vaccine 86.8% (n=393/453) do not have information about availability of HBV vaccine and 72.0% (n=326/453) also not know the treatment of HBV and HCV at all (Table 6.6).

6.6.2. Attitude of participants

In this study 8.2 %(n=37/453) refugees believed that HBV and HCV can transmit through food and 11.5% (n=52/453) participants were believed that HBV and HCV are curable diseases. About 75.7% (n=343/453) participants were believe that HBV and HCV is not serious public health problems and 72.8% (n=330/453) participants were believed that vaccine of HBV is not safe(Table 6.6).

6.6.3. Practice of participants

With regard to vaccination, 98.5% (n=446/453) were not vaccinated to HBV. Screening of HBV and HCV, 87.2% (n=395/453) were not screened, 99.8% (n=452/453) of refugees did not have practice of exchange of injection drugs (Table 6.6).

Table 6.6.Knowledge, Attitude and Practice assessment on HBV and HCV among refugees at Pugnido-I camp Gambela, Ethiopia, January to May 2018 (N=453), 2018.

Knowledge assessment questions	**(n=453) Yes (%)**	**(n=453) No (%)**
Have you ever heard about HBV and HCV infection?	94(20.8)	359(79.2)
Is Hepatitis transmitted through sex?	61(13.5)	392(86.5)
Can you get hepatitis infection through body fluid contact?	39(8.6)	414(91.4)
Do HBV and HCV cause liver cancer?	50(11.0)	403(89.0)
Does HBV have vaccine?	60(13.2)	393(86.8)
Is there effective treatment for HBV and HCV?	127(28.0)	326(72.0)
Attitude assessment questions		
Do you believe HBV &HCV transmitted through food?	37(8.2)	416(91.8)
Do you think hepatitis infection is curable disease?	52(11.5)	401(88.5)
Do you believe hepatitis infection is serious public health problem?	110(24.3)	343(75.7)
Do you think taking HBV vaccine is safe?	123(27.2)	330(72.8)
Practice assessment questions		
Have you received HBV vaccination?	7(1.5)	446(98.5)
Have you screened for HBV and HVC?	58(12.8)	395(87.2)
Have you exchange of injection drug uses	1(0.2)	452(99.8)

HBV-Hepatitis B Virus, HCV-Hepatitis C virus.

7. Discussions

Hepatitis viral infection due to HBV and HCV are widespread infectious diseases representing major health problems. It is well known that refugees constitute a special social group in a geographical area in which, they often live under conditions that facilitate the spread of infectious diseases [11].

The prevalence of HBV among refugees in this study was 7.3%(n=33/453) which was a lower finding compared to previous studies done in different African migrants those done in different countries. In Mali (11.7%)[28], in USA African migrants: Eritrea(15.5%), Ethiopia(9.1%), Somalia(8.3%)[26]. This finding was also a lower finding compared to studies done in different parts of the World immigrants such as: In Bari-Italy (8.3 %)[3], in Athens (15.4 %)[11],in South-Europe(11.9%)[12], in Pakistan-Afghan refugees (8.3%)[14], in India-Tibetan refugees(8.9%)[15],in Naples-Italy immigrants from different origin of countries such as; Eastern-Europe(15.7%),Asia(27.3%),Cambodia(8%) Albania (11.7%), Vietnam(9.3%)[19],in South-Italy(9.6%)[20],in Italy(10.7%)[21],in Athens (15.4%)[23], in Chicago(8.0%)[32].This might be because our study populations are all volunteer refugees and most of them could be confident of their sero-status, and difference in the study period, study subjects, sample size, might also be the cause of such differences.

In differently to the above comparison the prevalence of HBV in the current study was also higher than studies in USA African migrants such as: Ruanda (5.9%),Kenya(4.1%),Tanzania (3.1%0),Burundi(3.0%),Sudan(6.8%)[26].Around the World; in UK(5.0 %)[6], Canada (3.0%)[9], Malta(0.68%), India (6.2%) [10],NW-Europe(3.8%)[13],Kosovo (2.9%)[17],in Italy China-migrants (6.0%),Turkey-migrants (5.0%)[19].The differences might be due to variations in geographical distribution as well as population differences in terms of lifestyle, awareness.

HCV prevalence in the present study was 2.0 % and our finding was lower than the studies done in different refugees; for instance; in Bari-Italy(4.5%)[3],in Canada(3.0%)[9],in Athens (2.3%)[11],in Izmir(4.5%)[7] in Roma (23.4%) [2],in Spain(3.1%)[2],in Albania(2.3%)[2],in Sub-Saharan Africa(2.2%)[2].This study finding on HCV prevalence was higher than previous studies from Netherlands (1.5%)[2], Kosovo(0.7%) [2],Turkey(0.1%) [2],Malta(0.6%) [10], Germany

(1.9%) [22]. This difference might be due to difference in geographical location and sample size of participants.

Among female participants 6.8% (n=25/370) were positive for HBV and 9.6% (n=8/83) were males, however, the difference was not statistically significant (COR=0.679, CI 95%=0.295-1.565, P=0.364). From the total of female participants 1.4% (n=5/370) were positive for anti-HCV and 4.8 %(n=4/83) males were positive for anti-HCV but the difference was not statistically significant (COR= 0.271, CI 95%=0.071 1.030,P=0.055).This finding was incomparable to a study conducted in Barry Italy from the total of 442 male participants 9.7% (n=43/442) were positive for HBsAg and from the total of 87 female 1.1% (n=1/87) were positive for HBsAg where as 48.4%(n=214/442) males were positive for anti-HCV and 31%(n=27/87) females were positive for HCV [3]. The difference might be due to sex proportion and sample size.

In relation to marital status, prevalence of HBV was higher among married participants, which was 6.4 %(n= 29/453) but the difference was not statistically significant (P>0.05),in Pakistan 15.6% (58/903) was married higher than our study[14].Regards to education refugees who were elementary level were 4.4 %(20/453) positive for HBV but there was no statistically significant difference between educational status and HBV positivity(P>0.05).In Cebu, Philippine2% (9/450) was elementary level[36].And also Participants who have exchange of sharp materials with other person were 11.3% (12/106) positivity, however there was no statistically significant difference between associated risk factors HBV reactivity(P>0.05)[15].

The age group 36-45 was highly prevalence of HCV which was 12.3% (n=7/57).The study showed that Age group 18-25 was prevalence 1.2% for HCV and 26-35 was prevalence 1.0% for HCV and statistically significant with HCV positivity which was (P=0.003 and P=0.002) respectively. The study conducted in Minnesota the same age group prevalence was HCV 5.4 %(n= 48/883) was higher than our study [28].

This study also assessed the knowledge, attitudes and practices of participants towards to HBV and HCV infection. Majority of participants for instance 79.2% (n=359/453) were responded that they have never heard about HBV infection in other ways they had low knowledge of hepatitis infection. The study in Cebu, Philippine (84%) the study participants replied that they do not have knowledge in terms of: hepatitis B and C viral diseases [36]. In terms of mode of transmission 86.5% (n=392/453) and 91.4% (n=414/453) were never know how the HBV and HCV infection transmitted through sex and body fluid respectively. In Cebu, Philippine (78%), that the diseases

could be transmitted by unsafe sex [36].In case of vaccine 86.8% (393/453) participants did not have information about availability of HBV vaccine, in Philippine (90%) participants answered that there is a vaccination available for these diseases[36]. The above data showed that all most all the majority of participants have limited knowledge about HBV and HCV infection, transmission and protection. Therefore, the magnitude of HBV and HCV will be increased in the refugees as well as in the local society unless we have making awareness with formulating health education program in the refugees and as the same time in the local society.

With regard to participants attitude, 8.2 %(n=37/453) were believe that HBV and HCV can transmit through food, in Western Washington Participants expressed that the contamination of food sources and the sharing of used utensils were the most significant sources of hepatitis B transmission [25]; about 72.8% (n=330/453) participants believed that vaccine of HBV is not safe which is in line with study in Philippine (72%)[36].

In relation to participants practice, screening and vaccination of HBV and HCV was assessed using questions of which 87.2% (n=395/453) responded not screened and 98.5% (n=446/453) were not vaccinated respectively. In Philippine majority of the respondents did not do screening for hepatitis B or C (94%)[36].In Ethiopia the study performed by different group the overall prevalence of hepatitis B virus (HBV) was 7.4%,in community based studies 8.0% in blood donors, 8.4%[37] which was higher than our study result(7.3%). The difference might be due to large sample size in their study and different target group. Anti -HCV prevalence conducted in kemissie and Omo at community level which was 1.3%, this was lower than our finding (2.0%) [37].The difference might be due to high risk target group in our study.

8. Strength and Limitation of the study

8.1. Strength of the study

- The research is conducted for the first time.
- The research was performed among high risk groups

8.2 Limitation of the Study

- Unable to perform all hepatitis panels
- We did ELISA only for those sera which were positive by rapid tests

9. Conclusion and Recommendation

9.1. Conclusion

In general the prevalence of hepatitis B and C viruses was intermediate in Gambela refugee camp. The age category of 18-25, 26-35 and 36-45 years had significant association with hepatitis C infection. Majority of the participants had limited knowledge about the transmission and protection of HBV and HCV infection. There was no significant association between proposed risk factors and hepatitis B and hepatitis C.

9.2. Recommendations

- National surveillance screening for Hepatitis B and C among Ethiopian refugees is required.
- Conducting regular health education for refugees and the local population to prevent the transmission of hepatitis.
- Formulate the screening policy of HBV and HCV among refugees especially urban refugees is vital.
- Large scale study is important to make generalization among refugee in Ethiopia.

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Annexes

Annex-I. Information sheet

Purpose: To estimate prevalence, KAP and associated risk factors of HBV and HCV infections among refugees at Pugnido-I refugee camp.

Participation: We are asking you to voluntarily participate in this study. And expected from everyone is to respond some question and give 5 ml of venous blood. The blood samples are collected using sterile materials.

Risks: The risks associated with this study could be some discomforts/pain/ when we draw 5ml of venous blood from you. However, if in case any problems arise during and following sample collection, we shall offer you necessary medical interventions in this regard.

Benefits: If your status will be positive for HBV and HCV during investigation, result will be declared to you by investigator to get integrated health education and follow up by contacting the health institution. If your status will be negative for HBV you can prevent future HBV infection with dealing to concerned body.

Confidentiality: Information that we will collect from you during this study will be kept Confidentiality and your identity will be put away after re-coding your file and kept in a secured place. Only the principal investigators will be able to link your identity with the code number, if this becomes necessary to assist you in any way.

Sharing the result: At the end of the study we will present the result to responsible bodies, the report will not bear any information relevant to your personality. We assure you the confidentiality of such information.

Right to refuse: Since participation in this study is entirely voluntarily, you can refuse to participate in this study at any time.

Any question regarding this study can be addressed to:

Principal Investigator: Abiyu Ayele

Contact Address: Addis Ababa University, College of Health Sciences, School of Allied Health Sciences, Department of Medical Laboratory Sciences. Phone (Cell Phone): +251-911 481319. E-mail:abiyuayele62@gmail.com.

Annex - II. Consent Form

I have been requested to participate in a research project that aims to determine the prevalence and associated risk factors of Hepatitis B and C viruses' infection in refugees. I have been informed that all information I will be giving will be kept confidential was provided the Opportunity to ask questions and given adequate time to rethink the issue. The aim and objectives of the study are sufficiently clear to me. I have not been pressurized to participate in any way. I understand that participation in this study is completely voluntary and that I may withdraw from it at any time and without supplying reasons. I am fully aware that the results of this Study will be used for scientific purposes and may be published. Agree to this, provided my privacy is guaranteed. And I confirm my agreement by putting my signature below. I hereby give my consent for giving of blood specimens.

Full Name: _____

Signature: _____

Date: _____

Address of the investigator

Name: Abiyu Ayele

Address: Addis Ababa University, College of Health Sciences, School of Allied Health Sciences, Department of Medical Laboratory Sciences.

E-mail: abiyuayele62@gmail.com

Annex - III. Questionnaire

For the investigation of the socio-demographic, associated risk factors and KAP of HBV and HCV infections in refugees at Pugnido-I refugee camps Gambella, Ethiopia.

Participant Code no----------

A. Socio-demographic oftheparticipant (circle your answers)

No	Question	Response Option
1.	Sex	1.Female 2.Male
2.	Age	……
3.	Marital status	1. Single 2. Married 3. Divorced, 4. Widowed
4.	Education	1. Illiterate 2. Elementary 3. High school, 4. Higher education.

B. Associated risk factors of hepatitis B and C viruses infection

1	Do you have history of multi sexual partner in life?	1. Yes.	2.No.
2	Do you have sharing of sharp materials with others?	1. Yes.	2.No.
3	Do you have history of tattooing?	1. Yes.	2.No.
4	Is there family history of liver disease?	1. Yes.	2.No.
5	Do you have history of tooth extraction?	1. Yes.	2.No.
6	Have you ever taken HBV vaccination?	1.Yes	2.No

C. Participant knowledge towards HBV and HCV infection

No	Question	Response Option		Remark
1	Have you ever heard about HBV and HCV	**1.yes**	**2.No**	
2	Is Hepatitis transmitted through sex?	1. Yes.	2. No.	
3	Can you get hepatitis infection through body fluid contact?	1. Yes.	2. No.	
4	Do HBV and HCV cause liver cancer?	1. Yes.	2. No.	
5	Does HBV have vaccine?	1. Yes.	2. No.	
6	Is there effective treatment for HBV and HCV?	1.Yes	2.No	
D. Participants attitude towards HBV and HCV infection				
1	Do you believe HBV &HCV transmitted through food?	1. Yes	2. No	
2	Do you think hepatitis infection is curable disease?	1. Yes	2. No	
3	Do you believe hepatitis infection is serious public health problem	1. Yes	2. No	
4	Do you think taking HBV vaccine is safe?	1. Yes	2.No	
E. Participants practice towards HBV and HCV infection				
1	Have you received HBV vaccination?			
2	Have you screened for HBV and HVC?			
3	Have you exchange of injection with drug users			

Participants name ---------- signature ----------Date----------

Annex - IV. Laboratory Results format:

Code ----------

1. HBs Ag Positive - ☐ Negative - ☐

2. Anti-HCV Positive - ☐ Negative - ☐

3. ELISA HBV Positive - ☐ Negative - ☐

4. ELISA Anti-HCV Positive - ☐ Negative - ☐

Remark._____

_____

Annex - V. principle and procedure of tests

A) HBsAg rapid test principle:

HBsAg rapid test that utilizes the principle of immunochromathography, unique two site immunoassay on a membrane. The test sample flows through the membrane assembly of the cassette, the color monoclonal anti-HBsAg colloidal gold conjugate complexes with HBsAg in the sample. This complex moves further on the membrane to the test region where it is immobilized by another monoclonal anti-HBsAg antiserum coated on the membrane leading to formation of a pink-purple color band which confirms a positive test result. Absence of this color band in the test region indicates a negative test result. Un reacted 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 color band. This control band serves to validate the test result [Test kit leaflet].

B) **Anti-HCV rapid test principle:**

Rapid test is utilizes the principle of immuno-chromathography, unique two site immunoassay on a membrane. As the sample flow through the membrane of the test strip, the test color HCV antigen colloidal gold conjugate complexes with anti-HCV in the sample. This complex moves further on the membrane to the test region where it is immobilized by another HCV antigen coated on the membrane leading to formation of a pink-purple color band which confirms a positive test result. Absence of this color band in the test region indicates a negative test result. Un react conjugate and unbound complexes, if any moves further on the membrane and are subsequently immobilize by the anti-HCV coated on the membrane at the control region, forming a pink/purple color band. This control band serves to validate the test result [Test kit leaflet].

C) Procedures for HBV and HCV rapid tests

1. Before sample collection introduce yourself and identify the patient
2. Take a time to wash your hands and wear gloves
3. Prepare the material required (needles, tubes, etc.)
4. Prepare the patient
5. Apply the tourniquet (do not let it on for extended period)
6. Choose a vein
7. Disinfect the draw site
8. Collect serum specimen in a clean test tubes.
    - Ensure that only sufficient quantity of the specimen is collected.
    - Exit the vein and apply pressure
    - Discard the needle(in appropriate biohazard container)
    - Label the specimen before leaving the patient
    - Check the patient apply a plaster if necessary
    - Allow the specimen for 30 minutes to facilitate clotting-
    - Centrifuge with medium speed for 10 minutes
    - Separate serum from the blood by Pasture pipette
    - Perform the lab test and store the remaining serum at -20^0c
9. Bring the sealed pouch to room temperature, open the pouch and remove the cassette/strip
   Once opened, the cassette must be used immediately.
10. Measure 50 micron serum with pipette and dispense the sample in sample well.
11. The cassette/strip should be left to horizon until the specimen flow evenly distributed.
12. At the end of the given time based on the manufacturer instruction read the result as follows:
    - NEGATIVE: Only one colored band appears on the cassette
    - POSITIVE: two distinct colored bands appear on the cassette.
13. The test should be considered invalid if no band appears. Repeat the test with a new cassette/strip.
14. Although, depending on the concentration of HBsAg in the specimen, positive results may be start appearing as early as 2min, negative results must be confirmed only at the end of given time.
15. In case of a doubtful result at final time, the test may be extended up to 30min to get a clear back ground.

D)HBsAg ELISA test principle

This is a Sandwich Enzyme linked Immunosorbent assay method in which polystyrene microwell strips are pre-coated with monoclonal antibodies specific to HBsAg. Donor's serum or plasma sample is added to the microwells 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, is captured on the solid phase. After washing to remove sample serum protein and unbound HPR-conjugate, chromogen solution containing tetramethylbenzidine (TMB) and urea peroxidase are added to the walls. In the presence of the antibody-antigen-antibody (HRP) sandwich immune-complex, the colorless chromogens are hydrolyzed by the bound HPR conjugate a blue colored product. The blue color turns to yellow after stopping the reaction with sulfuric acid. The amount of color can be measured and is proportional to the amount of antigen in the sample (Test kit leaflet).

E) Anti-HCV ELISA test principle

Polystyrene microwell 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 HRP-conjugate is then removed by washing. Chromogen solutions containing TMB and urea peroxidase are added to the wells and in presence of the antigen-antibody-anti-IgG (HRP) immune-complex; the colorless chromogens are hydrolyzed by the bound HRP-conjugated to a blue colored product. The blue color turns to yellow after stopping the reaction with sulfuric acid. The amount of color can be measured and is proportional to the amount of antibody in the sample (Test kit leaflet).

F) Test Procedures for ELISA

1. A micro titration well plate is coated with known antigen.

2. Add patent's serum. If the serum contains antibody it combine with antigen.

3. Wash carefully by using automatic washer more than 6 times.

4. Add enzyme labeled antihuman globulin, which attaches to the antibody.

5. Wash carefully.

6. Add the substrate, which is hydrolyzed (broken down) by the enzyme to give a color change.

7. Read the result.

Annex .VI. Thesis declaration

I the undersigned agree to accept all responsibilities for the scientific and ethical conduct of the research project. I was provided timely progress report to my advisor and seek the necessary advice and approval from my primary advisors in the course of the research. I was communicated timely to my advisors and all stakeholders involved in the study.

Name of the principal investigator:

Abiyu Ayele (BSc) Signature_____ Date _____

Name of Advisors:

1.Mr- Kasu Desta(Bsc, Msc, PhD fellow, Associate professor)

Signature _____Date_____

2.Mr. Melese Hailu (Bsc ,MSc, PhD fellow)

Signature _____Date_____