SEROPREVALENCE AND ASSOCIATED RISK FACTORS FOR HEPATITIS B AND C VIRUS INFECTIONS AMONG APPARENTLY HEALTHY MOTHERS IN ADDIS ABABA, ETHIOPIA

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# Table of contents

ACKNOWLEDGEMENTS............................................................................................................................ II  
LIST OF TABLES........................................................................................................................................... V  
LIST OF FIGURES.......................................................................................................................................... VI  
LIST OF ABBREVIATIONS............................................................................................................................. VII  
ABSTRACT......................................................................................................................................................... IX  
1. INTRODUCTION ........................................................................................................................................ 1  
   1.1 Background........................................................................................................................................ 1  
   1.2 Statement of the problem .................................................................................................................... 2  
   1.3 Significance of the Study .................................................................................................................... 3  
2. LITERATURE REVIEW ............................................................................................................................. 4  
   2.1 Hepatitis B Virus................................................................................................................................. 4  
      2.1.1 Virology ....................................................................................................................................... 4  
      2.1.2 Epidemiology ............................................................................................................................... 4  
      2.1.3 Transmission and risk factors ...................................................................................................... 5  
      2.1.4 Clinical outcome of Hepatitis B Virus infection .......................................................................... 6  
      2.1.5 Occult Hepatitis B Virus infection ............................................................................................. 8  
      2.1.6 Diagnosis of Hepatitis B Virus infection .................................................................................... 9  
      2.1.7 Treatment and prevention .......................................................................................................... 9  
   2.2 Hepatitis C Virus............................................................................................................................... 11  
      2.2.1 Virology ..................................................................................................................................... 11  
      2.2.2 Epidemiology ............................................................................................................................. 11  
      2.2.3 Transmission and risk factors .................................................................................................... 13  
      2.2.4 Clinical outcome of Hepatitis C Virus infection ...................................................................... 13  
      2.2.5 Diagnosis of hepatitis C virus infection .................................................................................... 14  
      2.2.6 Prevention .................................................................................................................................. 15  
      2.2.7 Treatment of Hepatitis C virus infection .................................................................................. 15  
3. OBJECTIVES ............................................................................................................................................. 18  
   3.1 General objective ............................................................................................................................... 18  
   3.2 Specific objectives ............................................................................................................................. 18  
4. METHODS AND MATERIALS ................................................................................................................. 19  
   4.1 Study Design and study period .......................................................................................................... 19  
   4.2 Study Area ......................................................................................................................................... 19  
   4.3 Population .......................................................................................................................................... 19  
      4.3.1 Target Population........................................................................................................................ 19  
      4.3.2 Source of Population................................................................................................................. 19  

LIST OF TABLES

Table 1: The prevalence of HBsAg and HBcAb in different population in the World ............... 10
Table 2: The prevalence of HCV in different population in the World........................................ 17
Table 3: Socio-demographic characteristics among apparently healthy mothers in Addis Ababa, Ethiopia (N =454) ......................................................................................................................... 24
Table 4: The prevalence of HBsAg, HBcAb and Anti-HCV in relation to Socio-demographic characteristics among apparently healthy mothers in Addis Ababa, Ethiopia (N=454)............. 26
Table 5: Association in bivariate and multivariate logistic regression analysis of explanatory variables and HBV infection among apparently healthy mothers in Addis Ababa, Ethiopia...... 28
Table 6: Association in logistic regression analysis of explanatory variables and HCV infection among apparently healthy Mothers in Addis Ababa, Ethiopia................................................................. 29
LIST OF FIGURES

Figure 1: Geographic distribution of hepatitis B virus infection ................................................... 5
Figure 2: Natural history of hepatitis B virus ................................................................................ 8
Figure 3: Geographic distribution of hepatitis C virus ................................................................. 12
Figure 4: Natural history and pathogenesis of hepatitis C Virus infection ................................. 14
LIST OF ABBREVIATIONS

AHRI- Armauer Hansen research institute
ALT-Alanine Transaminase
Anti-HBc-Anti-Hepatitis B Core Antigen
Anti-HBe- Anti-Hepatitis B envelope Antigen
Anti-HBs- Anti-Hepatitis B Surface Antigen
Anti-HCV-Anti-Hepatitis C Virus
AOR-Adjusted Odds Ratio
CDC-Center for Disease Control and Prevention
CHB-Chronic Hepatitis B virus
CIA-Chemiluminescence Immunoassay
CIs –Confidence Intervals
CS-Cross Sectional Study Design
DAAs-Direct Acting Antiviral Drugs
DDIs-Drug-Drug Interactions
DNA –Deoxy Ribonucleic Acid
ELISA/EIA-Enzyme Linked Immunosorbent Assay/Enzyme Immunosorbent Assay
HAV-Hepatitis A Virus
HBeAg-Hepatitis envelope Antigen
HBsAg-Hepatitis B surface Antigen
HBV-Hepatitis B Virus
HCC-Hepatocellular Carcinoma
HCV-Hepatitis C Virus
HCWs-Health Care Workers
HEV-Hepatitis E Virus
HIV-Human Immunodeficiency Virus
INF-Interferon
IRB-Institutional Review Board
IU-International Unit
Kb-Kilo Base
ml-milliliter
NANBNE-non-A, non-B, non-E
NAT/NAAT-Nucleic Acid Test/Nucleic Acid Amplification Test
nm-nanometer
NS-Non-Structural Protein
OBI-Occult Hepatitis B Virus Infection
ORF-Open Reading Frame
OR-Odds Ratio
PBMCs-Peripheral Blood Mononuclear Cells
PCR-Polymerase Chain Reaction
RBIA-Recombinant Immunoblot Assay
RNA-Ribonucleic Acid
SES-Socio-economic Status
Ss-single stranded
SVR-Sustained Viral Response
WHO-World Health Organization
ABSTRACT

Background: Viral hepatitis is a global public health problem affecting millions of people every year, causing disability and death. Hepatitis B and hepatitis C viruses are common causes of viral hepatitis. Studies in different parts of the world showed that viral hepatitis due to HBV and HCV causes considerable morbidity and mortality from both acute infection and chronic sequelae including chronic hepatitis, cirrhosis, and hepatocellular carcinoma (HCC). Community based studies about the prevalence of each viral infections among apparently healthy mothers in Ethiopia has not been conducted, particularly in Addis Ababa.

Objective: To determine the Sero-prevalence of HBsAg and HCV among apparently healthy mothers and to identify potential risk factors associated with the infections.

Methods: A community based cross sectional study was conducted among 454 apparently healthy mothers, in Addis Ababa, Ethiopia from June 2016 to May 2017. A systematic probability sampling method was employed. A structured questionnaire was used to collect data on socio-demographic characteristics and associated risk factors. All samples were tested, using a sandwich third generation enzyme linked Immunosorbent assay for HBsAg, HbcAb, and HCV by using Bio-Rad ELISA kits.

Results: Four hundred and fifty four apparently healthy mothers were involved in this study. Sero-prevalence of hepatitis B and C virus infections were found to be 3.7% and 2.0% respectively. One hundred sixty five (36.3%) were found to be positive for HbcAb. None of them was co-infected by these two viruses. Among the assessed variables and clinical presentations, previous history of liver disease (AOR=5.5 CI (1.8-16.5), history of jaundice (AOR=17.8 CI (4.0-75.5), and family history of liver disease (AOR= 3.2 CI (1.0-10.4) were significantly associated with HBV infection which were important predictors of HBV infection. Marital statuses, consumption of alcohol (AOR=6.9 (1.3-37.0) and history of jaundice (AOR=19.2 (CI 3.5-104.9) were significantly associated with the occurrence of HCV infection.

Conclusion: Hepatitis B and C appear to be a major health problem in our community. Our study finding indicated that an intermediate level of hepatitis B and C virus infection among the study groups and routine screening and vaccine schedules (for HBV) may be important. Therefore, screening asymptomatic people is an important instrument in disease detection, prompt diagnosis and intervention.

Key words: Hepatitis B and C virus, sero-prevalence, apparently healthy mothers, risk factors
1. INTRODUCTION
1.1 Background

Hepatitis is an inflammation of the liver affecting millions of people every year (WHO, 2012). Hepatitis is among the most important causes of loss of healthy life years in women. Viral hepatitis is one of the important causes of hepatocellular malignancy, acute and fulminant hepatitis in developing countries (Shukla et al., 2011). There are different types of viruses which are responsible for viral hepatitis (WHO, 2012). Of these viruses, hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV), hepatitis D virus and hepatitis E virus (HEV) are the most common types of viruses that infect the liver. These viruses are members of different viral families, but all display a strong hepatotropism and can cause acute or chronic infections. But, infection with hepatitis B and C viruses and hepatocellular carcinoma (HCC) is responsible for heavy diseases burdens (Saeed et al., 2014).

Hepatitis B virus (HBV) infection is one of the top 10 viral infections globally (Perez et al., 2006, WHO, 2009). Infection with HBV could result in outcomes ranging from an acute, self-limiting disease through chronic hepatitis B (CHB) to cirrhosis and hepatocellular carcinoma (HCC) (Gerlich, 2007). HBV is one of the main causes of hepatic decomposition, cirrhosis, hepatocellular carcinoma (HCC) and acute disease usually occurs when the immune response is well preserved, while patients with an immunodeficiency are more likely to develop a chronic disease (Zanetti et al., 2008, WHO, 2012).

Hepatitis C virus has been considered to be one of the most potential pathogens that have hindered the medical community all over the world. HCV has been recognized as a major cause of chronic liver disease worldwide and due to the surpassing hepatitis B virus (Shepard et al., 2005, Alter, 2007). Such infection increases tremendously among the developing countries particularly at those categories that were considered to be at a potential risk of acquiring HCV.

All HCV genotypes have a common ancestor virus. However, HCV genotypes 1, 2, and 4 emerged and diversified in Central and Western Africa, genotype 5 in South Africa, and genotypes 3 and 6 in China, South-East Asia and the Indian subcontinent. In these areas, a large number of subtypes of these genotypes are found. The rest of the world, in particular industrialized areas, harbor a small number of HCV subtypes that could widely spread because
they met an efficient route of transmission, such as blood transfusion or the intravenous use of drugs. They include genotypes 1a, 1b, 2a, 2b, 2c, 3a, 4a and 5a (Simmonds et al., 2006).

1.2 Statement of the problem

Viral hepatitis places a heavy burden on the health care system because of the costs of treatment of liver failure and chronic liver disease. In many countries, viral hepatitis is the leading cause of liver transplants. Such end-stage treatments are expensive, easily reaching up to hundreds of thousands of dollars per person. Chronic viral hepatitis also results in loss of productivity. Hepatitis B and C infections are seen more often in recipients of organs, blood, and tissue, along with persons working or receiving care in health settings, and in vulnerable groups. Viral hepatitis has not received the attention it deserves from the global community. Although the burden of disease is very high, the problem has not been addressed in a serious way for many reasons, including the relatively recent discovery of the causative viruses, the mostly silent or benign nature of the disease in its early stages, and the insidious way in which it causes chronic liver disease (WHO, 2012).

HBV infection occurs all over the world. The WHO has estimated that there are more than 2 billion HBV infected people and about 378 million chronic carriers worldwide. There are approximately 620,000 HBV related deaths and 4.5 million new HBV infections occur worldwide each year, of which a quarter progresses to liver disease (WHO, 2002). In Sub-Saharan Africa, the prevalence of HBV surface antigen (HBsAg) is 3 - 20% and markers of past exposure ranging from 60 - 99% (Ayoola, 1988). In Asia and sub-Saharan Africa, HBV infection is endemic and thought to be the main etiological factor in over 75% of the chronic liver disease (Dawaki et al., 2006). Since Ethiopia is located in Sub-Saharan Africa, it is considered an area of high endemicity for HBV infection.

Hepatitis C virus (HCV) infects approximately 170 million individuals worldwide. Chronic HCV infection has been estimated to be responsible for approximately 250 000 to 350 000 deaths per year (WHO, 2002). In Ethiopia only few community-based studies on sero-epidemiology of HBV and HCV prevalence have been previously done and they indicated that hepatitis infections are endemic in Ethiopia with regional variation. However, community based studies are not conducted about the prevalence of each viral infections. By considering the existing scarcity of information about viral hepatitis infection especially mothers and children, the current study was
planned to be undertaken to measure the level of each viral hepatitis infection and contributing factors.

1.3 Significance of the Study

Viral hepatitis is key public health problems that pose an enormous risk for disease transmission in the general population, especially in children and women. Reliable epidemiological data are essential for planning health programs and facilitating the scaling up of hepatitis treatment as well to identify highly risk groups. It is important to know the number of persons infected with and dying from hepatitis related liver disease, the prevalence of hepatitis related morbidity, and the distribution of genotypes and fibrosis stages. This is because the selection of appropriate treatments can depend upon the genotype, and the presence or absence of cirrhosis, while the urgency with which to initiate treatment depends largely on the degree of liver fibrosis. Unfortunately, estimates of these key epidemiological parameters are limited by the lack of data from some parts of the world. This condition is much worse in developing countries like Ethiopia. There is a shortage of concrete data that reveals the actual figure of viral hepatitis sero-prevalence among apparently healthy mothers.

The prevalence of viral hepatitis infection varies greatly in different regions of the world and it is high in endemic areas. Although different studies on the prevalence of viral hepatitis have been conducted in different parts of Ethiopia (especially HBV and HCV), most of them focused on investigating the prevalence among pregnant mothers, blood donors, HIV infected individuals, health care workers and medical waste handlers. There is very limited data about the magnitude of viral hepatitis (B and C) prevalence among apparently healthy mothers. Therefore,

- It was important to define the prevalence of these viral infections among apparently healthy mothers.
- It was important to evaluate their associated risk factors so as to adopt effective preventive strategies, guidelines and educational programmes.
- It was also important to generate a baseline data among apparently healthy mothers in the study area.
- In general, screening asymptomatic people is an important instrument in disease detection, prompt diagnosis and intervention.
2. LITERATURE REVIEW

2.1 Hepatitis B Virus

2.1.1 Virology
In 1963, HBV was accidently discovered by Baruch Blumberg during his research on Australia antigen. Hepatitis B virus (HBV) belongs to the genus orthohepadnavirus in the family Hepadnaviridae (Schaefer, 2007). HBV is an enveloped virus with a diameter of ~42 nm. Within the core of the virus is a protein-linked, ~3.2 kb DNA genome that is partly double stranded. The HBV genome has four open reading frames (ORFs) (X, S, P and C) (Schaefer, 2007). Four major serotypes (adw, ayw, adr and ayr) and nine minor subtypes have been serologically identified at the hepatitis B surface antigen (HBsAg) level. The complete sequencing of DNA from HBV isolates worldwide has led to the identification of eight genotypes (from A to H) and a number of sub genotypes, showing different ethno/geographic distributions. HBV genotypes have also been associated with different clinical outcomes and response to interferon therapy (Zanetti et al., 2008, WHO, 2012). Genotypes A -H of HBV have been described (Schaefer, 2007) with members of a genotype not differing by more than 8% of their genome (Okamoto et al., 1988). Sub-genotypes have also been described with members not differing by more than 4% of their genome (Norder et al., 2004). The most circulating genotypes of HBV are A (78%) and D (22%) in Ethiopia. Phylogenetic analysis also revealed that one subgenotypes (A1) within genotype A, and 4 subgenotypes within genotype D (D1; 1.3%, D2; 55%, D4; 2.5%, and D6; 8.8%) were isolated and a novel hepatitis B virus subgenotypes D10 circulating in Ethiopia (Hundie et al., 2016).

2.1.2 Epidemiology
On the basis of the HBV carrier rate, the world can be divided in to high, medium and low endemicity regions (WHO, 2002, Elsheik et al., 2007) as shown in Figure 1.
In high endemic areas, like central Asian republics, Southeast Asia, Sub-Saharan Africa and the Amazon basin, the HBV carrier rate is over 8%. In low endemic regions, like the United States, Northern Europe, Australia and parts of South America, HBsAg prevalence is less than 2%. The Middle East, some Eastern European countries and the Mediterranean basin are considered areas of intermediate endemicity with a carrier rate between 2% and 8% (WHO, 2002).
2.1.3 Transmission and risk factors

Recent data in developing countries showed that the most common route of infection is still vertical transmission from mother to child and horizontal transmission between children, particularly siblings (Elsheik et al., 2007).

Globally, perinatal HBV transmission accounts for an estimated 21% of HBV related deaths, while regionally it ranges from 13% in the Eastern Mediterranean region to 26% in the Western Pacific region. Recent studies in Africa confirm the relatively high HBsAg Sero-prevalence in pregnant women, irrespective of age, parity, gestational age, and residence, history of blood transfusion, dental manipulation, tattooing and circumcision (Elsheik et al., 2007). The maternal-neonatal transmission was studied in Libya where HBsAg positivity was 1.5% and transmission 60.9% and in Ghana with an HBsAg prevalence of 16% but a materno-fetal transmission only in 8.4% of neonates (Candiotti et al., 2007, El-Magrahe et al., 2010).

In high endemic areas, other important modes of HBV transmission concern some high risk groups such as health care workers (HCWs), sexual contacts (Ganju et al., 2000, Zago et al., 2007, Perez et al., 2009) and intravenous drug use (Lama et al., 2010, Ashraf et al., 2010). Parenteral or percutaneous routes of HBV transmission, such as needle stick injury and mucus membrane splash in healthcare setting, tattooing, piercing, sharing razors or toothbrushes, are also important in spreading the virus (Lin et al., 2010, Mahfoud et al., 2010). Surgery and dental
care may be a source of infection. Transfusion-related infections have currently become very rare in developed countries thanks to the improved serology and advances in molecular blood screening but can be an important source of infection in the poorest countries (Tessema et al., 2010). In India, an intermediate endemic zone where the estimated prevalence rate of HBV in the healthy general population is around 4.7%, a recent study showed 5% HBsAg positivity in HCWs, but a highest seropositivity of around 40% among laboratory technicians (Kosgeroglu et al., 2004). In Taiwan, among HCWs who were exposed to high risk patients, nearly 16% had HBV (Pereira et al., 2009).

2.1.4 Clinical outcome of Hepatitis B Virus infection

The outcome of infection depends on both properties of the virus and the host. Several studies found an association between variants of the virus and genetic variations found in the host (Thursz et al., 1997). Hepatitis B infection is characterized by four dynamic stages (Pan et al., 2005).

The first stage, the “immune tolerant” phase is characterized by, high levels of HBV DNA replication, HBeAg positivity, normal serum Transaminase levels, little or no symptoms, and minimal histological activity in the liver. In the acutely infected child or adult, this stage represents the incubation period before immune response to HBV. It usually lasts for 2-4 weeks, but can last for decades in those who acquired the infection during the perinatal period. Individuals in this group are highly contagious and can transmit HBV easily. Active viral replication is known to continue despite little or no elevation in the aminotransferase levels and no symptoms of illness (Pan et al., 2005).

When the tolerogenic effect is lost during the immune tolerant phase, immune-mediated lysis of infected hepatocytes becomes active. This stage reflects the “immune response,” which is the inflammatory process that results in the destruction of HBV-infected cells, elevating Transaminase levels (ALT), HBeAg can be detected but the HBV DNA level decreases. The duration of this stage for patients with acute infection is approximately 3-4 weeks (symptomatic period). For patients with chronic infection, 10 years or more may elapse before cirrhosis develops. Persistence of the immune response phase beyond six months is considered chronic HBV infection. This stage carries the highest risk of progression to cirrhosis and hepatocellular carcinoma (Schaefer, 2005).
The third stage, the “inactive carrier” state, is thought to mark the end of active viral replication. HBeAg becomes negative, anti-HBe appears (Seroconversion), and Transaminase levels normalize. A low level of HBV DNA still may be present. Reflecting very low or no replication of HBV and mild or no hepatic injury the majority of adults with, acute HBV infection enter this stage rapidly. An inactive carrier forms the largest group in chronic HBV infected patients. Around 300 million people are inactive carriers. The inactive carrier stage may last for years or even lifetime. They may already have progressed to cirrhosis or may have insignificant fibrosis. Ten to 30% of carriers will have disease flares similar to acute HBV infection (Locarnini, 2000).

The fourth or “immune” stage is characterized by the clearance of HBsAg and development of HBsAb. HBV DNA is usually undetectable, and reactivation or re-infection is uncommon. Progression from the third to the fourth stage occurs in approximately 3 percent of HBV-infected persons per year (Sharma et al., 2005).

Clinical manifestations of HBV infection are a balance between viral and host factors. Possible clinical outcomes of HBV infection are described in Figure 2. Acute HBV infection is subclinical in 70 percent of adults and 90 percent of children younger than five years. The incubation period after infection lasts one to four months. Acute HBV infection leads to fulminant hepatic failure from massive hepatocellular necrosis in about 1 percent of infections. HBV infection is termed as chronic if it continues to be HBsAg positive for ≥ 6 months. Chronicity is dependent mostly upon age at exposure. Thus, 90% of children infected before their first birthday become chronic carriers compared to 5%-10% of adults (Sharma et al., 2005).

Chronic HBV infection is a dynamic process with a wide spectrum of affliction. Median progression rate from chronic hepatitis to cirrhosis is 27.9% after 8-12 years and HCC is other sequelae (Lavanchy, 2005). Chronic carriers often lack symptoms. Acute as well as chronic infections by HBV can be associated with extra hepatic diseases. The pathogenesis of both conditions involves the deposition of circulating immune complexes (Shim and Han, 2006).
2.1.5 Occult Hepatitis B Virus infection

Occult hepatitis B virus infection (OBI) is a challenging clinical entity (Lledo et al., 2011). Occult Hepatitis B infection (OBI) is defined by the absence of HBsAg despite the presence of HBV DNA in the liver, blood serum, or peripheral blood mononuclear cells (PBMCs), irrespective of the presence of other hepatitis B viral antibodies and antigens (Candiotti and Allain, 2009).

OBI poses a significant risk to those receiving blood transfusions or tissue transplants because conventional donor screening with HBsAg and HBcAb may yield serologically negative results despite the presence of HBV DNA (Liu et al., 2006). It has been shown that 20% of OBI infections are negative for all HBV serological markers while HBV DNA is present (Torbenson and Thomas, 2002) although the HBV viral load is often low (Candiotti and Allain, 2009). This means detection of OBI can be challenging due to the extremely low levels of viral DNA (<200IU/mL) in infected individuals without detectable HBsAg. At times, HBcAb can be used as a less than ideal surrogate marker for identifying potential seropositive OBI (Raimondo et al., 2008).
Despite numerous studies of OBI, its prevalence is unclear. This is due to the varying prevalence of OBI in cohort studies, small sample sizes, a lack of appropriate controls, and varying assay sensitivities used in detection across testing centers (Schmeltzer and Sherman, 2010). Although, there have been studies aiming to identify OBI prevalence in some populations, there has been no study characterizing the prevalence of OBI cases in Ethiopia.

The underlying immunological and molecular mechanisms of OBI are not completely understood. The failure of assays to detect HBsAg in OBI infection is attributable to mutations of the S and pre-S1/2 genes which may cause modifications to hepatitis B surface antigenicity (Fang et al., 2009, Yuan et al., 2010). Mutations of the S gene may be responsible for negative results in individuals tested with standard assays (Katsoulidou et al., 2009, Squadrito et al., 2012). The exact mechanism by which HBsAg remains undetected is poorly understood, due to difficulties of full-length DNA sequencing with low levels of viral DNA typical in the sera of OBI infected individuals (Squadrito et al., 2012).

2.1.6 Diagnosis of Hepatitis B Virus infection

The diagnosis of hepatitis B virus (HBV) infection was revolutionized by the discovery of Australia antigen, now called hepatitis B surface antigen (HBsAg). HBV diagnosis is accomplished by testing for a series of serological markers of HBV and by additional testing to exclude alternative etiological agents such as hepatitis C viruses. Serological tests are used to distinguish acute, self-limited infections from chronic HBV infections and to monitor vaccine-induced immunity. Nucleic acid testing for HBV-DNA is increasingly being used to quantify HBV viral load and measure the effectiveness of therapeutic agents. Given the multitude of available tests and the complexity of clinical management, there is a critical need for greater coordination among clinicians, diagnostic laboratory personnel and researchers to define optimal laboratory diagnostic and monitoring assays so that the appropriate tests are used to maximize prevention and optimize treatment outcomes (Krajden et al., 2005).

2.1.7 Treatment and prevention

Acute hepatitis B infection does not usually require treatment because most adults clear the infection spontaneously (Hollinger and Lau, 2006). Early antiviral treatment may only be required in less than 1% of patients, whose infection takes a very aggressive course (fulminant hepatitis) or who are immune compromised. On the other hand, treatment of chronic infection
may be necessary to reduce the risk of cirrhosis and liver cancer. Chronically infected individuals with persistently elevated serum alanine aminotransferase, a marker of liver damage, and HBV DNA levels are candidates for therapy (Lai and Yuen, 2007). Vaccination is the most effective measure to reduce the global incidence of hepatitis B. To date, global hepatitis B vaccine coverage is estimated at 69% (WHO, 2012). To mention some literatures on the sero-prevalence of HBsAg and HBcAb in different population groups, especially mothers in Ethiopia, African countries and in the world was summarized in the following Table 1.

Table 1: The prevalence of HBsAg and HBcAb in different population in the World

<table>
<thead>
<tr>
<th>Authors</th>
<th>study Area</th>
<th>design</th>
<th>setting</th>
<th>sample size</th>
<th>%HBsAg</th>
<th>%HBcAb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Awole, 2005</td>
<td>Jimma, Ethiopia</td>
<td>Cross sectional (CS)</td>
<td>Hospital</td>
<td>493 pregnant women</td>
<td>3.7</td>
<td></td>
</tr>
<tr>
<td>Abebe et al., 2002</td>
<td>Addis Ababa</td>
<td>CS community</td>
<td>4736</td>
<td></td>
<td>6.2</td>
<td>36.6</td>
</tr>
<tr>
<td>Gelaw and Mengitsu, 2007</td>
<td>Tigray and Amhara</td>
<td>CS Hospital</td>
<td>600 blood donors</td>
<td></td>
<td>6.2</td>
<td></td>
</tr>
<tr>
<td>Tegegne et al., 2012</td>
<td>Addis Ababa</td>
<td>CS Hospital</td>
<td>265 pregnant women</td>
<td></td>
<td>3.0</td>
<td></td>
</tr>
<tr>
<td>Negero et al., 2011</td>
<td>Shashemen, Ethiopia</td>
<td>CS Hospital</td>
<td>384 VCTs clients</td>
<td></td>
<td>5.7</td>
<td></td>
</tr>
<tr>
<td>Molla et al., 2015</td>
<td>Bahir Dar, Ethiopia</td>
<td>CS Hospital</td>
<td>384 pregnant</td>
<td></td>
<td>4.4</td>
<td></td>
</tr>
<tr>
<td>Agera et al., 2017</td>
<td>Gojam, Ethiopia</td>
<td>CS community</td>
<td>481 adult</td>
<td></td>
<td>3.1</td>
<td></td>
</tr>
<tr>
<td>Sharew et al., 2015</td>
<td>Dessie, Ethiopia</td>
<td>retrospective Hospital</td>
<td>8908 blood donors</td>
<td></td>
<td>4.66</td>
<td></td>
</tr>
<tr>
<td>Cho et al., 2012</td>
<td>Ghana</td>
<td>CS Hospital</td>
<td>1,500 pregnant women</td>
<td></td>
<td>10.6</td>
<td></td>
</tr>
<tr>
<td>Rusine et al., 2013</td>
<td>Kigali, Rwanda</td>
<td>prospective cohort</td>
<td>community</td>
<td>416 HIV</td>
<td>5.2</td>
<td>42.9</td>
</tr>
<tr>
<td>Terence et al., 2011</td>
<td>Hong Kong, China</td>
<td>CS Hospital</td>
<td>10808 subjects</td>
<td></td>
<td>7.5</td>
<td></td>
</tr>
<tr>
<td>Ding et al., 2013</td>
<td>China</td>
<td>CS Hospital</td>
<td>4,536 pregnant</td>
<td></td>
<td>5.5</td>
<td>58.5</td>
</tr>
<tr>
<td>Mehta et al., 2013</td>
<td>India</td>
<td>CS Hospital</td>
<td>1038 pregnant</td>
<td></td>
<td>2.9</td>
<td></td>
</tr>
</tbody>
</table>
2.2 Hepatitis C Virus

2.2.1 Virology

In 1989, HCV was identified by Choo et al. as a positive stranded RNA molecule related to Togaviridae or Flaviviridae (Saeed et al., 2014). Now, HCV is classified as the type member of the genus Hepaciviruses within the virus family Flaviviridae (Pawlotsky, 2004). It measures 30 to 60 nm in diameter, with a positive-sense RNA genome and is enveloped. The genome of HCV encodes 10 proteins including 2 glycoproteins (E1, E2) that undergo variation during infection due to hyper variable regions within their genes (AL avian, 2009). HCV has been suggested to have six genotypes and a large number of subtypes (1a, 1b, 1c, etc.) have been identified so far (Simmonds et al., 2005), which differ from each other by 31 –33% at the nucleotide level and are further classified into several subtypes (Pawlotsky, 2004, AL avian, 2009) the genotype 1, 2, and 3 appears to have a worldwide distribution and their relative prevalence varies from one geographic area to another. HCV genotype 4 appears to be prevalent in North Africa and the Middle East, and genotype 5 and 6 seems to be confined to South Africa and Hong Kong respectively (AL avian, 2009). Phylogenetic analysis revealed that the predominant genotype in Ethiopia were 4 (77.6%), followed by 2 (12.2%), 1 (8.2%), and 5 (2.0%). Seven subtypes were identified (1b, 1c, 2c, 4d, 4l, 4r and 4v), with 4d (34.7%), 4r (34.7%) and 2c (12.2%) as the most frequent subtypes (Hundie et al., 2017).

2.2.2 Epidemiology

Epidemiological studies of HCV are challenging since most cases of HCV infection are asymptomatic and indistinguishable clinically from other causes of hepatitis. A laboratory diagnosis is therefore essential, but not always available, particularly in resource-limited settings. There are consequently few population-based epidemiological studies of HCV. Most studies are based on selected high risk (e.g. intravenous drug users) or low risk (e.g. blood donor) populations which either overestimate or underestimate the true prevalence respectively (Norderstedt et al., 2010). HCV is endemic worldwide, with an estimated global prevalence of 3% (170 million) (Shepard et al., 2005).

There is great variation in the geographical distribution of HCV, with the highest prevalence in Africa and Asia, and lower prevalence in industrialized countries as illustrated in Figure 3. HCV
is four to five times more prevalent than HIV globally and is the commonest chronic blood-borne infection in the United States with three to four million people infected by HCV every year (Shepard et al., 2005). Even within developing nations, there is great variation in prevalence. Some of this variation may be explained by differences in study and reporting methods. Actual differences in HCV prevalence may be due to variation in risk factors in different parts of the world. Injection drug use is the single most important risk factor in developed regions of the world. Unsafe therapeutic injections and blood transfusions are important risk factors in developing nations where these practices occur (Norderstedt et al., 2010).

![Geographic Distribution of Hepatitis C Virus Infection](image)

Figure 3: Geographic distribution of hepatitis C virus (Norderstedt et al., 2010)

Sub-Saharan Africa has the highest prevalence of HCV (5.3%) among the WHO world regions. Within this region there are remarkable differences in prevalence: the central African region has the highest prevalence (6%), and Southern and East Africa, the lowest (1.6%). Cameroon has the highest national prevalence of 12% and South Africa the lowest (0.1%) among blood donors (Madhava et al., 2005). Limited data are available in Ethiopia. The overall seroprevalence of HCV in 1,580 Ethiopian subjects representing urban and rural populations was reported to be 2.0% (Frommel et al., 1993). Most of the studies revealed a low overall prevalence of HCV infection. These include: 0.3% in Health professionals (Yimer, 2003), 0.7% in 6361 consecutive blood donors (Tessema et al., 2010), 0.9% in inhabitants of Addis Ababa (Ayele et al., 2002), 1.3% in pregnant women attending antenatal clinic (Tiruneh, 2008) and 1.7% in Tigray and Amhara regions (Gelaw and Mengitsu, 2008).
2.2.3 Transmission and risk factors

HCV is a blood-borne which has multiple routes of transmission. It is transmitted by unsafe sexually intercourse, vertically, horizontally, iatrogenically (Unsafe transfusions and therapeutic injections, needle-stick injuries and acupuncture), occupational, cultural and recreational activities (Intravenous drug use, tattooing, scarification and ear-piercing (Hughes and Mahy, 1998).

2.2.4 Clinical outcome of Hepatitis C Virus infection

Patients infected with HCV have an 80 to 85% chance that the HCV infection will persist and they will go on to have a chronic HCV infection. Chronic HCV infection has multiple manifestations, the most common of which is chronic progressive liver disease associated with inflammation, fibrosis and cirrhosis. Cirrhosis or end–stage liver disease is associated with multiple complications including hepatocellular carcinoma. These conditions are common causes of morbidity and mortality in HCV infected patients (Weinstock, 1999).

HCV infection causes an acute or chronic disease of the liver. Only relatively small fractions of HCV infections are symptomatic, most infected individuals remain asymptomatic and undiagnosed (Sultan et al., 2009). Major clinical manifestation is progressive hepatic fibrosis, which leads to cirrhosis and an increased risk of hepatocellular carcinoma (Feinstone et al., 1975). Primary infection with HCV leads to persistent viremia in 85% of patients with development of chronic liver disease in >60% of cases. Approximately 20% of individuals with chronic hepatitis C eventually develop medically significant sequelae including cirrhosis, end stage liver disease or HCC (Wolfgang et al., 2000).
2.2.5 Diagnosis of hepatitis C virus infection

Diagnostic tests used for the detection of HCV infection include the HCV antibody enzyme immunoassay, recombinant immunoblot assay, and quantitative HCV RNA polymerase chain reaction (PCR) (Ghany et al., 2009).

The most widely used initial assay for detecting HCV antibodies is the enzyme immunoassay. A positive enzyme immunoassay should be followed by a confirmatory test. Recombinant immunoblot assays a confirmatory test for a positive enzyme immunoassay, detects antibodies to individual HCV antigens and has a greater specificity. It is used in conjunction with viral load tests to distinguish between a resolved infection and a false positive enzyme immunoassay (Scott and Gretchen, 2007).

Now a day the diagnosis of hepatitis C infection uses two assays: 1) serologic assays that detect human antibodies generated as a response to hepatitis C virus (HCV) infection. A positive HCV antibody test indicates HCV infection at some point in time, but it does not differentiate whether the person has resolved or current HCV infection. This assay includes Enzyme Immunoassay
(EIA), Chemiluminescence Immunoassay (CIA), Point-of-Care Rapid Immunoassays and Recombinant Immunoblot Assay (RIBA) (Gretch, 1997).

2) Molecular HCV RNA Tests: Molecular diagnostic tests for hepatitis C specifically detect HCV RNA and the process is commonly referred to as a Nucleic Acid Test (NAT) or Nucleic Acid Amplification Test (NAAT). The HCV NAT becomes positive approximately 1 to 2 weeks after initial HCV infection. The NAT test has become the gold standard supplemental test for patients who have a positive HCV EIA screening test. The NAT can determine whether a patient with a positive HCV antibody test has current (active) or resolved HCV infection. In addition, the NAT can be used to diagnose individuals with acute HCV infection. It could be Qualitative HCV RNA and Quantitative HCV RNA (Gretch, 1997). NAT for the detection of HCV RNA remains the gold standard for diagnosing active HCV infection (Hosseini-Moghaddam et al., 2012).

Quantitative viral load tests measure the amount of virus in blood. Quantitative studies provide information on initial viral load, viral load reduction with therapy, and a sustained virologic response, defied as undetectable HCV by PCR six months after stopping therapy (Ghany et al., 2009).

2.2.6 Prevention

There is no vaccine against HCV. Research is in progress but the high mutability of the HCV genome complicates vaccine development (Simmonds, 2006). In absence of a vaccine, all precautions to prevent infection of HCV should target reduction of transmission of the virus. The only means of protection are the implementation universal precautions and safe injection practices. Screening and treatment of blood products is the only way to prevent transfusion associated cases (Van, 1999). HCV carriers should be strongly discouraged from drinking alcohol because there is evidence that acts as a cofactor in developing more severe liver injury (Madhava et al., 2002).

2.2.7 Treatment of Hepatitis C virus infection

Treatment of acute hepatitis is mainly supportive, consisting of bed rest and balanced diet with small frequent nitrous meals and hospitalization reserved only for cases of sever disease (Madhava et al., 2002). The goal of treatment is to achieve a sustained viral response (SVR), as
defined by the absence of viremia 6 month after stopping the medications; SVR is associated with improved histology and decreased risk of morbidities (Backmund et al., 2005).

The complications of HCV infection can be prevented by antiviral therapy based on the use of a combination of pegylated interferon and ribavirin that yields a sustained eradication of infection in 40% to 50% of cases. The use of serological and virological tests has become essential in the management of HCV infection in order to diagnose infection, and most importantly guide treatment decisions and assess the virological response to antiviral therapy (Gessoni and Manoni, 2005).

A dramatic improvement in HCV therapy followed the introduction of oral medicines that directly inhibited the replication cycle of HCV. These medicines, called direct acting antiviral drugs (DAAs), target three important regions within the HCV genome: NS3/4A protease, NS5A and NS5B RNA-dependent polymerase. These medicines have led to higher sustained virological responses (SVRs) than interferon-based regimens, are shorter in treatment duration, are orally administered and have fewer side-effects. Individual DAAs vary in therapeutic efficacy, genotypic efficacy, adverse events and drug–drug interactions (DDIs), and must be used in combination with at least one other DAA (WHO, 2016).

The first-generation DAAs that were marketed were the protease inhibitors boceprevir and telaprevir, which were co-administered with interferon and ribavirin. However, they were only effective in treating patients with genotype 1 infection; moreover, they caused frequent and sometime severe side-effects, particularly among persons with more advanced disease (WHO, 2016).

Second-generation DAAs have higher rates of SVR, are safer and can be used in combinations that obviate the need for interferon and ribavirin. Thus, these are referred to as “interferon-free” treatment regimens. The combination of two or three subclasses of these DAAs have demonstrated excellent efficacy in general, although cure rates among certain patient subgroups are lower (WHO, 2016). To mention some literatures on the sero-prevalence of HCV in different population groups, special women in Ethiopia, African countries and the world was summarized in the following Table 2.
Table 2: The prevalence of HCV in different population in the World

<table>
<thead>
<tr>
<th>Authors and Year</th>
<th>study Area</th>
<th>design</th>
<th>setting</th>
<th>sample size</th>
<th>%HCV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ayele et al., 2002</td>
<td>Addis Ababa</td>
<td>Cross sectional</td>
<td>community</td>
<td>4593</td>
<td>0.9</td>
</tr>
<tr>
<td>Gelaw and Mengitsu, 2007</td>
<td>Tigray and Amhara</td>
<td>Cross sectional</td>
<td>Hospital</td>
<td>600 blood donors</td>
<td>1.7</td>
</tr>
<tr>
<td>Molla et al., 2015</td>
<td>Bahir Dar, Ethiopia</td>
<td>Cross sectional</td>
<td>Hospital</td>
<td>384 pregnant</td>
<td>0.26</td>
</tr>
<tr>
<td>Sharew et al., 2015</td>
<td>Dessie, Ethiopia</td>
<td>retrospective</td>
<td>Hospital</td>
<td>8908 blood donors</td>
<td>0.61</td>
</tr>
<tr>
<td>Abera et al., 2017</td>
<td>Gojjam, Ethiopia</td>
<td>Cross sectional</td>
<td>Community</td>
<td>481 adult</td>
<td>1.0</td>
</tr>
<tr>
<td>Belyhun et al, 2016</td>
<td>Ethiopia</td>
<td>a systematic review</td>
<td>Pooled population</td>
<td>68 studies reviewed</td>
<td>3.1</td>
</tr>
<tr>
<td>Ndong-Atome et al., 2008</td>
<td>Gabon</td>
<td>Cross sectional</td>
<td>Hospital</td>
<td>947 pregnant</td>
<td>2.1</td>
</tr>
<tr>
<td>Esan et al., 2014</td>
<td>Nigeria</td>
<td>Cross sectional</td>
<td>Facility based</td>
<td>649 pregnant</td>
<td>1.39</td>
</tr>
<tr>
<td>Rusine et al., 2013</td>
<td>Kigali, Rwanda</td>
<td>prospective cohort</td>
<td>Community</td>
<td>416 HIV</td>
<td>5.2</td>
</tr>
<tr>
<td>Mehta et al., 2013</td>
<td>India</td>
<td>Cross sectional</td>
<td>Hospital</td>
<td>1038 pregnant</td>
<td>0.19</td>
</tr>
</tbody>
</table>
3. OBJECTIVES

3.1 General objective

- To determine the sero-prevalence of HBV and HCV infection and to identify associated risk factors with the infection among apparently healthy mothers.

3.2 Specific objectives

✓ To determine the Sero-prevalence of HBV and HCV infection among apparently healthy mothers
✓ To determine co-infections between HBV and HCV infections among apparently healthy mothers
✓ To assess potential risk factors associated with HBV and/or HCV infections.
4. METHODS AND MATERIALS

4.1 Study Design and study period

A community based cross sectional study was conducted from June 2016 to May 2017.

4.2 Study Area

The study was conducted in Addis Ababa City Administration which is the capital city of the country, with a population of 3,384,569 according to the 2007 population census with annual growth rate of 3.8% and the population density was 5,165.1/km$^2$; all of the population is urban inhabitants. For the capital city 662,728 households were counted living in 628,984 housing units, which results in an average of 5.3 persons to a household. All Ethiopian ethnic groups are represented in the city (CSA, 2007).

According to the 2007 national census, 98.64% of the housing units of Addis Ababa had access to safe drinking water, while 14.9% had flush toilets, 70.7% pit toilet, and 14.3% had no toilet facilities. Values for other reported common indicators of the standard of living for Addis Ababa as of 2012 showed that 0.1% of the inhabitants fall into the lowest wealth quintile. Adult literacy for men is 93.6% and for women 79.95%, the highest in the nation for both sexes and the civic (urban) infant mortality rate is 45 infant deaths per 1,000 live births, which is less than the nationwide average of 77; at least half of these deaths occurred in the infants’ first month of life(CSA, 2007). HIV prevalence in Addis Ababa mothers was (5.2%) (EDHS, 2016).

4.3 Population

4.3.1 Target Population

All mothers aged above 18 years and lived in the study area were targeted.

4.3.2 Source of Population

All healthy mothers who have children aged between 5-9 years were recruited. This was important to determine vertical transmission rate and seroprotection level of children aged 5-9 years old.
4.3.3 Study Population

All healthy mothers whose child was recruited for the assessment of antibody level against hepatitis B virus after hepatitis B vaccine and those women who have unvaccinated child for HBV.

4.3.4. Eligibility

4.3.4.1. Inclusion

All healthy mothers who have either vaccinated or unvaccinated children for HBV and recruited for the assessment of antibody level against hepatitis B virus after hepatitis B vaccine were included.

4.3.4.2. Exclusion

- Women who have no children
- Individuals with drug history of immunosuppressive therapy
- Critically ill were excluded from the study population.

4.3.5 Sampling method

Administratively, Addis Ababa is divided into 10 Subcities and 116 Woredas. These Woredas are the smallest unit of administration. According to 2012 CSA there was around 700,000 households. The households from each Woredas were selected using systematic random sampling technique after getting the proportional allocation value by dividing the total number of households in the study area to the total number of selected households. The study subjects were recruited from three subcities (Gulele, Kirkos and Lideta) and seven Woredas were selected randomly. In each Woredas 50-70 households were recruited by health extension workers.

4.3.6 Sample Size Determination

The sample size was calculated by using single proportional formula. It was determined by taking a proportion of 0.5 by assuming that the mean population for each parameter was known to be 50%, 4.8% marginal error and 95% confidence level.

\[ n = \frac{z^2 \alpha^2}{d^2} \left( p(1 - p) \right) \]
Therefore, the sample size was around 458 including 10% non-responding rate.

4.5 VARIABLES
4.5.1 Dependent Variables
Sero-prevalence of HBV and HCV infection

4.5.2 Independent Variables
Demographic data (age, educational status, marital status etc.) and other associated risk factors

4.6 Data collection
4.6.1 Specimen collection and processing
Five milliliter of venous blood was collected and transported to the laboratory. The blood was allowed to clot and serum was separated by centrifugation at room temperature at 3000 rpm for four minutes and stored in the freezer at -20 °C before being tested.

4.6.2 Demographic data collection
A structured questionnaire was used to collect socio-demographic variables and associated risk factors of the study participants by face to face interview. The data was collected by principal investigator.

4.6.3 Laboratory investigations
All samples were tested, using a sandwich third generation Enzyme Linked Immunosorbent Assay (ELISA) for HBsAg, Anti-HBc, and anti-HCV by using Bio-Rad ELISA kits (Hwang et al., 2006).

Monolisa™ HBs Ag ULTRA assay (Bio-Rad, Marnes-la-Coquette, France): is a one-step Enzyme Immunoassay based on the principle of the "sandwich" type using monoclonal antibodies and polyclonal antibodies selected for their ability to bind to the various subtypes of HBs Ag and the variant HBV strains. The Monolisa™ HBs Ag ULTRA solid phase is coated with monoclonal antibodies. The Monolisa™ HBs Ag ULTRA conjugates are based upon the use of monoclonal antibodies from mouse and polyclonal antibody from goat against the HBs Ag which are bound to the peroxidase.

Monolisa™ Anti-HBc PLUS ELISA kit (Bio-Rad, Marnes-la-Coquette, France): is an enzyme immunoassay (indirect ELISA type) for the simultaneous detection of total antibodies to hepatitis
B virus core in human serum or plasma. It is based upon the use of a solid phase prepared with recombinant HBc antigen.

**Monolisa™ HCV Ag-Ab ULTRA assay** (Bio-Rad, Marnes-la-Coquette, France): is an enzyme immunoassay for the detection of HCV infection, based on the detection of capsid antigen and/or antibodies associated with an infection by Hepatitis C virus in patient serum or plasma. The Monolisa™ HCV Ag-Ab ULTRA Microplates solid phase is coated with:

- Monoclonal Antibodies against capsid protein of Hepatitis C virus.
- Two recombinant proteins
- One recombinant antigen
- A mutated peptide from the capsid of structural area of the hepatitis C virus genome.

There are two conjugates:

Conjugated 1: Mouse biotinilated monoclonal antibodies against hepatitis C capsid which does not react against the hepatitis C capsid mutated peptide coated on the microplate.

Conjugate 2: A Mouse peroxidase-labeled antibody to human IgG and peroxidase-labeled streptavidin.

All tests were carried out according to the manufacturer’s instructions as outlined in the package inserts. All laboratory tests were performed at Immunology Laboratory of Armauer Hansen Research Institute.

### 4.7 Quality Assurance

Standard operating procedures were followed during blood sample collection processing and analysis of data. All sera were screened for each viral hepatitis biomarkers using Bio-Rad ELISA kits screening test kits according to the manufacturer's instruction. The performances of Monolisa™ HBsAg ULTRA has been determined by testing samples and was recorded a sensitivity of 100% and the specificity of 99.94%. The Monolisa™ Anti-HBc PLUS test has a Sensitivity of 99.53% and specificity of 99.5%. The Monolisa™ HCV Ag-Ab ULTRA assay has a sensitivity of 100% and a specificity of 99.83%. According to the manufacturer’s instructions, positive samples should be retested in duplicate before the final interpretation. As indicated in the package inserts, positive and negative controls were run as the test runs.
4.8 Data Processing and Analysis

Data entry and analysis was done using SPSS version 20.0 computer software. Data was summarized and presented in descriptive measures such as a table, figures, mean and percentage. Chi-square test was used to establish association between serological results and different risk factors considered in the study. It was used to compare categorical data, and to evaluate the difference in prevalence between groups in the bivariate logistic analysis as well as the statistical significance between relevant variables. The result at p-value < 0.05 was considered as statistically significant. To determine the correlation between the data obtained from the questionnaire and the laboratory results, odds ratios (ORs) and their corresponding 95% confidence intervals (CIs) was calculated using logistic regression analysis. This is to determine whether a variable was associated with the infections.

4.9 Ethical Considerations

The study was carried out after it was approved by Addis Ababa University, College of Health Sciences, Department of Microbiology, Immunology and Parasitology Ethical Review Committee and Institutional Review Board (IRB). It was also be approved by AHRI Ethical Review Committee then a support letter was obtained from Addis Ababa Health Bureau. The purpose of the study was explained to each participant and sample was obtained only after each participant gives her written consent. All information obtained held securely and stored on paper and computer files with a unique identification number. No one except the interviewers knew the participant took part in the study and the answers were given by the participant marked with a especial study number only, and not the name.
5. RESULTS

5.1. Socio-demographic Characteristics

A total of 454 mothers were involved in this study and the mean age of the study subjects were 32.75±5.79 (SD) years, (range: 20 to 57 years). Majority of the mothers belonged to age group 30-34 years which accounts 33.5%, followed by age group 25-29 (25.1%) and 35-39 (25.1%). Regarding marital status of the participants, most of the mothers were married 371 (81.7%), followed by divorced which accounts 67(14.8%). Majority of the participants were housewives 249 (54.9%), followed by private workers 93(20.8%). Fifty seven (11.5%) were illiterate, 260 (57.3%) had primary educations and 106 (23.3%) the study participants studied above secondary schools as illustrated in Table 3.

Table 3: Socio-demographic characteristics among apparently healthy mothers in Addis Ababa, Ethiopia (N =454)

<table>
<thead>
<tr>
<th>Socio-demographic characteristics</th>
<th>Numbers</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age group</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20-24</td>
<td>21</td>
<td>4.6</td>
</tr>
<tr>
<td>25-29</td>
<td>114</td>
<td>25.1</td>
</tr>
<tr>
<td>30-34</td>
<td>152</td>
<td>33.5</td>
</tr>
<tr>
<td>35-39</td>
<td>114</td>
<td>25.1</td>
</tr>
<tr>
<td>40-44</td>
<td>34</td>
<td>7.5</td>
</tr>
<tr>
<td>45-49</td>
<td>14</td>
<td>3.1</td>
</tr>
<tr>
<td>50-54</td>
<td>3</td>
<td>0.7</td>
</tr>
<tr>
<td>55-59</td>
<td>2</td>
<td>0.4</td>
</tr>
<tr>
<td><strong>Education</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Illiterate</td>
<td>52</td>
<td>11.5</td>
</tr>
<tr>
<td>1-8</td>
<td>260</td>
<td>57.3</td>
</tr>
<tr>
<td>9-12</td>
<td>106</td>
<td>23.4</td>
</tr>
<tr>
<td>≥College</td>
<td>36</td>
<td>7.9</td>
</tr>
<tr>
<td><strong>Marital status</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Married</td>
<td>371</td>
<td>81.7</td>
</tr>
<tr>
<td>Unmarried</td>
<td>9</td>
<td>2.0</td>
</tr>
<tr>
<td>Divorced</td>
<td>67</td>
<td>14.8</td>
</tr>
<tr>
<td>widowed</td>
<td>7</td>
<td>1.5</td>
</tr>
<tr>
<td><strong>Occupation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Housewife</td>
<td>249</td>
<td>54.9</td>
</tr>
<tr>
<td>Employed</td>
<td>57</td>
<td>12.5</td>
</tr>
<tr>
<td>Dailylaborer</td>
<td>55</td>
<td>12.1</td>
</tr>
<tr>
<td>Private</td>
<td>93</td>
<td>20.8</td>
</tr>
<tr>
<td><strong>Family size</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-4</td>
<td>194</td>
<td>42.5</td>
</tr>
<tr>
<td>5-6</td>
<td>210</td>
<td>46.2</td>
</tr>
<tr>
<td>≥7</td>
<td>50</td>
<td>11.0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>454</td>
<td>100</td>
</tr>
</tbody>
</table>
5.2 Magnitude of HBV and HCV Infection

Overall, 42.3 % (95% CI: 39.5-46.1%) of apparently healthy mothers were positive for HBsAg, HBcAb or HCV antibody. The sero-prevalence for HBsAg was 17/454 (3.7%), for HBcAb was 165/454 (36.3%) and anti-HCV was 9/454 (2.0%) among the study participants’ respectively. Seventeen (3.7%) of the study participants were positive both HBsAg and HBcAb which were acute or chronic infections. One Hundred forty nine (32.9%) of the study participants had HBcAb only.

Age specific prevalence of HBsAg was different across various age groups but the difference is not statistically significant (p = 0.348). Majorities were detected among those with age group 25-29 years which accounts 8 (7.0%), followed by age group 30-34 years 7(4.6%) as illustrated in Table 4. The highest prevalence of HCV infection was found in age group 50-55 years 1/3 (33.3%). Followed by among age groups 30-34 years 4/152 (2.6%) and 35-39 years 3/114 (2.6%). But it was 0.0% among age groups 40-44, 45-49 and 55-60 years as shown in Table 4. The different was no statistical significant among different age groups (p =0.24).

Age specific prevalence of HBcAb was also different across various age groups. Within the age groups, majorities were detected among those with age group 50-54 years which accounts for 2/3 (66.7 %), followed by age group 45-49 years 8/14 (57.1%), 40-44 years 19/34(55.9) but the prevalence was heterogeneous among different age groups which was slightly increased across varies age groups (p=0.091) as indicated in Table 4.

Withn educational status of participants, among those who attended primary school, secondary school and college and above, the prevalence of HBsAg was 12/260 (4.6%), 3/106 (2.8%), and 2/36 (5.6%) respectively while 0.0% in illiterate (p=0.2) as shown in Table 4.

The prevalence of HCV was 1/36 (2.8%) among those who attended college and above, 3/106 (2.8%) among those who attended secondary schools, 1/52 (1.9%) among those who had no formal education and 4/260 (1.5%) among those who attended primary educations.

There was a high prevalence of 1/9 (11.1%) HBsAg among unmarried apparently healthy mothers (those give birth without legal marriage) and 16/371 (4.3%) among married mothers within marital status (p=0.069). Within occupational status of the study participants, there was
4/55 (7.3%) HBsAg prevalence among daily laborers, 11/249 (4.4%) of housewives and 2/93 (2.2%) among private workers ($p=0.15$) as revealed in Table 4.

With regard to the family size of the study participants, there was 3/194 (1.5%) prevalence of HBsAg among 1-4 households, 9/210 (4.3%) prevalence of HBsAg among 5-6 households and 5/50 (10%) among more than seven households.

There was 2/194 (1.0%) prevalence of HCV among 1-4 households, 7/210 (3.3%) prevalence of HCV among 5-6 households and 0.0% among those who had more than seven family sizes as shown in Table 4.

**Table 4**: The prevalence of HBsAg, HBcAb and Anti-HCV in relation to Socio-demographic characteristics among apparently healthy mothers in Addis Ababa, Ethiopia (N=454)

<table>
<thead>
<tr>
<th>Socio-demographic characteristics</th>
<th>Numbers</th>
<th>HBsAg Positive (%)</th>
<th>HBcAb Positive (%)</th>
<th>HCV Positive (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age group</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20-24</td>
<td>21</td>
<td>0(0.0)</td>
<td>6(28.6)</td>
<td>0(0.0)</td>
</tr>
<tr>
<td>25-29</td>
<td>114</td>
<td>8(7.0)</td>
<td>34(29.9)</td>
<td>1(0.9)</td>
</tr>
<tr>
<td>30-34</td>
<td>152</td>
<td>7(4.6)</td>
<td>55(36.2)</td>
<td>4(2.6)</td>
</tr>
<tr>
<td>35-39</td>
<td>114</td>
<td>2(1.8)</td>
<td>40(35.1)</td>
<td>3(2.6)</td>
</tr>
<tr>
<td>40-44</td>
<td>34</td>
<td>0(0.0)</td>
<td>19(55.9)</td>
<td>0(0.0)</td>
</tr>
<tr>
<td>45-49</td>
<td>14</td>
<td>0(0.0)</td>
<td>8(57.1)</td>
<td>0(0.0)</td>
</tr>
<tr>
<td>50-54</td>
<td>3</td>
<td>0(0.0)</td>
<td>2(66.7)</td>
<td>1(33.3)</td>
</tr>
<tr>
<td>55-59</td>
<td>2</td>
<td>0(0.0)</td>
<td>1(50.0)</td>
<td>0(0.0)</td>
</tr>
<tr>
<td><strong>Education</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Illiterate</td>
<td>52</td>
<td>0(0.0)</td>
<td>11(21.2)</td>
<td>1(2.0)</td>
</tr>
<tr>
<td>1-8</td>
<td>260</td>
<td>12(4.6)</td>
<td>97(37.3)</td>
<td>4(1.5)</td>
</tr>
<tr>
<td>9-12</td>
<td>106</td>
<td>3(2.8)</td>
<td>43(41.0)</td>
<td>3(2.8)</td>
</tr>
<tr>
<td>≥College</td>
<td>36</td>
<td>2(5.6)</td>
<td>14(38.9)</td>
<td>1(2.8)</td>
</tr>
<tr>
<td><strong>Marital status</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Married</td>
<td>371</td>
<td>16(4.3)</td>
<td>137(37.2)</td>
<td>4(1.1)</td>
</tr>
<tr>
<td>Unmarried</td>
<td>9</td>
<td>1(11.1)</td>
<td>2(22.2)</td>
<td>0(0.0)</td>
</tr>
<tr>
<td>Divorced</td>
<td>67</td>
<td>0(0.0)</td>
<td>24(35.8)</td>
<td>4(6.0)</td>
</tr>
<tr>
<td>widowed</td>
<td>7</td>
<td>0(0.0)</td>
<td>1(28.6)</td>
<td>1(14.3)</td>
</tr>
<tr>
<td><strong>Occupation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Housewife</td>
<td>249</td>
<td>11(4.5)</td>
<td>92(36.9)</td>
<td>4(1.6)</td>
</tr>
<tr>
<td>Employed</td>
<td>57</td>
<td>0(0.0)</td>
<td>23(40.4)</td>
<td>1(1.8)</td>
</tr>
<tr>
<td>Dailylaborer</td>
<td>55</td>
<td>4(7.3)</td>
<td>16(29.1)</td>
<td>2(3.6)</td>
</tr>
<tr>
<td>Private</td>
<td>93</td>
<td>2(2.2)</td>
<td>34(36.6)</td>
<td>2(2.2)</td>
</tr>
<tr>
<td><strong>Family size</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-4</td>
<td>194</td>
<td>3(1.5)</td>
<td>66(34.0)</td>
<td>2(1.0)</td>
</tr>
<tr>
<td>5-6</td>
<td>210</td>
<td>9(4.3)</td>
<td>77(36.7)</td>
<td>7(3.3)</td>
</tr>
<tr>
<td>≥7</td>
<td>50</td>
<td>5(10.0)</td>
<td>22(44.0)</td>
<td>0(0.0)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>454</td>
<td>17(3.7)</td>
<td>165(36.3)</td>
<td>9(2.0)</td>
</tr>
</tbody>
</table>
With regard to the HBV vaccination status, based on self-reporting of the study participants, it was only 4/454 (0.9%) of the participants were vaccinated.

5.3 Risk factors associated with HBV and HCV Infections
A positive association was noted between participants’ number of households ($x^2=8.2$, $p=0.017$), diagnostic history of liver diseases ($x^2=11.6$, $p=0.01$), History of Jaundice ($x^2=27.1$, $p =0.00$), and family history of liver disease ($x^2 =4.3$, $p =0.038$) and HBsAg seropositivity at 95% confidence interval. Of the total with history of liver disease (n= 31), history of jaundice (n=9) and family history of hepatitis (n=38), the prevalence of HBsAg was 13.9%, 30.8% and 10.5% respectively. Age group, education level, marital status, occupation, alcohol consumption habits, caring for hepatitis patients, history of operation and cesarean section, history of female genital mutilation, history of sharp injury, history of blood transfusion, history of ear-piercing, dental procedure, having multiple sexual partner and history of abortion, on the other hand, did not impact significantly on the likelihood of having hepatitis B virus infection.

In addition to that, the associated risk factors were assessed for their association with HBV infection using binary and multivariate logistic regression. As a result, binary and multivariate logistic regression analysis indicated that few predictor variables showed statistically significant association with HBV infections. Mothers who had a diagnosis history of hepatitis were 5.5 times more likely of being positive by HBV than mothers who had no history of diagnosis (AOR = 5.5 CI (1.8-16.5); $p=0.003$. Mothers who had having history of jaundice (AOR=17.8 CI (4.0-75.5); $p=0.03$ were more likely to be infected than their counterparts at 95% confidence interval. The odd of having HBsAg was 17.8 times with those without history of jaundice. Mothers who had previous history of house hold contact or family history of hepatitis were 9.3 %, of which 9.5 % were positive for HBsAg. Statistically significant association was detected between having previous contact with members of the house hold with HBV infection ($p = 0.049$). Mothers who had previous history of house hold contact were 3.2 times more likely to have infection with HBV than those without previous history of house hold contact (AOR= 3.2 CI (1.0-10.4); $p=0.049$ at 95% confidence interval as illustrated in Table 5.
Table 5: Association in bivariate and multivariate logistic regression analysis of explanatory variables and HBV infection among apparently healthy mothers in Addis Ababa, Ethiopia

<table>
<thead>
<tr>
<th>s.n.o</th>
<th>Variables</th>
<th>HBsAg test result</th>
<th>COR (CI) 95%</th>
<th>AOR (CI) 95%</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Positive %</td>
<td>Negative</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>Diagnosis history of hepatitis</td>
<td>Yes</td>
<td>5(13.9)</td>
<td>31</td>
<td>5.4(1.8-16)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No</td>
<td>12(2.9)</td>
<td>406</td>
<td>1.0(reference)</td>
</tr>
<tr>
<td>2.</td>
<td>Caring of hepatitis patients</td>
<td>Yes</td>
<td>2(4.8)</td>
<td>40</td>
<td>1.3(0.3-6.0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No</td>
<td>15(3.6)</td>
<td>397</td>
<td>1.0(reference)</td>
</tr>
<tr>
<td>3.</td>
<td>Operation</td>
<td>Yes</td>
<td>4(2.5)</td>
<td>153</td>
<td>1.7(0.6-5.0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No</td>
<td>13(4.4)</td>
<td>284</td>
<td>1.0(reference)</td>
</tr>
<tr>
<td>4.</td>
<td>Sharp injury</td>
<td>Yes</td>
<td>10(4.2)</td>
<td>227</td>
<td>1.3(0.5-3.6)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No</td>
<td>7(3.2)</td>
<td>210</td>
<td>1.0(reference)</td>
</tr>
<tr>
<td>5.</td>
<td>Blood transfusion</td>
<td>Yes</td>
<td>2(2.7)</td>
<td>25</td>
<td>2.3(0.5-10.6)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No</td>
<td>15(3.5)</td>
<td>412</td>
<td>1.0(reference)</td>
</tr>
<tr>
<td>6.</td>
<td>History of jaundice</td>
<td>Yes</td>
<td>4(30.8)</td>
<td>9</td>
<td>14.6(4.0-54)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No</td>
<td>13(3.0)</td>
<td>428</td>
<td>1.0(reference)</td>
</tr>
<tr>
<td>7.</td>
<td>Tattoo</td>
<td>Yes</td>
<td>3(3.4)</td>
<td>86</td>
<td>1.2(0.33-4.13)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No</td>
<td>14(3.8)</td>
<td>351</td>
<td>1.0(reference)</td>
</tr>
<tr>
<td>8.</td>
<td>Ear-piercing</td>
<td>Yes</td>
<td>16(3.6)</td>
<td>425</td>
<td>1.2(0.06-3.7)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No</td>
<td>17(7.7)</td>
<td>12</td>
<td>1.0(reference)</td>
</tr>
<tr>
<td>9.</td>
<td>Dental procedure</td>
<td>Yes</td>
<td>5(3.2)</td>
<td>152</td>
<td>1.2(0.27-2.3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No</td>
<td>12(4.3)</td>
<td>285</td>
<td>1.0(refernece)</td>
</tr>
<tr>
<td>10.</td>
<td>Multiple sexual partner</td>
<td>Yes</td>
<td>4(5.8)</td>
<td>65</td>
<td>1.3(0.25-7.1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No</td>
<td>13(3.4)</td>
<td>374</td>
<td>1.0(refernece)</td>
</tr>
<tr>
<td>11.</td>
<td>Family history of hepatitis</td>
<td>Yes</td>
<td>4(9.5)</td>
<td>38</td>
<td>3.2(1.0-10.4)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No</td>
<td>13(3.2)</td>
<td>399</td>
<td>1.0(refernece)</td>
</tr>
<tr>
<td>12.</td>
<td>History of abortion</td>
<td>Yes</td>
<td>3(2.2)</td>
<td>136</td>
<td>2.0(0.2-3.4)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No</td>
<td>14(4.4)</td>
<td>301</td>
<td>1.0(refernece)</td>
</tr>
<tr>
<td>13.</td>
<td>Vaccine</td>
<td>Yes</td>
<td>0</td>
<td>4</td>
<td>0.0(0.1.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No</td>
<td>17</td>
<td>433</td>
<td>1.0(refernece)</td>
</tr>
</tbody>
</table>

Participants' age group ($x^2=17.9$, $p=0.012$), marital status ($x^2=12.7$, $p=0.05$), caring of hepatitis patient ($x^2=6.3$, $p=0.012$), blood transfusion history ($x^2=4.6$, $p=0.03$), history of jaundice
(X²=12.4, p= 0.00), as well as family history of liver disease (X²=6.3, p=0.012), were significantly associated with HCV infection at 95% confidence interval as shown in Table 6. However, previous history of dental procedure, body tattooing, having multiple sexual partner, body piercing with sharp objects as well as history of surgical procedure, showed no significant impact on the likelihood of having Hepatitis C virus infection among these mothers. In addition, associated risk factors were assessed for their association with HCV infection using binary and multivariate logistic regression. As a result, history of jaundice (AOR=19.2 (CI 3.5-104.9); p=0.02 and Alcohol consumption habits (AOR =6.9 (1.3-37.0); P=0.024 were significantly associated with the occurrence of HCV positivity at 95% confidence interval respectively. In the multivariate analysis, after adjustment for all other confounding variables, age group, marital status, history of blood transfusion and family history of liver disease has no impact on the acquisition of HCV infection as shown in Table 6.

Table 6: Association in logistic regression analysis of explanatory variables and HCV infection among apparently healthy Mothers in Addis Ababa, Ethiopia

<table>
<thead>
<tr>
<th>Variables</th>
<th>Positive (%)</th>
<th>Negative (%)</th>
<th>COR(CI) 95%</th>
<th>AOR(CI) 95%</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marital status</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Married</td>
<td>4(1.1)</td>
<td>367</td>
<td>1.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unmarried</td>
<td>0(0.0)</td>
<td>9</td>
<td>NA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Divorced</td>
<td>4(6.0)</td>
<td>63</td>
<td>5.8(1.4-23.8)</td>
<td>9.3(1.5-58.8)</td>
<td>0.01</td>
</tr>
<tr>
<td>Widowed</td>
<td>1(14.3)</td>
<td>6</td>
<td>1.5(1.48-15.4)</td>
<td>34.5(1.2-100.0)</td>
<td>0.004</td>
</tr>
<tr>
<td>Caring of hepatitis patient</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>3(7.1)</td>
<td>39</td>
<td>5.2(1.25-21.6)</td>
<td></td>
<td>0.59</td>
</tr>
<tr>
<td>No</td>
<td>6(1.5)</td>
<td>406</td>
<td>1.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood transfusion</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>2(7.7)</td>
<td>24</td>
<td>5.0(1.0-25.4)</td>
<td></td>
<td>0.21</td>
</tr>
<tr>
<td>No</td>
<td>7(1.7)</td>
<td>421</td>
<td>1.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>History of jaundice</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>2(8.3)</td>
<td>11</td>
<td>11.3(2.1-60.6)</td>
<td>19.2(3.5-104.9)</td>
<td>0.022</td>
</tr>
<tr>
<td>No</td>
<td>7(1.6)</td>
<td>434</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Family history of liver hepatitis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>3(7.1)</td>
<td>39</td>
<td>5.2(1.2-21.6)</td>
<td></td>
<td>0.72</td>
</tr>
<tr>
<td>No</td>
<td>6(1.5)</td>
<td>406</td>
<td>1.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alcohol consumption</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>4(6.7)</td>
<td>56</td>
<td>5.6(1.5-21.3)</td>
<td>6.9(1.3-37.0)</td>
<td>0.025</td>
</tr>
<tr>
<td>No</td>
<td>5(1.3)</td>
<td>389</td>
<td>1.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>9(2.0)</td>
<td>445</td>
<td>1.0</td>
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</tbody>
</table>
6. DISCUSSION

HBV and HCV infections are significant health problems around the globe. Both infections are associated with a broad range of clinical presentations ranging from acute hepatitis to chronic infection that may be clinically asymptomatic or may progress to chronic hepatitis and liver cirrhosis (WHO, 2016). Population based serological studies of viral hepatitis have demonstrated the diversity of epidemiological patterns with regard to the risk of acquiring infection related to personal attributes, place and risk distribution over time. Screening asymptomatic people is an important instrument in disease detection, prompt diagnosis and intervention, particularly at an early stage of the disease. This may improve the health outcome as well as better understanding of the transmission pattern of the disease (Wolfgang et al., 2000).

The results from this present study in Addis Ababa, Ethiopia, revealed a sero-prevalence rate of 3.7% for HBsAg, this lies within the established standard intermediate endemicity of hepatitis B prevalence (Simmonds, 2006). Even though, these findings were in agreement with the WHO intermediate level of endemicity which did not represent the whole community. Because, this study mainly focus among apparently healthy mothers who represent only female populations; it needs more inclusive sample from both sexes in order to argue the WHO established endemicity classifications. This result was coinciding with a study conducted in Jimma among 493 pregnant women 3.7% (Awole and Gebre-Selassie, 2005) and also in agreement with the study conducted in Addis Ababa among delivering women 3.0 % (Tegegne, et al.,2014), Woldia, South Gondar among diabetes and non-diabetic patients 3.7% (Mekonnen et al.,2014).The reported HBsAg prevalence of this study was higher than the research conducted in Dessie among healthy female blood donors 1.5% (Sharew et al.,2015),in Addis Ababa among Public Health Centers cleaners was 3.57% (Mekonnen et al., 2015) and in Jimma blood donors 2.1% (Yami et al., 2011). In contrast, it was lower than the study documented in Dessie among pregnant women 4.9% (Seid et al., 2014), Gondar, Bahir Dar, Dessie and Mekelle blood donors 6.2% (Gelaw and Mengitsu, 2007), Shashemene, Southern Ethiopia 5.7% (Negero et al., 2011),Ghana 10.6% (Cho et al., 2012) and in Addis Ababa community 6.2% (Abebe et al., 2013). The observed discrepancies in HBV distribution across different geographical location might be attributed by variation in socio-demographic characteristics of the study population such as socio-cultural environment, traditional practices, sexual practices and medical exposure and the difference in hepatitis
epidemiology and other underlined diseases like HIV/AIDS. Moreover, the variation might be
due to circulating genotypes which is responsible disease severity as well as treatment responses,
methodological difference (test method, study design etc.), the level of awareness and behavioral
differences for the potential risk factors of HBV infection.

The overall seroprevalence of HCV (2.0%) in this study is similar to the study conducted in
Gabon, central Africa 2.1% (Ndong-Atome et al., 2008), in Sudan 1.9 % (Indris and Elamin,
2015), in general population of Ethiopia 2.0% (Frommel et al., 1993), in Poland 1.9% (Flisiak et
a., 2011) and in northern Ethiopia volunteer testing and counseling center 2.0 % (Atsbaha et al.,
2016). This founding was also comparable with other reports found in Addis Ababa health center
cleaners 1.59 % (93) and Gondar, Bahir dar, Dessie and Mekele Blood Banks 1.7 % (60).

In contrast, this finding was higher in a report from Dessie blood bank 0.39% (Mekonnen et al.,
2015), Jimma adult blood donors 0.2 % (Seid et al.,2014), Debretabor hospital among HIV
patients 1.3% (Zenebe et al., 2014), Arba Minch blood bank, in southern Ethiopia 0.0 %
(Frommel et al., 1993), in Dessie among pregnant mothers was 0.8 % (Wondimeneh et al.,
2013), 0.19% in Indian women (Mehta et al., 2013), and in Nigerian pregnant women 1.39%
(Esan et al., 2014). Moreover, our finding was lower than the study conducted in Gondar 5 %
(Balew et al 2014), 6% were reported in Hawassa, southern Ethiopia, 5.2 % in Kigali, Rwanda
(Rusine et al., 2013) and 7.7% were reported in Ghana ( Ephraim et al., 2015). These differences
may be attributed by methodological differences (test methods e.g ELISA vs Rapid test methods
which differ in their sensitivity and specificity. Study design: usually facility based study designs
have overestimation because people who have some complain come and visited those health
facilities. Thus, pose to recruit those who have already underlined cases. Sampling technique:
those convenient sampling techniques usually are subjected to bias etc.), population variation,
types of risk exposure and sample size which have a great effect on the result different studies.

In this study all of the socio-demographic variables were not statistically significant. In this
study, caring for hepatitis patients and history of jaundice were significantly associated with the
occurrence of HCV infection. These findings were supported by a study conducted in Ethiopian
public hospitals (Balew et al., 2014). In the present study, no statistical significant differences
were observed for HBV and HCV infections in terms of age, sex, occupation and educational
status. This study was supported by a study reported in Amhara regional state general
populations (Abera et al., 2017) and in Felege Hiwot Referral Hospital, northwest Ethiopia (Molla et al., 2015).

The highest prevalence of HBsAg was detected among apparently healthy mothers who were on age 25-29 and 30-34 years and this was in agreement with study conducted in Shashemene General Hospital, southern Ethiopia Shenyang, China, Debretabor hospital, South Gondar, Northwest Ethiopia and Addis Ababa Ethiopia, (Negero et al., 2011, Ding et al., 2013, Balew et al., 2014, Dessalegn et al., 2016) and Nigeria pregnant mothers (Esan et al., 2014). The observed high prevalence of HBV positivity among younger age group could be defined with the high probability of exposure for high risk health behavior.

The present study tried to address the prevalence HBV infection and the level of education. As per the finding, high positivity was recorded among those with primary level of education. This finding was in agreement with previous study conducted in Ethiopia among pregnant women (Dessalegn et al., 2016). However, history of liver disease, history of jaundice and family history of liver disease were significantly associated with HBV infection and were important predictors of HBV infection. These findings were supported by a study conducted in Bahir dar (Zenebe et al., 2014), in Debretabor and Gondar hospital (Wondimeneh et al., 2013, Balew et al 2014) and in Karachi, Pakistan (Jafri et al., 2006).
7. LIMITATIONS OF THE STUDY

- In this study viral hepatitis biomarkers were screened by using Bio-Rad ELISA test kits. This test kits are screening tests that need a confirmatory tests like molecular techniques (RNA or DNA detections).
- The seromarkers used for assessment of HBV infection is not complete, like HBeAg and IgM for HBcAb which enable to determine acute infections.
- Direct comparison group was not available to the study subjects, which makes difficult to estimate an accurate prevalence and relative risk of the study groups.
8. CONCLUSION AND RECOMMENDATION

8.1 Conclusion

The present study showed an intermediate prevalence of HBV and HCV infection among apparently healthy mothers according to World Health Organization’s classification. A 3.7 % and 2.0% overall prevalence of HBV and HCV infection respectively in our study setting among healthy mothers that the need for timely intervention strategies to alleviate the burden of HBV and HCV infection in the community. This prevalence rate also calls for additional efforts regarding active screening and vaccination for young adults and public health education campaigns in the media to promote better awareness of risk factors.

In this study, age groups 24-29 and 30-34 had prevalence of 7.0% and 4.6%, respectively which was the highest prevalence. It may be at high risk and serves as a reservoir which requires routine screening and vaccine schedules (for HBV) may be important for those high risk groups. This implies that high level of carrier state in mothers at reproductive age would suggest that there is a high risk of mother-to-infant transmission in the study areas.

Among the assessed variables and clinical presentations, previous history of liver disease, history of jaundice and family history of liver disease were significantly associated with HBV infections which were important predictors of HBV infection. Marital statuses, consumption of alcohol and history of jaundice were significantly associated with the occurrence of HCV infection.

8.2 Recommendation

In the future large-scale study for the assessment of hepatitis B and C prevalence as well as to establish any statistical differences among pregnant and non-pregnant mothers should be conducted. Thus, scaling up of the screening of pregnant and non-pregnant mothers for HBV and HCV infections and provision of health education about the risk factors, the mode of transmissions and prevention is recommended. Population based studies with additional serological markers and molecular techniques are required so as to design a working strategy for evidence based intervention and implement control measure. Therefore, screening asymptomatic people is an important instrument in disease detection, prompt diagnosis and intervention strategies. Since, HBV and HCV treatment guideline is absent in Ethiopia, the responsible stake holders should take the responsibility to develop and implement treatment modalities in the country.
9. REFERENCES


Central Statistical Agency of Ethiopia demographic and Health Survey. (2016).Key Indicators Report


10. ANNEXES
Annex I: ELISA Laboratory Test Procedures and Protocols

1. **Monolisa™ HBsAg ULTRA assay**

**Principle of the test**
Monolisa™ HBs Ag ULTRA assay is a one-step enzyme immunoassay based on the principle of the "sandwich" type using monoclonal antibodies and polyclonal antibodies selected for their ability to bind themselves to the various subtypes of HBs Ag now recognized by the WHO and the most part of variant HBV strains.

The Monolisa™ HBsAg ULTRA solid phase is coated with monoclonal antibodies. The Monolisa™ HBsAg ULTRA conjugates are based upon the use of monoclonal antibodies from mouse and polyclonal antibody from goat against the HBs Ag which are bound to the peroxidase.

### PREPARATION OF THE REAGENTS

<table>
<thead>
<tr>
<th>Conjugate working solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>□ Carefully remove the cap and pour the content of a conjugate diluents vial 1 into the lyophilized conjugate vial 2. Put the cap on and let stand for 10 minutes while gently shaking and inverting from time to time to ease dissolution.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Enzyme development solution :</th>
</tr>
</thead>
<tbody>
<tr>
<td>□ Dilute the chromogens 1:11 using substrate solution. 10 ml are necessary and sufficient for 1 to 12 strips. Homogenize.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Concentrated washing solution (20X) :</th>
</tr>
</thead>
<tbody>
<tr>
<td>□ Dilute 1:20 in distilled water to obtain the ready-for-use washing solution. Prepare 800 ml for one plate of 12 strips.</td>
</tr>
</tbody>
</table>

### PROCEDURE

<table>
<thead>
<tr>
<th>□ Take out from the protective packing the support frame and the necessary number of strips.</th>
</tr>
</thead>
<tbody>
<tr>
<td>□ Add 100μl of negative control serum in well A1,B1,C1 and D1</td>
</tr>
<tr>
<td>□ Add 100 μl of positive control serum in wells E1</td>
</tr>
<tr>
<td>□ Add 100μl of the serum sample in each well(from F1 to H12)</td>
</tr>
<tr>
<td>□ Add 50μl of conjugate working solution into each well</td>
</tr>
<tr>
<td>□ Cover the plate with new adhesive film and incubate for 1 hour and 30 ± 5 minutes at 37±1°C.</td>
</tr>
</tbody>
</table>
- Remove the adhesive film, empty all wells by aspiration and tap plate face down on the absorbance tissue paper.
- Wash with 400μl washing solution minimum of 5 times.
- Add quickly 100 μl of the developer solution into all wells. Allow the reaction to develop in the dark for 30 ± 5 minutes at room temperature (18 - 30°C). Do not use adhesive film during this incubation.
- Add 100 μl stopping solution in each wells. Homogenize the reaction mixture.
- Wait at least 4 minutes and read within 30 minutes of stopping the reaction, read the optical density(OD) at 450/620-700 nm using a plate reader.

**Interpretation of the result**

- Samples with ratio values greater than 1 are considered to be positive by the Monolisa™ HBs Ag ULTRA.
- Samples with ratio values lower than 1 are considered to be negative by the Monolisa™ HBs Ag ULTRA.
- Samples ratio between 0.9 and 1 should however, be interpreted with caution.

It is advisable to retest in duplicate the corresponding samples when the systems and laboratory procedures permit.

Cut off value = mean of negative control +0.05

Sample ratio = \( \frac{OD \text{ of sample}}{cut-off \text{ value}} \)

2. Monolisa™ Anti-HBc PLUS

**Principle:** It is an enzyme immunoassay (indirect ELISA type) for the simultaneous detection of total antibodies to hepatitis B virus core in human serum or plasma. It is based upon the use of a solid phase prepared with recombinant HBe antigen.

**Procedure**

**PROCEDURE**

- Take out from the protective packing the support frame and the necessary number of strips.
- Add 20 μl of negative control serum in well A1, B1
- Add 20 μl of positive control serum in C1, D1, E1
Add 20 μl of the serum sample in each well (from F1 to H12)

Add 200 μl of diluent into each well

Cover the plate with new adhesive film and incubate for 30 ± 5 minutes at 37±1°C.

Remove the adhesive film, empty all wells by aspiration and tap plate face down on the absorbance tissue paper.

Wash with 400 μl washing solution minimum of 5 times.

Add quickly 200 μl of the conjugate solution into all wells. The conjugate must be shaken gently before use. Cover it with a new adhesive film and incubate for: 60 ± 5 minutes at 37°C ± 1°C.

Remove the adhesive film, empty all wells by aspiration and wash 5 times as previously described.

Quickly dispense into each well 100μl of freshly prepared development solution.

Allow the reaction to develop in the dark for 30 ± 5 minutes at room temperature (18 - 30°C). Do not use adhesive film during this incubation.

Add 100 μl stopping solution in each wells. Homogenize the reaction mixture.

Wait at least 4 minutes and read within 30 minutes of stopping the reaction, read the optical density (OD) at 450/620-700 nm using a plate reader.

**Interpretation of the result**

- Samples with an optical density less than the cut-off value are considered to be negative with the Monolisa™ Anti-HBc PLUS test.
  - Samples with an optical density higher than, or equal to, the cut-off value are considered to be initially positive with the Monolisa™ Anti-HBc PLUS test and must be retested in duplicate before the final interpretation.
  - Results just below the cut-off value (cut-off -10% < OD) should be interpreted with care.

Cut-off value = \( \frac{\text{mean of positive control OD}}{5} \)

3. **Monolisa™ HCV Ag-Ab ULTRA Assay**

**Principle:** It is an enzyme immunoassay for the detection of HCV infection, based on the detection of capsid antigen and/or antibodies associated with an infection by Hepatitis C virus in patient serum or plasma.
The Monolisa™ HCV Ag-Ab ULTRA Microplates solid phase is coated with:

- Monoclonal Antibodies against capsid protein of Hepatitis C virus.
  - two recombinant proteins
  - One recombinant antigen
- A mutated peptide from the capsid of structural area of the hepatitis C virus genome.

There are two conjugates:

Conjugated 1: Mouse biotinilated monoclonal antibodies against hepatitis C capsid which does not react against the hepatitis C capsid mutated peptide coated on the microplate.

Conjugate 2: Mouse peroxidase-labeled antibodies to human IgG and peroxidase-labeled streptavidin.

**PROCEDURE**

- Take out from the protective packing the support frame and the necessary number of strips (R1).
- **Add 50 μl of negative control serum in well A1**
- Add 50 μl of Antibodies positive control serum in wells B1, C1, D1
- Add 50 μl of the working Antigen positive control solution in wells E1
- Add 50 μl of the serum sample in each well (from F1 to H12)
- **Add 100 μl of conjugate 1 into each well**
- Cover the plate with new adhesive film and incubate for 1 hour and 30 ± 5 minutes at 37±1°C.
- Remove the adhesive film, empty all wells by aspiration and tap plate face down on the absorbance tissue paper.
- Wash with 400μl washing solution minimum of 5 times.
- Add quickly 100 μl of the conjugate 2 solution into all wells. The conjugate must be shaken gently before use. Cover it with a new adhesive film and incubate for: 30 ±5 minutes at 37°C ± 1°C.
- Remove the adhesive film, empty all wells by aspiration and wash 5 times as previously described.
- Quickly dispense into each well 80μl of prepared development solution, freshly prepared before use. Allow the reaction to develop in the dark for 30 ± 5 minutes at room temperature
(18 - 30°C). Do not use adhesive film during this incubation.

☐ Add 100 μl stopping solution in each wells. Homogenize the reaction mixture.

☐ Wait at least 4 minutes and read within 30 minutes of stopping the reaction, read the optical density (OD) at 450/620-700 nm using a plate reader.

**Interpretation of the result**

- Samples with an optical density lower than the cut off value are considered to be negative (ratio < 1) by the Monolisa™ HCV Ag-Ab ULTRA.
- Results just below the cut-off value (CO-10 % < O.D. < CO, ratio between 0.9 and 1) should however, be interpreted with caution. It is advisable to retest in duplicate the corresponding samples when the systems and laboratory procedures permit.
- Samples with optical density greater or equal to the cut off (ratio ≥ 1) are considered to be initially positive by the Monolisa™ HCV Ag-Ab ULTRA. They should be retested in duplicate before final interpretation.

\[
\text{Sample ratio} = \frac{OD \text{ of sample}}{cut-off \text{ value}} \\
\text{Cut-off value} = \frac{\text{mean of positive controls}}{5} \quad \text{where OD=optical density and CO= cut-off value}
\]
Annex II: Information letter to participants of the study

1. Information Sheet
Hello, how are you? My name is _______________. This is an interview to be done with you for a study that is being conducted at Addis Ababa University, college of health Science, School of medicine.

Title of the study
The title of the study is the determination of sero-prevalence and risk factors for viral hepatitis (HBV and HCV) infections among non-pregnant healthy mothers in AddisAbaba city administration, in Addis Ababa, Ethiopia.

Propose of the study
The purpose of the study is to determine the prevalence of each viral infection and to assess the predisposing factors among non-pregnant healthy mothers.

What it will mean if you decide to take part in the study?
If you agree to participate in this study, you will participate in this interview in a private place. The interview will last for about 30-45 minutes and will be facilitated by me and my colleague. During the interview, you will be asked to respond questions related to hepatitis infections and their predisposing or risk factors. During the interview, my colleague will write down what you say. The recorded data will not contain your names or other identifying information. They will just be labeled with a study number.

The results will assist policy makers, planners and health service providers for making considerations regarding the risk factors, transmission and sero-prevalence of viral infections among women. It will also help to contribute in the subsequent efforts to improve prevention, diagnosis, treatment and support of viral hepatitis in relation to their family, children and fetus, at large in the community.

Risks and discomforts
There is no possible risk associated with participating in this study. But there is a little pain during drawing venous blood which will be collected by professional phlebotomists. You are free to decline answering any question that you do not wish to answer and you may leave our interview at any time you want to.

Confidentiality
All information obtained will be held securely and stored on paper, and computer files. No one except the interviewers will know that you took part in the study the answers that you give will be marked with a special study number only, and not your name. The data will protect information about you in this research to be the best of our ability.

**Voluntary participation**

Your participation is voluntary. You may withdraw from the interview at any time without giving a reason and without any penalty. If you have questions regarding this study or would like to be informed of the results after its completion, please do not hesitate to contact: Habtamu Biazin, School of Medicine Department of MIP, and Addis Ababa University

Cell phone: +251910446421

Email: [habtamu.biazin@aau.edu.et](mailto:habtamu.biazin@aau.edu.et)
እርሰዎንም በእናቶች በጉበትን በሽታን የሚያመጡ የሚያስከትለው የሚልነው፤ ይህ በጥናቱ ያላማ በረቂቅ የክርስ የሚመጣ የጉበትን በሽታን ላይ በሚልነው፣ ይህ በጥናቱ ያላማ በረቂቅ የከርስ የሚመጣ የጉበትን በሽታን የሚልነው፤ ይህ በጥናቱ ያላማ በረቂቅ የከርስ የሚመጣ የጉበትን በሽታን የሚልነው፤ ይህ በጥናቱ ያላማ በረቂቅ የከርስ የሚመጣ የጉበትን በሽታን የሚልነው፤ ይህ በጥናቱ ያላማ በረቂቅ የከርስ የሚመጣ የጉበትን በሽታን የሚልነው፤ ይህ በጥናቱ ያላማ በረቂቅ የከርስ የሚመጣ የጉበትን በሽታን የሚልነው፤ ይህ በጥናቱ ያላማ በረቂቅ የከርስ የሚመጣ የጉበትን በሽታን የሚልነው፤ ይህ በጥናቱ ያላማ በረቂቅ የከርስ የሚመጣ የጉበትን በሽታን የሚልነው፤ ይህ በጥናቱ ያላማ በረቂቅ የከርስ የሚመጣ የጉበትን በሽታን የሚልነው፤ ይህ በጥናቱ ያላማ በረቂቅ የከርስ የሚመጣ የጉበትን በሽታን የሚልነው፤ ይህ በጥናቱ ያላማ በረቂቅ የከርስ የሚመጣ የጉበትን በሽታን የሚልነው፤ ይህ በጥናቱ ያላማ በረቂቅ የከርስ የሚመጣ የጉበትን በሽታን የሚልነው፤ ይህ በጥናቱ ያላማ በረቂቅ የከርስ የሚመጣ የጉበትን በሽታን የሚልነው፤ ይህ በጥናቱ ያላማ በረቂቅ የከርስ የሚመጣ የጉበትን በሽታን የሚልነው፤ ይህ በጥናቱ ያላማ በረቂቅ የከርስ የሚመጣ የጉበትን በሽታን የሚልነው፤ ይህ በጥናቱ ያላማ በረቂቅ የከርስ የሚመጣ የጉበትን በሽታን የሚልነው፤ ይህ በጥናቱ ያላማ በረቂቅ የከርስ የሚመጣ የጉበትን በሽታን የሚልነው፤ ይህ በጥናቱ ያላማ በረቂቅ የከርስ የሚመጣ የጉበትን በሽታን የሚልነው፤ ይህ በጥናቱ ያላማ በɾɾɾ
Annex III: Consent Form

I have read the information sheet concerning this study (or have understood the verbal explanation) and I understand what will be required of me and what will happen to me if I take part in it. I also understand that any time I may withdraw from this study without giving a reason and without me or my families’ are being affected for my refusal.

May I continue the interview?
1. Yes ____________ Continue the interview
2. No ______________ Stop the interview and thank the respondent

Witness's signature certifying that the informed consent has been given
Witness's signature ______________ Date ______________

Introduction to the interview

Thank you for deciding to participate in the interview and for coming to this session, previously (on the statement of consent form), we have discussed briefly on the purpose of the research, how you were identified, and your part in the research study. Now I am going to have discussion with you on the relevant topic items. Before going to the discussion, would you tell me important backgrounds such as age, educational background etc.? There is no right or wrong answers. All answers /responses/ ideas you provide are equally important and you are requested to respond honestly from your experiences and beliefs. I may interrupt and probe your ideas. Once again I would like to tell you that what we are going to discuss is very confidential and it will be used only for the research.
Annex IV: Questionnaire

a. General Information
1. I.D No.
2. Subcity
3. Woreda
4. House no.
5. Phone no.

b. Demographic data
6. Age
7. Educational status
   1. No formal education  2. 1-8  3. 9-12 4. College and above
8. Marital status
9. Your occupation

Risk assessment for viral hepatitis (hepatitis B and C)
10. History of Jaundice or Diagnosed liver disease. 1. Yes 2. no
11. Number of House holding  1. 1-4  2.4-6  3.>7
12. Alcohol Consumption  1. Yes 2. No
15. History of sharp injury (cut) 1. Yes 1. No
17. Do you have history of hepatitis infection? 1. Yes 2. No 3. I don’t know
18. History of tattooing 1. Yes 2. No
19. History of ear piercing 1. Yes 2. No
20. History Tooth extraction (dental procedure) 1. Yes 2. No
21. History of Multiple sexual partners 1. Yes 2. No
22. Family history of liver disease 1. Yes 2. No 3. I don’t know
23. History of Abortion 1. Yes 2. No
24. Number of abortions  1. 1 2.2 3.3 4. > 4
25. Did you take vaccination for HBV? 1. Yes 2. No
IV. መጠይቅ

መጠይቅን ከተጠቀማቸው የሚቻለው ከእንጋት ከእንጋት የአማራ ከወይም የበኻት ሏይ የመለካ ያስታወቂያ ከማህ የሚመለከቱ ከሚያስፋ抱歉

v. የአማራ የጭፋ

1. ከረ: 
2. ከሆኔ ከጂጆነ
3. በወሩድ
4. የእስትወርስ
5. በሚለስ

A. የሚሆናት ከመፋቃ

6. የሚጠቅላላ ብጥያቄ

7. የሚሆናት ከሚደረጉ የሚጠቅላላ ብጥያቄ 1-8 3-9-12 4. ከእንጋት ከሚል
8. የሚሆናት ከሚደረጉ የሚጠቅላላ ብጥያቄ 1-8 3-9-12 4. ከእንጋት ከሚል
9. የሚሆናት ከሚደረጉ የሚጠቅላላ ብጥያቄ 1-8 3-9-12 4. ከእንጋት ከሚል
10. የሚሆናት ከሚደረጉ የሚጠቅላላ ብጥያቄ 1-8 3-9-12 4. ከእንጋት ከሚል
11. የሚሆናት ከሚደረጉ የሚጠቅላላ ብጥያቄ 1-8 3-9-12 4. ከእንጋት ከሚል
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13. ይወራል ከሚደረጉ የሚጠቅላላ ብጥያቄ 1-8 3-9-12 4. ከእንጋት ከሚል
14. የሚሆናት ከሚደረጉ የሚጠቅላላ ብጥያቄ 1-8 3-9-12 4. ከእንጋት ከሚል
15. የሚሆናት ከሚደረጉ የሚጠቅላላ ብጥያቄ 1-8 3-9-12 4. ከእንጋት ከሚል
16. የሚሆናት ከሚደረጉ የሚጠቅላላ ብጥያቄ 1-8 3-9-12 4. ከእንጋት ከሚል
17. የሚሆናት ከሚደረጉ የሚጠቅላላ ብጥያቄ 1-8 3-9-12 4. ከእንጋት ከሚል
18. የሚሆናት ከሚደረጉ የሚጠቅላላ ብጥያቄ 1-8 3-9-12 4. ከእንጋት ከሚል
19. የሚሆናት ከሚደረጉ የሚጠቅላላ ብጥያቄ 1-8 3-9-12 4. ከእንጋት ከሚል
20. የሚሆናት ከሚደረጉ የሚጠቅላላ ብጥያቄ 1-8 3-9-12 4. ከእንጋት ከሚል
21. የሚሆናት ከሚደረጉ የሚጠቅላላ ብጥያቄ 1-8 3-9-12 4. ከእንጋት ከሚል
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26. የሚሆናት ከሚደረጉ የሚጠቅላላ ብጥያቄ 1-8 3-9-12 4. ከእንጋት ከሚል
27. የሚሆናት ከሚደረጉ የሚጠቅላላ ብጥያቄ 1-8 3-9-12 4. ከእንጋት ከሚል

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Declaration

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

Name of PI: Habtamu Biazin (BSc, MSc. candidate)

Signature __________ date of submission ______________

This thesis has been submitted with our approval by Advisors.

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Signature: ___________ Date ______________

Tamrat Abebe (PhD: Addis Ababa University)

Signature: ___________ Date ______________