ADAMA SCIENCE AND TECHNOLOGY UNIVERSITY

OFFICE OF GRADUATE STUDIES

Assessment on seroprevalence and associated risk factors of hepatitis B surface antigen among pregnant women in Gandhi Memorial Hospital in Addis Ababa, Ethiopia

A Thesis Submitted to the Department of Biology

School of Applied Natural Science

in Partial Fulfillment of the Requirements for the Degree of Master in Biology.

By

Zemenu Abera

Adama, Ethiopia

September, 2017

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Id. No GSU/0413/05

Advisor: Dr. Daniel Getahun

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Approval of Board of Examiners

We, the undersigned, members of the Board of Examiners of the final open defense by Zemenu Abera have read and evaluated his thesis entitled “Assessment on Seroprevalence and Associated risk factors of hepatitis B surface Antigen among pregnant women in Gandhi Memorial Hospital in Addis Ababa” and examined the candidate. This is, therefore, to certify that the thesis has been accepted in partial fulfillment of the requirement of the Degree of Master in Biology.

Supervisor /Advisor	Signature	Date
Chairperson	Signature	Date
Internal Examiner	Signature	Date
External Examiner	Signature	Date

Declaration

I hereby declare that this MSc Thesis is my original work and has not been presented for a degree in any other university, and all sources of material used for this thesis have been duly acknowledged.

Name: Zemenu Abera

Signature:_____

This MSc Thesis has been submitted for examination with my approval as thesis advisor/ Supervisor.

Name: _____

Signature:_____

Date of submission: _____.

Supervisor's/Advisor's approval sheet

To: Biology department

Subject: Thesis Submission

This is to certify that the thesis entitled "Assessment on Seroprevalence and Associated risk factors of hepatitis B surface Antigen among pregnant women in Gandhi Memorial Hospital in Addis Ababa" submitted in partial fulfillment of the requirements for the degree of Master's in Biology, the Graduate program of the department of Biology, and has been carried out by Zemenu Abera Id. No GSU/0413/06, under my/our supervision. Therefore, I/we recommend that the student has fulfilled the requirements and hence hereby he/she can submit the thesis/dissertation to the department.

_____ _____ _____

Name of major Advisor/supervisor Signature Date

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ACKNOWLEDGMENTS

I would like to thank Dr. Daniel Getahun, for his unreserved advice and counseling throughout the whole course of this research. I would like also to extend my gratitude to the staff members of Gandhi Memorial Hospital Laboratory team for their cooperativeness and technical assistance during data collection and laboratory work. I would also like to acknowledge Adama Science and Technology University for funding this research. I have also a special thanks to Sululta town administration for partially funding this research project.

ABBREVIATIONS

ANC	Antenatal care
ANT	Antenatal treatment
ELISA	Enzyme linked immuno sorbent assay
FGM	Female Genital Mutilation
HBcAg	Hepatitis B core antigen
HBeAg	Extracellular hepatitis B antigen
HBIG	Hepatitis B Immune Globulin
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HepB	Hepatitis B (vaccine)
Ig	Immunoglobulin
PDV	Plasma derived vaccine
WGO	World Gastroenterology Organization
WHO	World Health Organization

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Abstract

Hepatitis B virus (HBV) is a major blood-borne and sexually transmitted infectious agent causing a significant health problem with more than 350 million people chronically infected worldwide and becoming a cause of 57% of liver cirrhosis and 80% of the world's primary liver cancer. The aim of this study was to determine the seroprevalence and risk factors of HBV among pregnant women attending antenatal care service in Gandhi Memorial Hospital located in Addis Ababa, Ethiopia. A cross-sectional study participated a total of 384 pregnant women attending antenatal care service in Gandhi Memorial Hospital from April to June, 2017 was conducted. A structured questionnaire was used to collect socio-demographic data and information regarding possible risk factors for Hepatitis B virus infection and screening was performed by immuno-chromatographic method using advanced quality one step hepatitis B surface antigen (HBsAg) test card. The seroprevalence of HBsAg among 384 pregnant women attending antenatal care service in Gandhi memorial Hospital in Addis Ababa during the study period was 2.3%, which is a moderate prevalence according to WHO classification. Higher age specific and ethnic group specific prevalence was observed in the study, but no statistical association was observed between other risk factors included in the study and HBV transmission in the study area. Screening pregnant women in all health facilities, providing HBV vaccines to neonates born to HBV positive mothers, improving of the community awareness about the transmission routes of HBV and pre-marriage screening of the couples are recommended to reduce transmission of the virus.

Key words: Chronic Hepatitis, Hepatitis B virus, pregnancy, vertical transmission

1. INTRODUCTION

1.1 Background of the study

Hepatitis B Virus is blood-borne and sexually transmitted infectious agent, approximately 50-100 times more contagious than human immunodeficiency virus (HIV) causing serious global public health problem. It is found in diverse populations and sub populations (Dessie *et al.*, 2014; Yeshi *et al*., 2016). It is estimated that more than 2 billion people have been infected with this virus worldwide. Of these, being at risk of serious illness and death from cirrhosis and hepatocellular carcinoma (HCC), approximately 350 million are chronically infected (Eke *et al*., 2011; Erena and Tefera, 2012).

HBV is the prototype member of the Hepadnaviridae (hepatotropic DNA virus) family measuring 40 to 42 nm in diameter with an outer lipoprotein envelope that contains three related envelope glycoproteins or surface antigens (WGO, 2007; Hinnachi *et al*., 2009). HBV replicates in the hepatocytes of humans and other higher primates but cannot be grown in artificial cell cultures. HBsAg is a lipoprotein of the viral envelope that is produced in conspicuous excess and circulates in the blood as spherical and tubular particles 22 nm in size (WHO, 2004).

Worldwide, HBV is a major cause of primary cancer of the liver, or hepatocellular carcinoma (HCC) (Block *et al*., 2003). According to Dessie *et al.* (2014), in 2012 alone, over 350 million chronic carriers and 1.2 million annual deaths were occurred. Besides, about 60 to 80% of the world's primary liver cancer is caused by HBV and infants infected due to vertical transmission (mother to child) are nearly 90% at higher risk of developing chronic liver disease and cancer. Infection with HBV causes not only acute illnesses, but chronic illnesses such as liver cirrhosis and HCC which accounts for more than 1 million deaths globally (Magrahe *et al*., 2010). About 2.7% of global deaths are due to acute hepatitis B, cancer and cirrhosis of the liver. Besides, an estimated 57% of liver cirrhosis is due to HBV infection (Eke *et al*., 2011).

Hepatitis B is preventable and universal vaccination is probably best certainly in countries with a high prevalence. In the developed world, hepatitis B is an illness that mainly affects high-risk adults. In the world's poorer areas however, it is highly endemic and widely present in children. All vaccination programs are best focused on immunization in infants and children, because at this age chronic infection can be prevented (WGO, 2007).

Diagnosis of HBV infection is usually through serological and virological markers. Hepatitis B surface antigen is the hallmark of HBV infection and is the first serological marker to appear in acute HBV infection, and persistence of HBsAg for more than 6 months after acute infection suggests chronic HBV infection (WHO, 2004).

1.2. Statement of the problem

Hepatitis B virus is a major blood-borne and sexually transmitted infectious agent, and represents a serious global public health problem. Africa has the second largest number of chronic carriers after Asia, and is considered as a region of high endemicity with over 8% prevalence. Although it is difficult to assess the exact burden of HBV in Africa, between 56% and 98% of the adult population show evidence of past exposure to HBV infection, and the seroprevalence of HBsAg has been estimated to range from 6% to 20%. In Ethiopia, studies among pregnant women from some parts of the country have shown moderate endemicity with prevalence of HBsAg positivity ranging from 3 % to 6.1 % (Yeshi *et al*., 2016). Cross-sectional community studies of serological markers of HBV infection have an important role in identifying population endemicity, possible routes of transmission and associated risk factors and such data help in the development of appropriate control measures, such as estimating the optimal age for vaccine delivery, the level of vaccine coverage required for elimination (Abebe *et al.,* 2003).

Since HBV virus is found in all body fluids, transmission could be vertically (from infected mother to the infant or to the child), sexually and through contact with different body fluids. However, mother to child transmission of HBV is of great importance as it is an important mode of transmission and the earlier an individual is infected, the more likely to develop chronic disease (Lavenchy, 2004). Up to 75% of chronic carriers acquire the infection through vertical transmission in highly endemic areas and transmission and infection during infancy increases the chance to develop chronic disease to 90%, while adults usually acquire acute hepatitis B and recover, and as a result the chance to develop chronic disease is only 6 to 10% (Dessie *et al.,* 2014). Therefore, having enough information about the prevalence of HBV specifically during pregnancy is vital, because

- Major mode of transmission in developing countries is perinatal or vertical because of lack of health facilities to screen all pregnant women.

- Studying HBV prevalence in pregnant mothers is the best way of assessing the prevalence of HBV in the general population.
- Data regarding HBV in pregnant women are fundamental for health planners, policy makers and caregivers in order to make evidence-based interventions. Therefore, the aim of this study is to assess the seroprevalence of HBsAg and associated risk factors among pregnant women attending antenatal care service at Gandhi Memorial Hospital in Addis Ababa, Ethiopia.

1.3 Research Questions

1. What is the current prevalence rate of HBsAg among pregnant woman in Gandi Memorial hospital, Addis Ababa? (low, moderate or high)?
2. What are the major risk factors associated to transmission of the disease?

1.4 Significance of the study

The prevalence of HBV infection in Ethiopia in general and in Addis Ababa region in particular was not sufficiently studied. Therefore, for any concerned bodies, policy makers, health planners and future researchers of this issue, the result of this study may provide updated information about the current sero prevalence of HBsAg infection and risk factors associated with transmission of HBV among pregnant women in Gandhi Memorial Hospital in Addis Ababa.

1.5 Objectives of the study

1.5.1 General objective

The general objective of this study was to assess the status of HBV infection and predisposing factors among pregnant women in Gandhi Memorial Hospital in Addis Ababa, Ethiopia.

1.5.2 Specific objectives:

- To assess the prevalence of HBV among pregnant women in the study area.
- To identify the possible risk factors associated with hepatitis B virus infection among pregnant women visiting Gandi Memorial Hospital.

2. LITERATURE REVIEW

2.1 Epidemiology

Hepatitis B virus is a small DNA virus and belongs to a group of hepatotropic DNA viruses. The virus consists of a nucleocapsid and an outer envelope composed mainly of three hepatitis B surface antigens that play a central role in the diagnosis of HBV infection. Hepatitis B core antigen, a DNA polymerase-reverse transcriptase, the viral genome as well as cellular proteins are contained in the nucleocapsid (Thomas *et al.*, 2007). The viruse belongs to the family of hepadnaviridae, a group of hepatotropic mammalian and avian DNA viruses, which replicate via reverse transcription of a genomic RNA intermediate. The virus contains a partially double stranded genomic DNA (Abel, 2012)

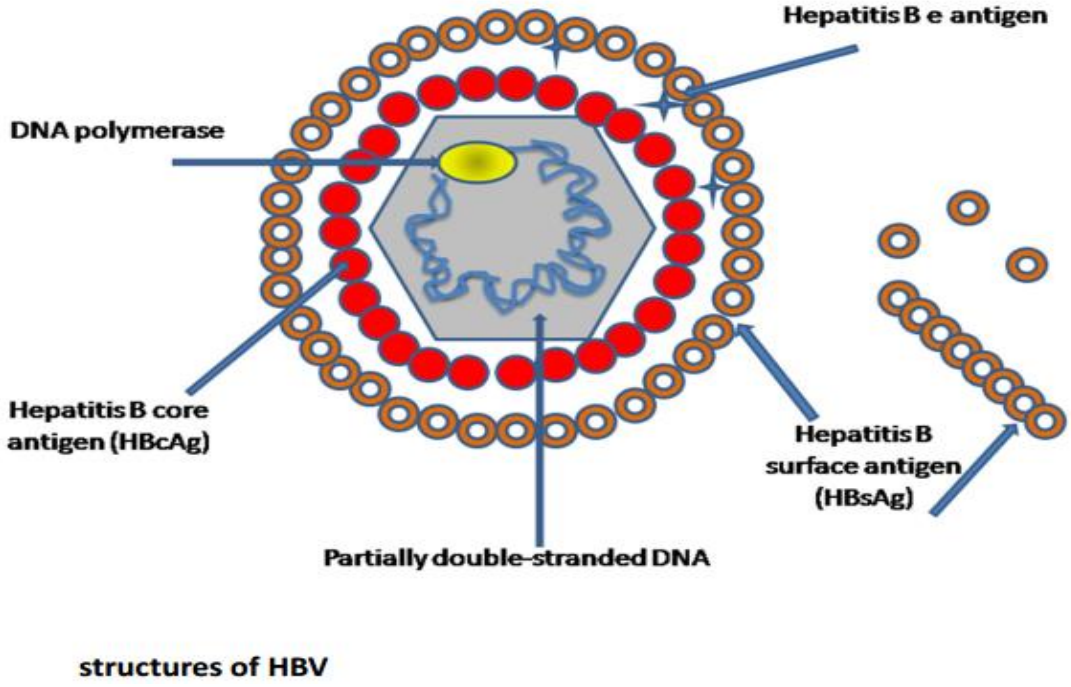

Figure 1. Structure of Hepatuitis B viruse. Source: Sisay kirba, (2014). Seroprevalence of HBsAg among pregnant woman and rate of vertical transmission in 3 teaching hospitals in Addis Ababa (unpublished).

HBV is a major cause of primary cancer of the liver or hepatocellular carcinoma (HCC) worldwide (Block *et al*., 2003). People who are chronically infected with the hepatitis B virus have a lifetime risk of death from end-stage liver disease or HCC of between 15% and 25%.

With more than 350 million people chronically infected worldwide, lives lost to HBV eventually could exceed 100 million (Cohen *et al*., 2010; Erena and Tefera 2014). In the South-East Asia region only, there are approximately 100 million hepatitis B carriers, and they account for more than 5.6% of the global population.

Acute hepatitis

The first contact of a body with HBV results in an acute hepatitis B infection which is most of the time without symptom. According to Bowden (2006) however, 20-30% of patients may exhibit symptoms of acute infection such as fever, malaise, anorexia, nausea followed by jaundice. In more than 95% of adult patients with acute HBV infection, the disease is self-limiting leading to complete recovery and life-long immunity. However, in 1-5% of immune competent adults the disease may progress to chronic hepatitis B infection (WGO, 2007).

Acute hepatitis B is characterized by hepatocellular necrosis and acute inflammation and occurs in approximately 1% of perinatal infections (in <1 years), 10% of early childhood infections (in children aged 1–5 years) and 30% of late infections (in persons aged >5 years).The incubation period for acute hepatitis B on average is about 75 days, but may vary from about 30 to 180 days. On the other hand, recent HBV infection is characterized by the presence of HBsAg and immunoglobulin M (IgM) against HBcAg in the blood. Besides, at the initial highly replicative phase of infection, patients may also be seropositive for HBeAg (WHO, 2017).

The age of acquisition of the infection plays an important role in determining the course of the infection, because 90% of those who acquire the infection during the perinatal period progress to chronic hepatitis B infection (Feld and Heathcote, 2006). Besides, according to Eke *et al*. (2011), acute hepatitis during pregnancy induces premature labor.

Chronic hepatitis

Persistence of high levels of HBsAg for 6 months or more following acute infection is an indicator of chronic hepatitis B infection (Bowden, 2006; WHO, 2017). Chronic infection with HBV is a major risk factor for development of end-stage liver disease, including cirrhosis, liver failure and primary liver cancer. According to World Health Organization (WHO) global

health impact report of viral hepatitis, more than 240 million populations were affected by chronic liver disease due to HBV (WHO, 2013).

According to WHO (2017) report, the endemicity of active HBV infection is reflected in the serologic prevalence of the HBsAg in the general population of a defined geographical area. Epidemiologically and according to HBsAg positivity, the globe is divided into three defined areas: highly endemic areas**,**(with ≥8% prevalence) and this include Africa, the Far East, the Middle East and parts of South America, intermediate or moderately endemic (with 2%–4% prevalence) including Japan, Iran, parts of South America, Eastern and Southern Europe, and parts of central Asia, Areas with low endemicity (with <2% prevalence) including Northern Europe, Australia, the southern part of South America, Canada and USA (Nima *et al.*, 2012; WHO, 2017*)*. As it is also indicated in Some studies, Africa has the second largest number of chronic HBV carriers after Asia and is considered as a region of high endemicity (Hwang and Cheung, 2011).

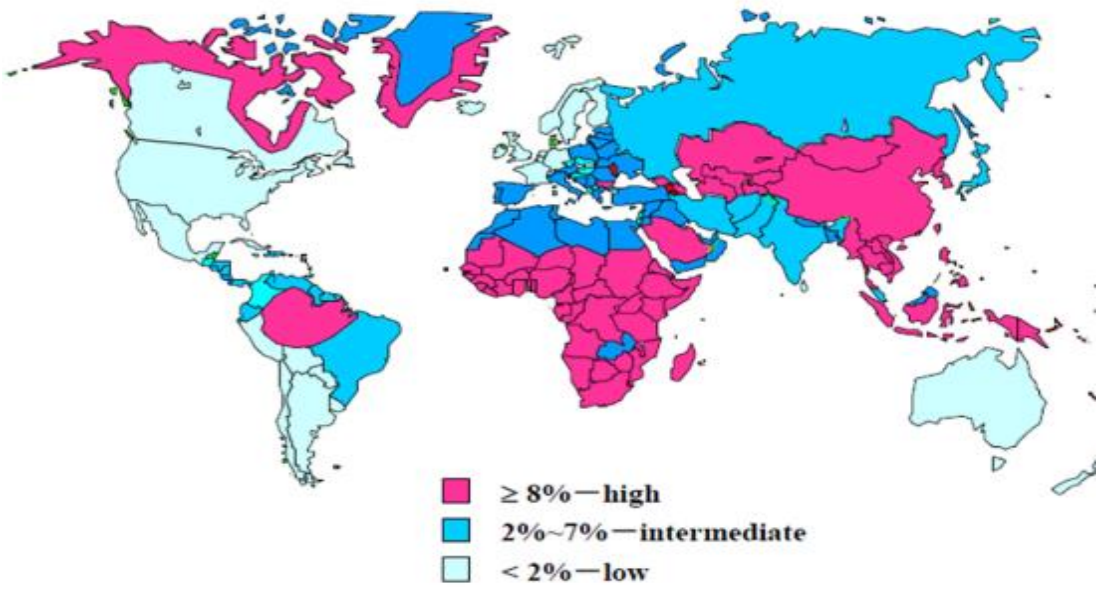

Figure 2. Geographic distribution of chronic hepatitis B infection. (Source: Jinlin *et al.*, 2005).

In Ethiopia the national hepatitis study among young males from all regions of the country showed that 10.8% prevalence for HBsAg (Kefene *et al.*, 1988). Another study which was done in the same year on seroepidemiology of HBV infection among pregnant women in Addis Ababa showed 5% prevalence (Tsega *et al.*,1988). Another study conducted on the

seroprevalence of HBsAg among pregnant women attended antenatal care at Debretabor Hospital showed 5.3 % prevalence (Walle *et al.,* 2004). Another study from Jimma which was done in 2005 showed a 3.7% seroprevalence of HBsAg during pregnancy (Mohammed *et al.*, 2005).

2.2 Transmission of HBV

Hepatitis B virus (HBV) is transmitted through exposure to infectious body fluids such as blood, semen and vaginal fluids. It can also be transmitted vertically from infected mothers to infants at the time of birth, or from family members to infants in early childhood. Transmission is also possible through transfusions of HBV-infected blood and blood products, contaminated injections during medical procedures, sharing of needles and syringes among injectable drug users and unsafe sexual intercourse (Yosef *et al*., 2016). According to Mohammed *et al*. (2005), HBV also poses a risk to healthcare workers who sustain accidental needle-stick injuries while caring for HBV infected people. Now days a safe and effective vaccine is available to prevent HBV infection since 1982 (Goldstein *et al.*, 2005). Currently, three major modes of HBV transmission have been recognized:

1. Perinatal or vertical transmission (from carrier mothers to their babies during the perinatal period)
2. un protected sexual intercourse
3. parenteral/percutaneous transmission including injection drug use (IUD), blood transfusions and dialysis, acupuncture, working in a health-care setting, tattooing and household contact *(*Dessie *et al.*, 2014).

Approximately about 45% of the world's population lives in regions that are highly endemic for HBV infection, where most infections are acquired prenatally or during early childhood. Another 43% live in regions of intermediate endemicity, where multiple modes of transmission (perinatal, household, sexual, injection drug use associated, and health care associated) are possible. In countries with low endemicity however, most infections are due to sexual practices and exposure to infectious body fluids among adolescents and adults and injection drug user, homosexual males, health care workers, patients who require regular blood transfusion or hemodialysis are targeted groups (Jinlin *et al*., 2005; Wasley *et al.,* 2010). World health organization recommends HBV vaccination at birth in order to prevent maternal-child

transmission. However, Less than half of the WHO Member States have a policy to provide HBV vaccine at birth and globally only 27 % of newborns received this vaccine (WHO, 2012).

The prevalence of HBV infection among pregnant women may be a reliable indicator of hepatitis B virus prevalence rate in the general population. Hence, screening of pregnant women for HBsAg can give reliable evidence about the prevalence of the disease in a general population and it can provide a guideline for preventing mother to child transmission of the virus (Eke *et al*., 2011). Perinatal transmission is the common route of transmission in developing countries. Infants born to mothers who are positive for both HBsAg and HBeAg are at a higher risk of acquiring infection vertically up to 70% –100 % while those born to HBsAg positive alone mothers can acquire the infection up to 5 % –30 %. The presence of HBeAg in the blood indicates that HBV replication is highly active and blood and other body fluids (vaginal fluids, semen and saliva) are highly contagious (WHO, 2017). The mother to fetus transmission rate of HBV also depends on the time of onset of hepatitis in mother. There is a higher rate of transmission (76 %) in the third trimester, compared to (10 %) transmission in the first or second trimester (WHO, 2012).

HBV infection acquired at an early age is more likely to become chronic than infection acquired later in life or the rate at which chronic HBV inversely related to the age at acquisition of the infection occurs. Approximately 80 % – 90 % of infants infected perinatally and 30 % – 50 % of children infected before the age of 6 years develop chronic HBV infection while only <5% chronic infection occurs if the infection is at the adulthood (WHO, 2017).

2.3. Prevention of HBV Infection

To prevent of HBV transmission in a community especially in developing countries, preventing vertical transmission is a very important step. Therefore maternal screening followed by providing HBV vaccination immediately after delivery in all infants born to HBsAg-positive mothers which is 75 % effective alone or providing HBV vaccine combined with HBV immunoglobulin which is 93 % effective are effective strategies for reducing the incidence of chronic infections. Both should be given within 12 hours of delivery (Lee *et al*., 2006). On the other hand according to WHO, screening of all blood products, vaccinating of partners of sero-discordant couples, non-recycling of injection needles and post exposure

prophylaxis with vaccine and immunoglobulin have a significant role in preventing HBV (WHO, 2012).

Three main strategies have also been approved to be effective in preventing HBV infection. These are behavioral modification, passive immune prophylaxis, and active immunization. Besides, the implement of mass HBV immunization program is recommended by the WHO since 1991, and it has dramatically decreased the prevalence of HBV infection and HCC in many countries (Jinlin *et al.*, 2005).

Behavioral Modification

Behavioral modifications in sexual practice and improved screening procedures of blood can reduce the risk of sexual and transfusion-associated HBV infections. Behavioral modification is thought to be more beneficial in people living in developed countries where infections are mainly through sexual practices and using injectable drugs than in people living in developing countries, where neonates and children in early childhood are at the greatest risk of acquiring infection.

Passive immune prophylaxis

Hepatitis B Immune Globulin (HBIG) is a sterile solution of ready-made antibodies against hepatitis B. HBIG is prepared from human blood from selected donors who already have a high level of antibodies to hepatitis B and used in passive immuno prophylaxis (WGO, 2007). Passive immuno prophylaxis can be used in four situations

1. Newborns of mothers infected with hepatitis B
2. After needle stick exposure
3. After sexual exposure
4. After liver transplantation (WHO, 2004).

Immune prophylaxis is recommended for all infants born to HBsAg positive mothers. Current dosing recommendations are 0.13ml/kg HBIG immediately after delivery or within12 hours after birth in combination with recombinant vaccine. The combination results in a higher than 90 % level of protection against perinatal acquisition of HBV. However between 3.7 % to 9.9 % of infants still acquire HBV infection perinatally from HBV infected mothers, despite immune prophylaxis. Failure of passive and active immuno-prophylaxis in this setting may be

the result of in utero transmission of HBV infection which is caused by a damage of placental barrier (Xu *et al.,* 2001).

According to Eke *et al.* (2011), administration of HBIG in a dose of 200 international units (IU), which is given intramuscularly every week from 28th weeks of gestation reduces mother to child transmission of hepatitis B virus. Lamivudine, which is a cytosine analogue drug that acts as a nucleoside reverse transcriptase inhibitor, is a potent inhibitor of HBV replication which brings rapid reduction of HBV DNA levels, with a median reduction of 97 % after 2 weeks and decreases the risks of vertical transmission if used with good safety and efficacy in the last four weeks of pregnancy (Wang *et al*., 2004). Though the role of caesarean section in reducing mother to child transmission of HBsAg is negligible in some studies), according WHO 2017 weekly epidemiological report, caesarean section reduces the perinatal transmission of HBV infection from HBsAg-positive women to their infants (WHO, 2017).

Active immunization

Hepatitis B vaccination which is available since 1982 is the most effective measure to prevent HBV infection and its consequences (Wasley *et al*., 2009). Hepatitis B immunization strategies are targeted to prevent chronic HBV infection and its serious consequences, including liver cirrhosis and hepatocellular cancer. Countries may use variety of schedules for hepatitis B immunization in their national programs, depending on the local epidemiological situation. However, in countries where a high proportion of HBV infections are acquired perinatally, the first dose of hepatitis B vaccine should be given within 24 hours after a birth. In countries of intermediate or low hepatitis B endemicity, catch up vaccination strategies targeted at older age groups or groups with risk factors for acquiring HBV infection should be considered rather infant vaccination, because in such settings, the disease burden may be attributable to infections acquired by older children, adolescents and adults. In countries of high endemicity however, large-scale routine vaccination of infants rapidly reduces the transmission of HBV. In these circumstances, since most of them have already been infected, catch up vaccination of older children and adults is of relatively little impact on chronic disease prevention (WHO, 2004).

The two types of hepatitis B vaccines available are:

1. The first-generation hepatitis B vaccine or Inactive human plasma-derived vaccines (PDVs) are prepared from purified HBsAg from the plasma of individuals with chronic HBV infection and are available since 1982.
2. The second generation HB vaccine or DNA Recombinant or genetically engineered vaccines made using HBsAg synthesized in yeast (Saccharomyces cerevisiae) or in mammalian cells into which the HBsAg gene has been inserted. Both of the vaccines consist of a suspension of HB surface antigen and proven to be safe and efficacious in preventing HBV infection (Franco, 2012). More than 15 different PDVs are licensed throughout the world and each country has different preparations (WGO, 2007).

Vaccination programs against hepatitis B are very effective as 3-dose vaccination (second dose after 1 month, third dose after 6 months) started with a birth dose in 2 highly endemic areas of China reduced chronic HBV infection rates from 9.3% to 0.8% and from 10.4% to 0% respectively (WHO, 2017). Besides, according to 2017 WHO global Hepatitis report, early vaccination of the baby against hepatitis B with a first dose within 24 hours of birth contributes to the prevention of mother-to-child transmission. The efficacy of the vaccine decreases with the concentration of HBV in the blood of the mother. For instance, HBeAg-negative, but HBsAg positive mothers have a near 0% risk of transmitting HBV to their offsprings vaccinated at birth while HBeAg-positive mothers have a 20% risk of transmitting the virus despite vaccination at birth. The study conducted in USA showed that a three dose plasma derived vaccine gives protection for at least for 30 years (WHO, 2017).

3. MATERIALS AND METHODS

3.1 Description of the study area

The study was conducted at Gandhi Memorial Hospital which is one of the six Hospitals running under Addis Ababa City Administration Health Bureau and located in Kirkos sub city. The hospital was established in 1951 E.C (1959 G.C) and providing service as a referral Hospital for gynecological cases and maternity services and also serves as a center of excellence for Addis Ababa University for those students who are specializing in gynecology and obstetrics. The Hospital also provides other services such as antiretroviral therapy (ART), voluntary counseling and testing (VCT), rape clinic and neonatology. The hospital is known by providing large number of delivery services, as high as 550 deliveries per month and recognized and prized by Ministry of Health in 2014 for its best obstetrics and gynecology services.

3.2 Study design and study population

A cross sectional study was conducted on pregnant women attending antenatal care service at Gandhi Memorial Hospital from April to June 2017 in Addis Ababa, Ethiopia. The population of the study was all pregnant women who visited Gandi memorial hospital to follow antenatal care service. Those pregnant women who ordered by health professionals to give blood sample at laboratory of the hospital for different hematological diagnostic purpose during the study period and those of them who were willing to give additional 2ml of blood sample for the purpose of this research and to fill the questionnaire were included.

3.3 Study variables

The dependent variable in the study was serological result for HBsAg of pregnant women involved in the study. The independent variables included in the study were Having multiple sexual partner in life, Blood donation history, History of abortion, History of FGM, History of teeth extraction, History of surgery. Similarly, History of contact with jaundiced person, History of using Barberry machine, History of nose piercing, History of ear piercing and Ethnic group of the study participants were independent variables.

3.4 Sample size determination and eligibility

Sample size was determined using Single population proportion formula and was based on 3.7 % prevalence of HBsAg during pregnancy from Mohammed Awole, Solomon Gebre-Selassie, Seroprevalence of HBsAg and its risk factors among pregnant women in Jimma, Southwest Ethiopia, 2005, 2% error of margin and 10% non-response rate.

Sample size:

$$n = \frac{(z\,\partial/2)\ 2\ p\ (1-p)}{d^2}$$

Where n = sample size

P= 0.037

d = 0.02 (2% error of margin)

z ∂/2 = 1.96 (standard normal probability for 95% CI)

n = 350 When 10% non-response rate is added, n = 384

3.4.1 Inclusion Criteria

Those pregnant women referred to the laboratory by the physicians to provide blood sample for different diagnostic purpose and were willing to sign a written consent, to provide additional 2ml blood sample and to respond to the questionnaire was participated in the study.

3.4.2 Exclusion Criteria

- Those pregnant women who were not willing to participate in the study.
- Those non pregnant women who came to the laboratory to give blood sample were excluded.

3.5 Ethical consideration

Ethical clearance was obtained from research and publication committee of the Addis Ababa Regional Health Bureau. Gandhi Memorial Hospital, where the study was conducted was notified by official letter from Addis Ababa regional Bureau.

Written consent was obtained from respondents and data were collected and handled confidentially throughout this study. No name of the respondents was written and only numerical codes were used on the questionnaire.

3.6. Data collection

Descriptive data and blood samples were collected at laboratory of Gandhi Memorial Hospital after explaining the objective of the study to the pregnant women by the principal investigator. It was also clearly explained to the pregnant women that they would not write their names on the questionnaire and confidentiality was protected.

3.6.1 Data collection Tools

3.6.1.1 Questionnaires

Once the pregnant woman agreed and signed the written consent to participate in the study, blood sample was taken and a structured questionnaire was filled to collect socio-demographic data and to find out possible risk factors for HBV infection.

3.6.1.2 Blood sample collection

Once written consent was obtained from the participants five milliliter of blood sample was taken from each pregnant woman, three ml of the collected blood was used for different diagnostic purpose based on the laboratory request they brought to the laboratory and the remaining two milliliter of blood was transferred to serum separator tube, centrifuged and stored in refrigerator in 2℃ - 8℃ for later analysis.

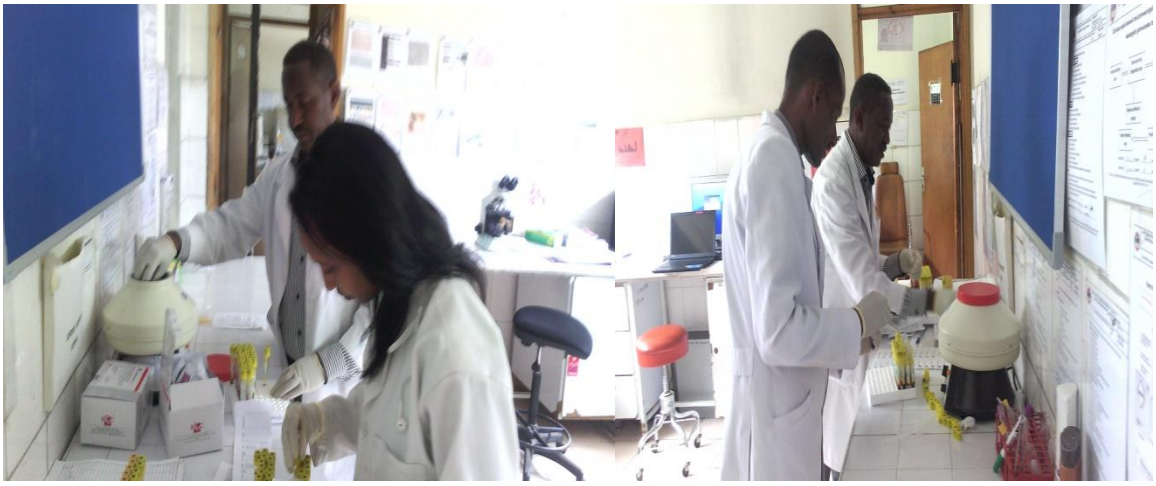

Figure 3. Bloodsample processing by laboratory technician in Gandhi Memorial Hospital during the study

Serum from each blood sample was used to test the presence of HBsAg according to standard operating procedure of HBsAg test of the Hospital using rapid diagnostic tests employing the

principle of immune chromatography using Advanced Quality One step HBsAg test card (Manufactured by: InTech products,inc.).

Figure 4. Blood sample collection by health professionals at Gandhi memorial hospital laboratory during the study.

Immune chromatography test for diagnosing HBsAg in the serum

The Advanced Quality One step HBsAg test is a colloidal gold enhanced immunoassay for determination of HBsAg in human whole blood, serum or plasma. Anti-HBsAg antibody is immobilized in the test region on nitrocellulose membrane. During the assay the specimen was allowed to react with the colored conjugate (antibody-colloidal gold conjugate); the mixture then migrates chromatographically on the membrane by the capillary action. An HBsAg positive specimen produces a distinct red or pink color band on the test region, formed by the specific antibody-HBsAg colored conjugate complex. Absence of this colored band on the test region suggests a negative result. The same colored band always appears in the control region serving as procedural control regardless of the test result.

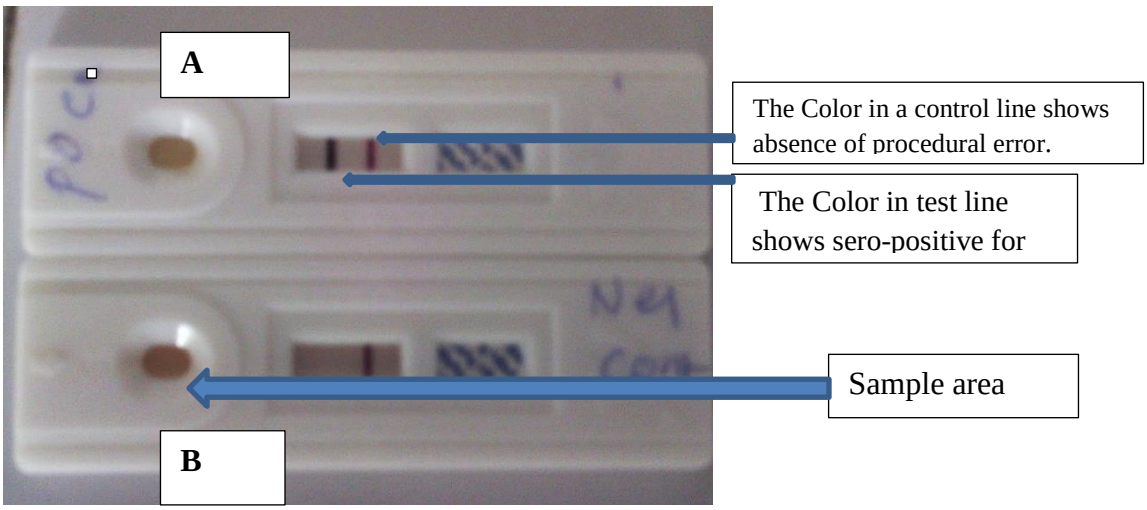

Figure 5. Performance of the Advanced Quality One step HBsAg test using the internal quality control of the test (control line formation) and by known positive and known negative blood serum control samples obtained from national blood bank. 'A'shows seropositive for HBV while 'B' shows seronegative result.

Quality control

The validity and completeness of the data were daily verified by the principal investigator and the performance of the Advanced Quality One step HBsAg test was evaluated using the internal quality control of the test and by known positive and known negative blood serum controls obtained from national blood bank which was ELISA-tested.

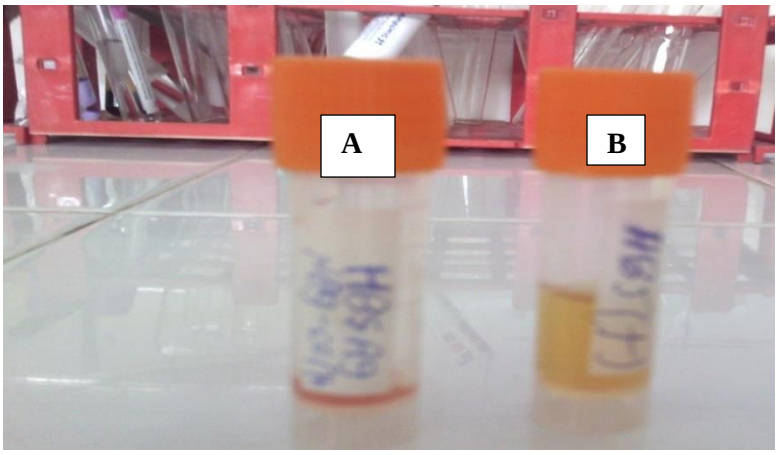

Figure 6. ELISA tested, known negative (A) and known positive (B) serum samples obtained from National Blood Bank and used in Gandhi Memorial Hospital as a control test.

Procedure

The whole screening process of the blood was done based on the instruction given by the manufacturer as follows

1. 2 ml of venous blood was taken, centrifuged and serum was separated.

2. A test cassette or card was removed from a foil pouch, and placed on a flat surface.

3. The test device was labeled with patient identification number or numerical code.

Figure 7. Test card in foil pouch (A). Test card with patient identification number (B)**.**

4. Three drops of the specimen (serum) was added to the sample area.

5. The results were seen within 15 minutes, because interpreting the test result after 15 minutes is not advisable.

Interpretation of the result

The result was interpreted as follows

If one red or pink line appears in control line and there is no line in test region, it shows the test has been carried out correctly and indicates negative result.

If a distinct red or pink color band appears in the test region, in addition to a red or pink colored control line, it indicates positive result. A positive result may develop even sooner at a high viral concentration of HBsAg in the serum. However, the lower the concentration of HBsAg, the longer time it takes to develop a color band on a test region; therefore, a negative result should be determined at 15 minutes to insure it is truly negative instead of weak positive.

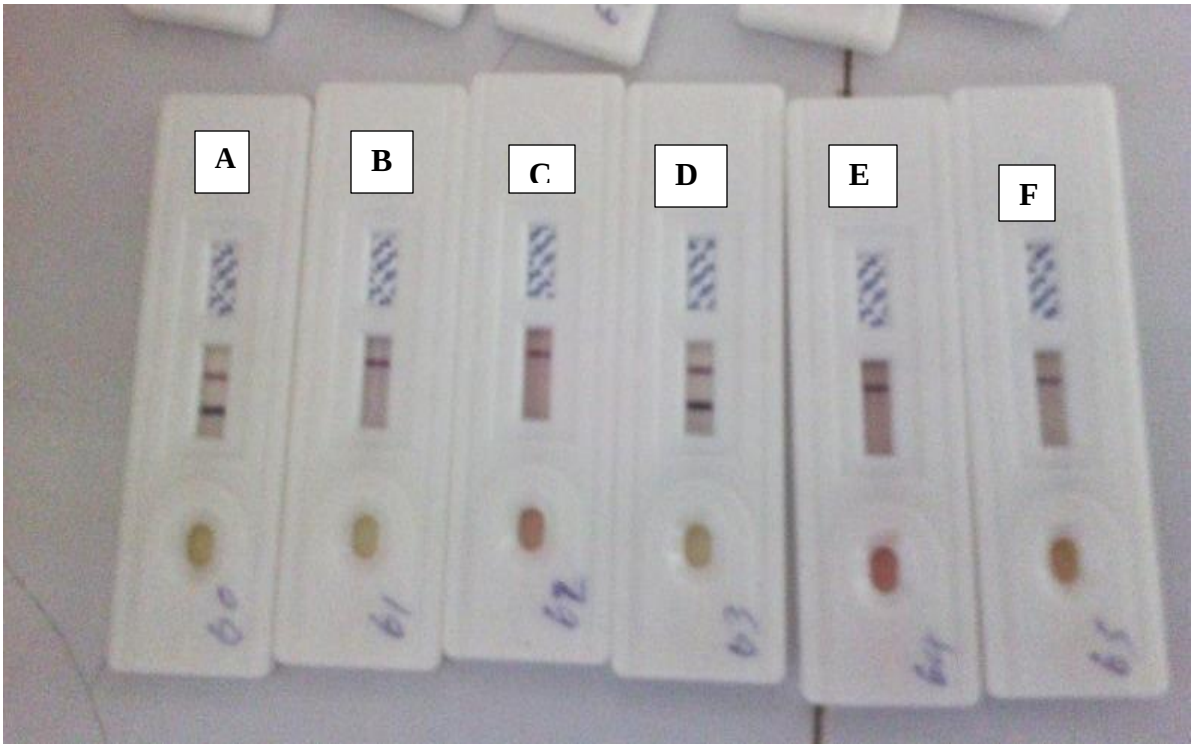

Figure 8. Some of the seropositive samples (A and D) and the seronegative samples (B, C, E and F) during the study.

A total absence of color in both regions or the formation of color in test region in the absence of color in control region is an indication of procedure error or the test reagent has deteriorated (invalid). The test should be repeated using a new test card.

Accuracy of the Advanced Quality One step HBsAg test cards or cassette.

HBsAg Advanced Quality One step HBsAg test was compared with a leading commercial Radio immunoassay (RIA) and an Enzyme immunoassay (EIA) test for Hepatitis B. There is 98% overall agreement between RIA and Advanced Quality One step HBsAg test and 97% between EIA and HBsAg Advanced Quality One step HBsAg test. Advanced Quality One step HBsAg test can detect HBsAg at concentration as low as 1ng/ml with sensitivity of 98.89% and specificity 98.87 for all HBsAg subtypes which are all common in Ethiopia (Yeshambel *et al*., 2016).

3.7. Data Analysis

All the collected data from participants of the study were entered into Microsoft office Excel and processed using SPSS version 24 statistical software for comparison and testing the relationship of HBsAg positivity and various variables included in the study. Descriptive statistics was employed to quantify the results in the study area. Chi-square test was used for bivariate analysis of the association between HBsAg seropositivity and the possible risk factors included in the study, P value < 0.05 at 95% CI was considered as statistically significant.

4. RESULTS

Socio demographic data

Large number of the pregnant women participated in the study 189 (49.2%), were in the age group of 25-29, followed by 94 (24.5%) in 30-34 years age group while only one percent of participant of the study were from 40 and above years age group(Table-1); (Figure 9).

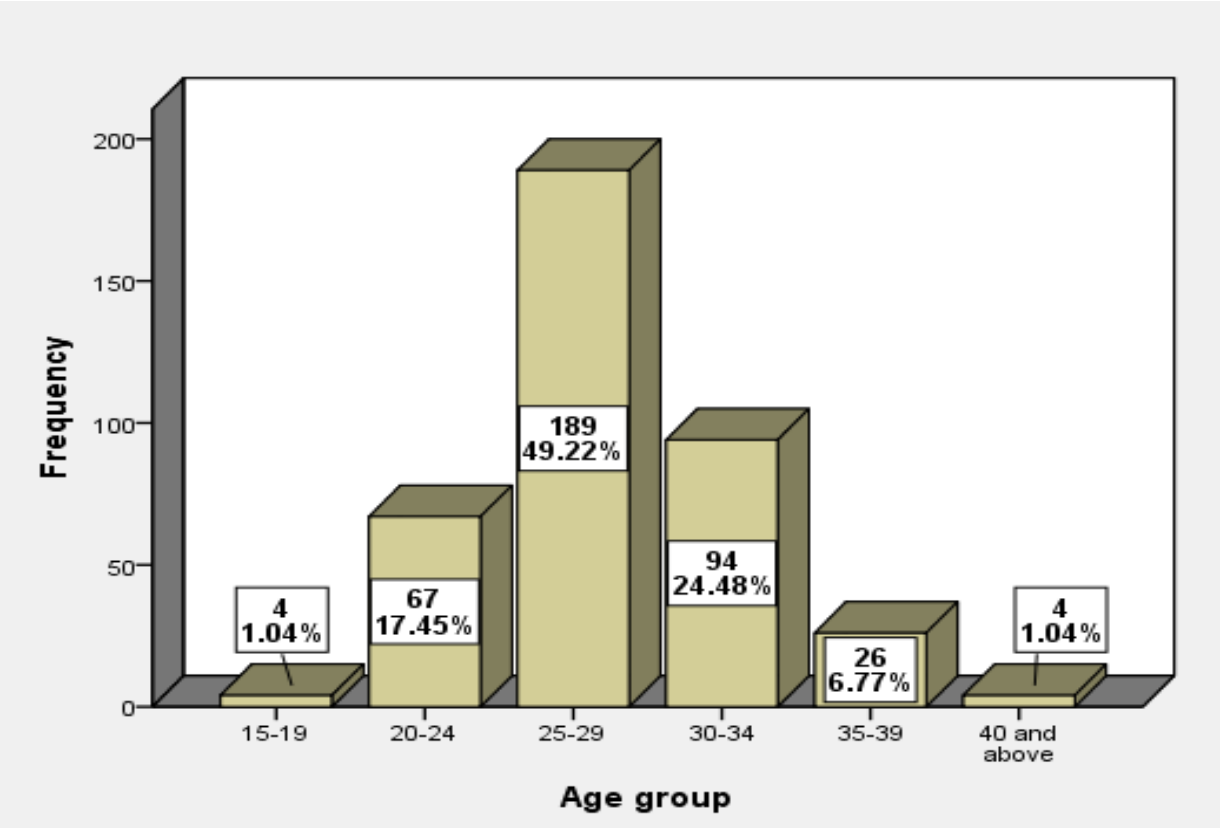

Figure 9. Age group of pregnant women attending antenatal care service at Gandhi Memorial Hospital Addis Ababa, April to June, 2017 (n=384).

Majority of the study participants 372 (96.9%) were married, 112 (29.2%) were educated up to college and above, 86 (22.4%) learned up to grade 9-10, 85 (22.1%) learned up to grade 5-8, while 32(8.3%) had no formal education. Regarding occupation of the study subjects (n=384), higher percentage, 195(50.8%) of the pregnant mothers involved in the study were house wives, followed by private employee 96 (25%). Government employees were 45 (11.7%) while 30 (7.8%), 14 (3.6%), 4 (1%) were merchants, unemployed, and students respectively.

Most of the study participants, 94.8% (364) were from Addis Ababa, and only 20 (5.2%) participants were from outside Addis Ababa. About, 172 (44.8%) of the pregnant women participated in the study earn <1000, 106 (27.6%) earn 1000-2000 birr, and 106 (27.6%) earn greater than 2000 Birr.

Of 384 pregnant women involved in the study, 71 (18.5%) had history of having more than one sexual partner in life while 313 (81.5%) had only one sexual partner in life. Regarding history of blood donation, 323 (84.1%) of the participants of the study have no history of blood donation, while the remaining 61 (15.9%) had either donated or received blood. On the other hand, only 80 (20.8%) of the study groups had history of tattooing, while the majority of the study subjects, 296 (77.1%) had no history of abortion. Besides, 199 (51.8%) of the pregnant women had history of FGM while the remaining 185(48.2%) had no history of FGM (Female genital mutilation) (Table.1).

Out of all pregnant women participated in the study, 140 (36.5%), 136 (35.4%), 14 (3.6%), 7 (1.8%) had history of teeth extraction and implantation, history of operation, history of contact with jaundiced person and history of using barberry machine respectively. All the pregnant women participated in the study 384 (100%) had history of ear piercing. On the other hand only 18 (4.7%) of the study groups had history of noes piercing.

The higher number of the study subjects 174 (45.3%) were from Amahara, 80 (20.8%) were from Oromo, 67 (17.4%) were from Gurage, 33 (8.6%) were from Tigre ethnic groups while 33 (7.8%) were from other ethnic groups of Ethiopia (Figure 10).

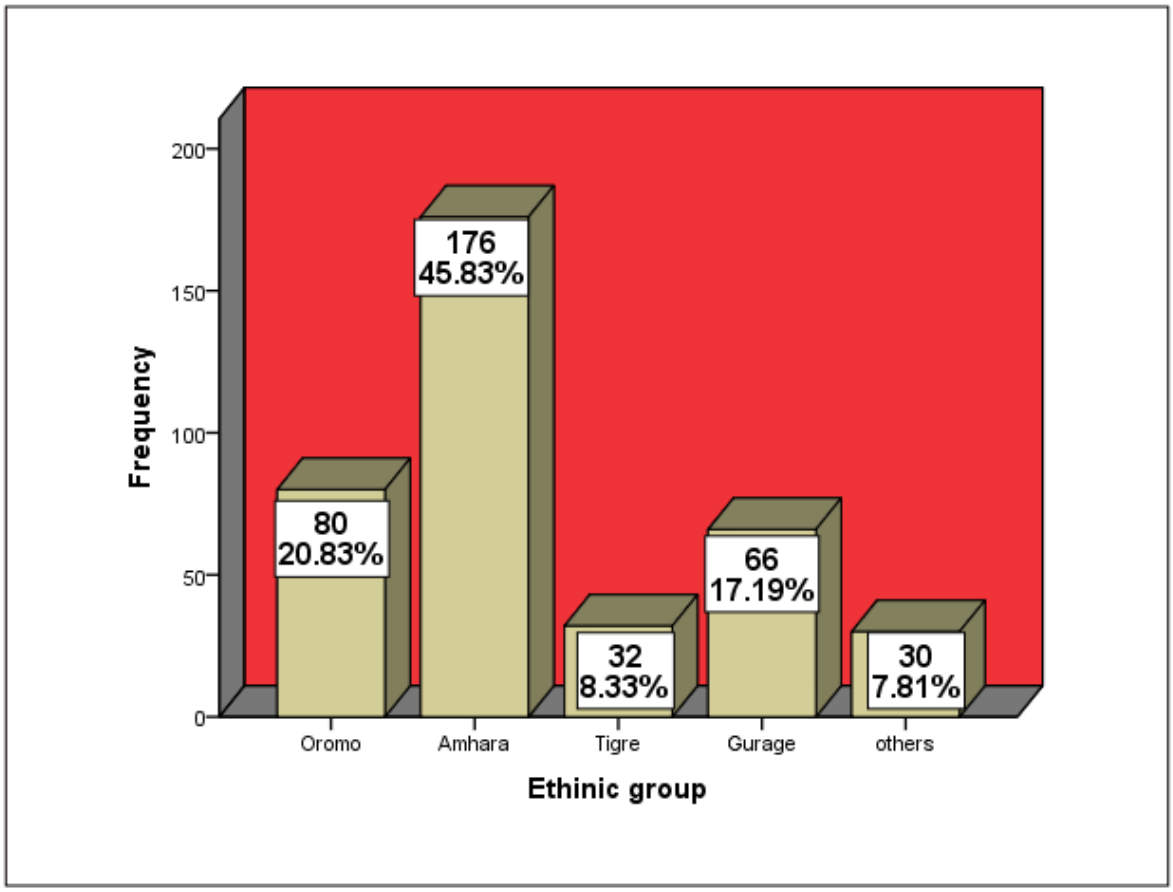

Figure 10. Ethnic group of pregnant women attending antenatal care service at Gandhi Memorial Hospital, April to June, 2017 (n=384).

Serological result

Out of the 384 pregnant women participated in the study 9 (2.3%) were positive for HBsAg, but none of them had acute clinical hepatitis. The remaining 375(97.7%) were negative for HBsAg.

The age group in which maximum seropositivity, 5 (55.5%) is observed was 30-34 followed by 25-29 age group with 3(33.3%) and only 1 (11.1%) of the participants was positive for HBsAg in 40 and above age group, and accordingly the age group of the study participants (p=0.031) showed statistical significance with their serological result for HBsAg, but none of the study participants from 15-19, 20-24 and 35-39 age groups were seropositive (Figure 11). Besides, all of the seropositive pregnant women 9 (100%) were married (Table 1).

From 364 pregnant women participated in the study from Addis Ababa, 8(2.2%) were seropositive for HBsAg and from 20 pregnant women participated in the study from outside Addis Ababa, 1(5%) participant was seropositive for HBsAg, but there was no statistical significance between serological result and address of the participants of this study. Although the majority of the seropositive pregnant women participated in the study, 5 (55.5%) earn below 1000 birr and the remaining 3(33.3%) earn above 2000 birr and only 1 (11.1%) earn 1000-2000 birr monthly, there was no statistical significance between monthly income of the participants (p = 0.664) and their serological result.

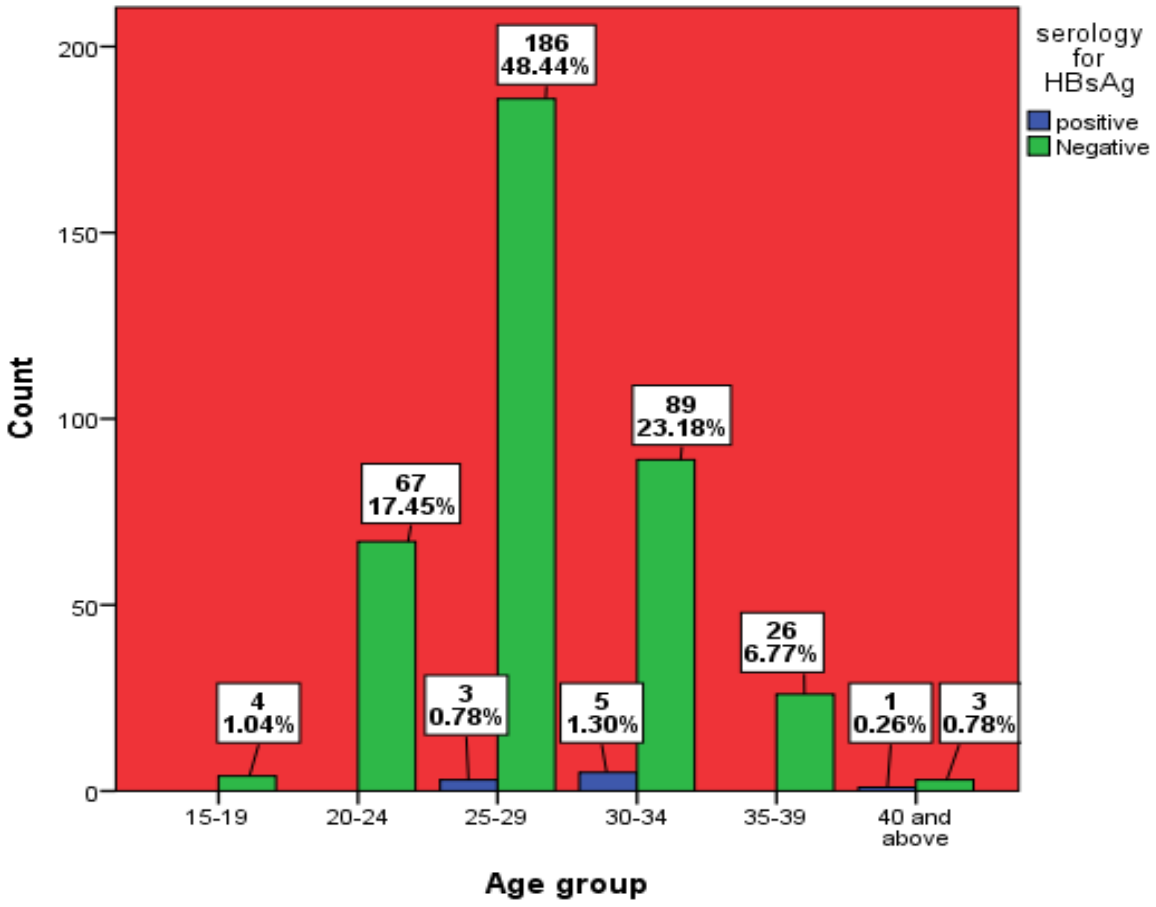

Figure 11. Age group based seroprevalence of HBV among pregnant women attending antenatal care service in Gandhi memorial Hospital, April to June, 2017 (n=384).

As it is shown in Table-2 and figure 12, from seropositive pregnant women (n=9), the higher seroprevalence of HBsAg, 6 (66.6%) was observed in pregnant women from Gurage ethnic group and another similar 1(11.1%) seroprevalence was observed in Oromo, Amhara and Tigre

ethnic groups, but none of the pregnant women from other ethnic group of the country was seropositive for HBsAg. Accordingly, ethnic group of the participants (p=0.023) showed statistical significance with serological result for HBsAg

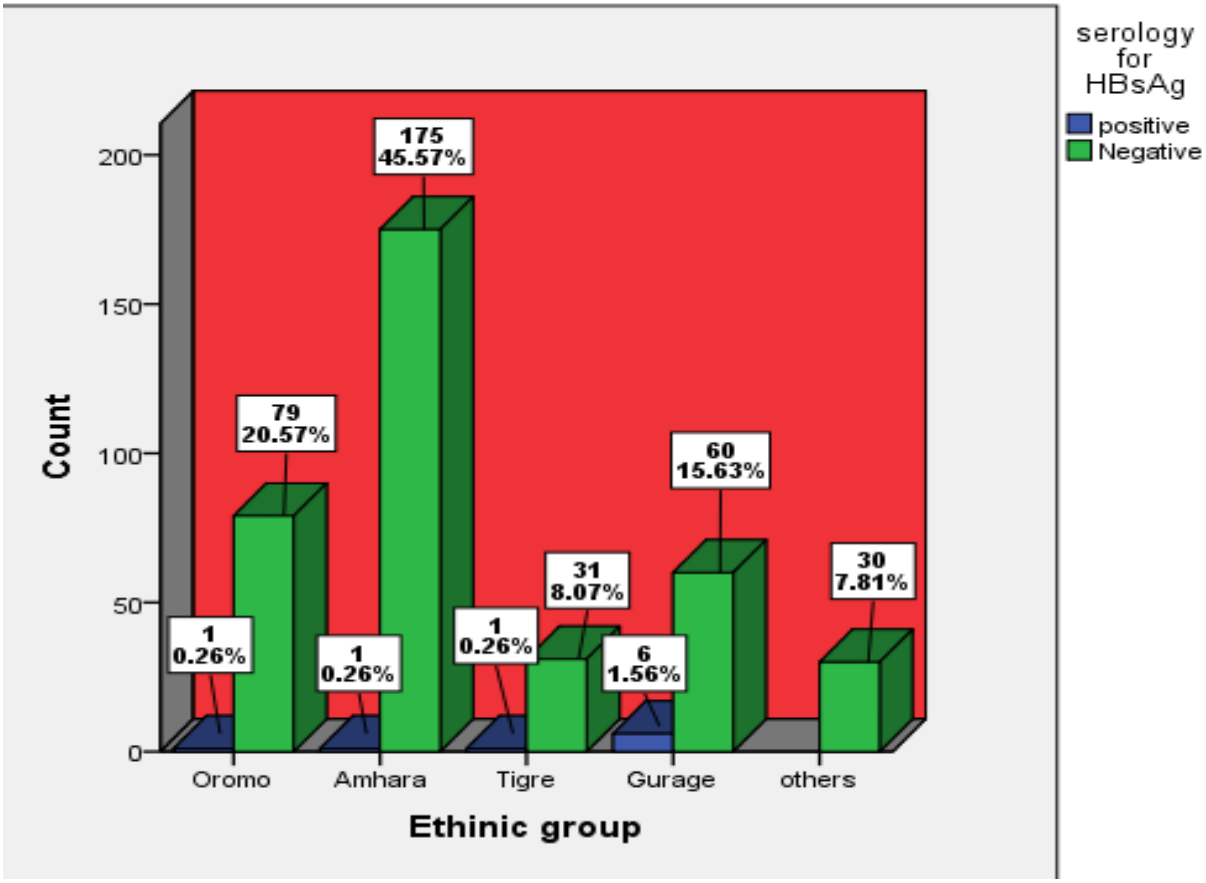

Figure 12. Seroprevalence of HBV among pregnant women of different ethnic group attending antenatal care service at Gandhi Memorial Hospital, April to June , 2017 (n=384).

Table 1. Seroprevalence of HBV and socio demographic characteristics of pregnant women attending antenatal care service at Gandhi Memorial Hospital in Addis Ababa, April to June 2017 (n=384).

Variables	Catagories	HBsAg Positive(n=9) No	%	Negative (n=375)	Total	P-value
Age	15-19	0	0	4	4	0.02*
	20-24	0	0	67	67	
	25-29	3	33.3	186	189	
	30-34	5	55.6	89	94	
	35-39	0	0	26	26	
	40 and above	1	11.1	3	4	
	Total	9	100	375	384	
Marital status	Married	9	100	363	372	1.00
	Unmarried	0	0	12	12	
	Total	9	100	375	384	
Education level	Illiterate	2	22.2	30	32	0.599
	Grade 1-4	0	0	41	41	
	Grade 5-8	2	22.2	3	85	
	Grade 9-10	1	11.1	85	86	
	Grade11-12	1	11.1	27	28	
	College and above	3	33.3	109	112	
	Total	9	100	375	384	
Occupation	Government employee	1	11.1	44	45	0.362
	private employee	2	22.2	94	96	
	Unemployed	1	11.1	13	14	
	Student	0	0	4	4	
	Merchant	2	22.2	28	30	
	House wife	3	33.3	192	195	
	Total	9	100	375	384	
Participants' address	Addis Ababa	8	88.9	356	364	0.391
	Outside AddisAbaba	1	1.1	19	0	
	Total	9	100	375	384	
Monthly income	Below 1000	5	55.6	167	172	0.688
	1000-2000	1	11.1	105	106	
	Above 2000	3	33.3	103	106	
	Total	9	100	375	384	
Status of having private house.	Yes	3	33.3	110	113	1.00
	No	6	66.7	265	271	
	Total	9	100	375	384	

*statistically significant at $p < 0.05$

Table 2. Risk factors and prevalence of HBsAg among pregnant women attending antenatal care at Gandhi Memorial Hospital in Addis Ababa, April to June 2017

Variables	Categories	HBsAg Positive(n=9) No	%	Negative (n=375)	Total	P value
History of multiple sexual partner	Yes	1	11.1	70	71	0.74
	No	8	88.9	305	313	
	Total	9	100	375	384	
History of blood donation	Never	8	88.9	315	323	1.00
	Donated	1	11.1	52	53	
	Received	0	0	8	8	
	Total	9	100	375	384	
History of tattooing	Yes	4	44.4	76	80	0.070
	No	5	55.6	299	304	
	Total	9	100	375	384	
History of abortion	Never	6	66.7	290	296	0.474
Yes	At health station	3	33.3	79	82	
	Outside H.station	0	0	6	6	
	Total	9	100	375	384	
History of FGM	Yes	7	77.8	192	199	0.188
	No	2	22.2	183	185	
	Total	9	100	375	384	
History of teeth extraction or implantation	Yes	2	2.2	138	140	0.503
	No	7	77.8	237	244	
	Total	9	100	375	384	
History of surgery	Yes	3	33.3	133	136	1.00
	No	6	66.7	242	248	
	Total	9	100	375	384	
History of contact with jaundiced person	Yes	1	11.1	13	14	0.281
	No	8	88.9	362	370	
	Total	9	100	375	384	
History of using barberry machine	Yes	1	1.1	6	7	0.151
	No	8	88.9	369	377	
	Total	9	100	375	384	
History of nose piercing	Yes	0	0	18	18	1.00
	No	9	100	357	366	
	Total	9	100	375	384	
History of ear piercing	Yes	9	100	375	384	
	No	9	100	384	384	
	Total	9	100	375	384	
Ethnic group	Oromo	1	11.1	79	80	0.023*
	Amhara	1	11.1	175	176	
	Tigre	1	11.1	31	32	
	Gurage	6	66.7	60	66	
	Others	0	0	30	30	
	Total	9	100	375	384	

5. DISCUSSION

In this study, the overall seroprevalence of HBsAg in pregnant women was 2.3%. According to WHO (2017) criteria of classification the level of endemicity of HBV infection in this study showed moderate prevalence, but there was no pregnant woman with acute clinical hepatitis. This prevalence was lower compared to former studies conducted in different regions of Ethiopia, such as 5.3% among pregnant women in Debre Tabor Hospital (Walle *et al.,* 2008), 3.7% among pregnant women in Jimma and its neighboring towns (Mohammed *et al.,* 2005), 5.7% among visitors of voluntary counseling center of Shashemene Hospital (Negero *et al.,* 2011), 3.8% among pregnant women in Bahir Dar city (Yohanes *et al.,* 2014), 7.8% among pregnant women in southern Ethiopia (Yeshi *et al.,* 2016). This prevalence was also lower than former results such as 4.7% among blood donors in Arbaminch, (Ayele and Gebru *et al*., 2016), 3.73 % among blood donors in Dire Dawa (Yosef *et al.,* 2016), 7.4% among individuals visiting Goba General Hospital, South East Ethiopia (Erena and Tefera, 2012). This slightly lower prevalence of HBV among pregnant women from this study area in Addis Ababa could be because of availability of relatively better health facilities and community awareness compared to other parts of the country.

The prevalence observed in this study was also lower than the result of some former studies conducted in Addis Ababa such as 7% in community-based sero epidemiological survey in Addis Ababa (Abebe *et al.,* 2003), 3% among delivering women in St. Paul's Hospital Millennium Medical College and Selam Health Center in Addis Ababa (Dessie *et al.,* 2014). The prevalence rate of the present study was also lower than those reported in cities of different African countries such as 16.6% in Niger (Nigeria), 8.7% in Birazzavil (Congo), 8% in Mali (Brunel *et al*., 2014), 5.6% in Sudan (Alegbeleye *et al.,* 2013). However, it was higher than 0.59% that was reported in Tehran (Iran) (Kheiri *et al*., 2015), 1.5% in Tripoli (Libya) (Magrahl *et al*., 2010). This difference in the prevalence can be due to differences in cultural and behavioral characteristics of the participants studied.

Though further study is required to determine the burden of HBV infection in general population in Addis Ababa, the prevalence of HBV virus seems to be slightly declining from 7% in community-based study in 2003 (Abebe *et al.,* 2003), 3% among delivering women in 2014 (Dessie *et al*., 2014) to 2.3% among pregnant women in this study. A recent systematic

review and meta-analysis about Hepatitis viruses in Ethiopia also so showed a decreasing pattern of overall HBsAg prevalence rate in general population (Belayhun *et al*., 2016). This might be because of prevention and control measures done for prevention of HIV as both infections are sexually transmitted and this could indicate a need for integrating HBV management to the HIV prevention and control programs in Ethiopia. However, the prevalence of HBV infection in Addis Ababa is still moderate which requires significant intervention of concerned bodies mainly to prevent vertical transmission and infections at early childhood which cause chronic HBV infection and its consequences such as Cirrhosis and Liver cancer (HCC).

The socio demographic status of the study population in this study showed that the age specific prevalence is highest in the age group 30-34 and 25-29 which comprises 5 (55.6%) and 3 (33.3%) of those positives for HBsAg respectively which is contrary to the former study conducted among pregnant women in southern part of Ethiopia in which higher seroprevalence (10%) was observed in 35-40 age group and lower serprevalence (1.9%) was observed in 25-29 age group (Yeshi *et al.,* 2016).

The result is also contrary to the result of the study conducted in Dire Dawa among blood donors in which higher prevalence was observed in 33- 40 age group (Yosef *et al.,* 2016) and the result of the previous study conducted in Jimma in which almost similar seroprevalence was observed in all age groups (Mohammed *et al*., 2005). Besides, in this study age group of the study participants (p=0.031) showed statistical association with serological result for HBsAg. Based on the fact that all the seropositive pregnant women participated in the study were married, it is predictable that at the age levels of 29-34, most of the women become sexually active with great probability of practicing unprotected sex with their spouse. As a result this age related serological result of the study indicated that sexual intercourse mainly in marriage might be the main route of transmission of HBV in the study area which is comparable to the result of the study conducted in Jimma town and its surrounding in which sexual contact and vertical transmission were identified as a means of transmission (Mohammed *et al*., 2005).

Considering unprotected sexual contact as a means of transmission in the study area, the reason why less number of HBV seropositive women was observed in 40 and above age group could be because of small sample from this age group or it could be associated with the fact that HBV is self-limiting and the majorities of individuals infected at adulthood can clear the virus after some time. As a result this study participant could either be infected when she was in age group of $\leq$ 34 or she might have come to marriage and become sexually active later at age of 40 and above.

Although level of education is known as the determining factor for HBV infection which might be due to the level of awareness, according to the finding of the present study, higher seroprevalence among seropositive participants (33.3%) was observed among pregnant women having college and above education level. This could indicate lack of awareness among study participants regardless of their education level. Accordingly, illiteracy and participants income had no association with HBsAg positivity in this study which is contrary to the study conducted among pregnant women in Jimma (Mohammed *et al.,* 2005) and the study conducted among pregnant women in southern part of Ethiopia (Yeshi *et al*.,2016) which showed high HBsAg positivity rate among the illiterate and those with low income. This could be because of relatively better community awareness in in Addis Ababa city about sexually transmitted infection than in Jimma and its surrounding towns.

Regarding the occupation of the study subjects, 3 (33.3%) of the seropositive participants were house wives followed by 2 (22.2 %) merchants and private employee, but only 1(11.1%) was among unemployed pregnant women, with zero seroprevalence among students. This result is contrary to the result of the study reported in southern part of Ethiopia in which higher seroprevalence was reported among daily laborer and students and this could be because of difference in life style of and awareness of the population studied, but in both studies no statistical significance was observed between occupation of the participants and HBV infections. Another study conducted among blood donors in Dire Dawa also reported that higher seroprevalence among government employees or civil servants (Yosef *et al.,* 2016).

Compared to those pregnant women who have been living in Addis Ababa (n=364) in which 8(2.19%) seroprevalence of HBV was observed in the present study, relatively higher seroprevalence for HBsAg 1 (5%) was observed in pregnant women who came from outside of

Addis Ababa city (n=20). This result of the study seems to be an indicator of high seroprevalence of HBsAg outside Addis Ababa city. However, these data are not sufficient enough and further study is required to provide accurate information on this issue.

Although being single is expected to be exposing condition to HBV infection because of a probability to have multiple sexual partner, considering the marital status of participants of the study, all seropositive pregnant women participated in this study were married and no one of the unmarried pregnant women were seropositive for HBV infection.

According to Mohammod *et al*. (2005), pregnant women who experienced abortion had a higher prevalence of HBsAg in Jimma (7.3%). In this study however, abortion is with minimal contribution in transmitting the HBV infection, because 6 (66.7%) of the seropositive pregnant mothers had no history of abortion. This could be because of availability of relatively better health facilities and services in Addis Ababa city than in Jimma city to perform safe abortion.

Although other studies conducted in Ethiopia have reported that blood transfusion (Yohanes *et al.,* 2014), body tattooing (Eke *et al.,* 2011), circumcision (Elsheikh *et al.,* 2007), and surgery; (Walle *et al.,* 2008) were potential risk factors for HBV infection, in this study in general, risk factors such as history of multiple sexual partner, blood transfusion, history of surgery, tooth extraction and implantation, contact with jaundiced person, tattooing, nose and ear piercing, abortion and shaving hair at barberry were not associated with HBV infection (Table 2). This result is comparable with the former study conducted in pregnant women in southern Ethiopia (Yeshi, 2016), but contrary to the study conducted among visitors of Shashemene General Hospital voluntary counseling and testing center in which FGM, tooth extraction, abortion, ear piercing, unsafe injection and hospital admission were significantly associated to HBV infection (Asfaw *et al*., 2011). On the other hand from seropositive pregnant women in the study (n=9), higher ethnic based seroprevalence, 6 (66.7%) was observed in Gurage ethnic group which is against the previous study conducted in Jimma in which zero prevalence was reported among pregnant women from Gurage ethnic group, but higher prevalence among Oromo ethnic groups (Mohammed *et al.,* 2005).

The reason why higher HBV prevalence was observed among pregnant women from Gurage ethnic group in Addis Ababa city could probably be because of the fact that individuals from

this ethnic group are usually hardworking merchants and usually leave their birth place at their earlier ages or some times after marriage and move to bigger cities like Addis Ababa to make money. Most of the time these individuals visit their family once a year during Ethiopian meskel celebration. Until they become economically strong and come to marriage or bring their family or their spouse, they lead solitary life for prolonged time and as a result may be exposed to sexual contact with multiple sexual partners and may finally infect their spouses. However, further study is required to investigate the exact cause of this issue.

6. CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The prevalence of HBsAg among pregnant women in the present study was 2.3%, which is a moderate prevalence according to the WHO criteria. This moderate prevalence implies the need for routine screening of pregnant women in order to identify and treat the infection, so that risk of vertical transmission of the virus is prevented. In this study, except age group and ethnic group of the participants of the study, none of the risk factors included in the study was associated with HBV infection. However, higher age specific and ethnic group specific prevalence of HBV infection was observed, as about 8(88.8%) of the seropositive participants of the study (n=9) were in 25-34 age group. Besides, 6(66.6%) of the seropositive pregnant women were from Gurage ethnic group. This high prevalence of HBV infection in 25-34 age group, where married women most probably practice unprotected sex with their spouse indicates that the main route of HBV transmission in the study area could be sexual contact. However further study is required to clarify why higher seroprevalence for HBV was observed within a single ethnic group.

6.2 Recommendations

Based on the results of the present study, the following recommendations are given:

- Priority has to be given to improve knowledge and awareness among the general population about the cause and ways of the transmission of HBV.
- HBV screening or testing has to be arranged for pregnant women at all health stations to prevent vertical transmission of HBV infection.
- HBV vaccine has to be a part of national vaccination program at all levels of the health facilities in the country and has to be given to all neonates born to HBV infected mothers in medically recommended period of time in order to prevent vertical transmission.
- Pre- marital screening of HBV infection has to be promoted to prevent transmission of the virus through sexual contact.

REFERENCES

Abebe A, Nokes D J., Dejene A., Enquselassie F., Messele T. and Cutts FT. (2003). Seroepidemiology of hepatitis B virus in Addis Ababa, Ethiopia: transmission patterns and vaccine control. *Epidemiol. Infect*. (2003), **131**: 757–770

Abel Girma (2012). Sero-prevalence of HBV and HCV among chronic liver disease patients visiting OPD in public hospitals in Addis Ababa. ISRN 2013(563821):1-7.

Ahizechukwu C. Eke, Uzoamaka A. Eke, Charles I. Okafor, Ifeanyichukwu U. Ezebialu, and Chukwuanugo O. (2011). Prevalence, correlates and pattern of hepatitis B surface antigen in a low resource setting, *Virol J* **8**: 12. doi: 10.1186/1743-422X-8-12

Annemarie W., Deanna K., Wendi K., Edgar P. Simard, Lyn F., Geraldine M., and Beth B. (2010). The Prevalence of Hepatitis B Virus Infection in the United States in the Era of Vaccination. *Jour. Infec Disea* 202 **(2)**:192–201

Asfaw Negero, Zufan Sisay, Girmay Medhin (2011). Prevalence of Hepatitis B surface antigen (HBsAg) among visitors of Shashemene General Hospital voluntary counseling and testing center. *BMC Research Notes 4:35*

Ayele Adato, kabato and Gebru Mulugeta Weldearegay (2016). Prevalence and associated risk factors of Hepatitis B and Hepatitis C virus among volunteer blood donors in Arba Minch Blood Bank SNNPR, Ethiopia; *J.Med Lab and Diag* **7**(4):20-27

Bowden S. (2006). Serological and molecular diagnosis. Semin Liver Dis; **26** (2):97-103.

Angounda B M., Dzia A B., Luc B M., Itoua A C., Ahombo G., Moukassa D., Ibara J., Ennaji MM. (2014). Prevalence of Serologic Markers and Risk Factors for Hepatitis B Virus among Pregnant Women in Brazzaville, Congo. *Int J Sci and Resea* (IJSR) ISSN (Online): 2319-7064

Cohen C., Holmberg SD., McMahon BJ., Block JM., Brosgart CL., Gish RG., London WT., and Block TM.(2010). Is chronic hepatitis B being undertreated in the United States? *Jour Vir Hepat*,doi:10.1111/j.1365-2893.2010.01401.x

Dessie Tegegne, Kassu Desta, Belete Tegbaru and Tesfaye Tilahun (2014). Seroprevalence and transmission of hepatitis B virus among delivering women and their new born in selected health facilities, Addis Ababa, Ethiopia: a cross sectional study. BMC Res Notes **7**:239.

Eke AC., Eke UA., Okafor CI., Ezebialu IU., Ogbuagu C. (2011). Prevalence, correlates and pattern of Hepatitis B surface antigen in a low resource setting. *Virol J* **8**:12.

Elisabetta F., Barbara B., Maria G., Cristina M., Laura S., Laura Z. (2012). Hepatitis B: Epidemiology and prevention in developing countries. *World J Hepatol* **4**(3): 74-80

Magrahe H., Furarah AR., Figih K., Urshfany S., Ghenghesh KS. (2010). Maternal and neonatal seroprevalence of Hepatitis B surface antigen (HBsAg) in Tripoli, Libya. *J Infect Dis* Dev Ctries **4**:168-170.

Elsheikh RM., Daak AA., Elsheikh MA., Karsany MS., Adam I. (2007). Hepatitis B virus and hepatitis C virus in pregnant Sudanese women. *Virol Jour* **4**:104.

Erena and Tefera (2012). Prevalence of hepatitis B surface antigen (HBsAg) and its risk factors among individuals visiting Goba General Hospital, South East Ethiopia. *BMC Research Notes, 7,* 833. http://doi.org/10.1186/1756-0500-7-833

Feld JJ. and Heathcote EJ.(2006). Hepatitis B antigen positive chronic hepatitis B: natural history and treatment Semin Liver Dis **26**(2):116-29.

Goldstein ST., Alter MJ., Williams IT, Moyer L A, Judson F N, Mottram K (2002). Incidence and risk factors for acute hepatitis B in the United States, 1982-1998: implications for vaccination programs. *J Infect Dis* **185**:713-719.

Goldstein ST., Zhou F., Hadler SC., Bell BP., Mast EE., Margolis HS. (2005). A mathematical model to estimate global hepatitis B disease burden and vaccination impact. *Int J Epidemiol* **34**:1329–1339 http://www.who.int/wer

Hwang EW., Cheung R. (2011). Global epidemiology of hepatitis B virus (HBV) infection. *N Am J Med* Sci **4**:7–13.

Nyengidiki TK., Ikimalo JI. (2013). Maternal and neonatal seroprevalence of hepatitis B surface antigen in a hospital based population in South-South, Nigeria. *Int J Med Sci;* **5**(5):241-246

Jinlin H., Zhihua L., Fan G. (2005). Epidemiology and Prevention of Hepatitis B Virus Infection. *Int J Med Sci* **2**(1):50-57

Kefene H., Rapicetta M., Rossi GB. (1988). Ethiopian national hepatitis B study.*J Med Virol*, **24**(1):75-84.

Lavanchy D. (2004). Hepatitis B virus epidemiology, disease burden, treatment, and current and emerging prevention and control measures. *J Viral Hepat* **11**:97-107

Lee C., Gong Y., Brok J., Boxall EH., Gluud C. (2006). Effect of hepatitis B immunization in newborn infants of mothers positive for hepatitis B surface antigen: systematic review and meta-analysis. *BMJ* **332**:328-336.

Kheiri L., Makvandi S. (2015). The Prevalence of Hepatitis B Surface Antigen (HBsAg) and its Influencing factors in Pregnant Women Referring to Healthcare Centers of Dehloran, Iran in 2011-2012. *J Midwife Reprod Health* **3**(3): 424-429.

Melashu Balew, Feleke Moges, Gizachew Yismaw, Chandrashekhar Unakal (2014). Assessment of hepatitis B virus and hepatitis C virus infections and associated risk factors in HIV infected patients at Debretabor hospital, South Gondar, Northwest Ethiopia. *Asian Pac J Trop Dis **4**(1): 1-7*

Mohammed Awole, Solomon Gebre-Selassie (2005). Seroprevalence of HBsAg and its risk factors among pregnant women in Jimma, Southwest Ethiopia. Ethiop *J Health Dev* **19** (1):45-50.

Nima Mahboobi, Stephen Porter R, Peter Karayiannis, Alavian SM. (2012). Oral fluid and hepatitis A, B and C: A literature review. *J Oral Pathol Med* **41**: 505–516.

Sisay Kirba (2014). Seroprevalence of HBsAg among pregnant woman and rate of vertical transmission in 3 teaching hospitals in Addis Ababa.

Thomas F., Robert T., Fritz V. (2007). Pathogenesis of hepatitis B virus infection. *World J Gastroe*; **13**(1): 82-90

Tsega E., Tsega M., Mengesha B. (1988).Transmission of hepatitis B virus infection in Ethiopia with emphasis on the importance of vertical transmission. *Int J Epidemiol* **17** (4):874-9.

Walle Fisseha, Asrat Daniel, Kasu Desta, Alem Atnaf, Tadesse Enyew (2008). Prevalence of hepatitis B surface antigen among pregnant women attending antenatal care service at Debre-Tabor Hospital, Northwest Ethiopia. *Ethiopia J Health Sci* **17**(1)

WHO (2013). Global policy report on the prevention and control of viral hepatitis.WC: WHO member states; p. 536

WHO Global Immunization Data March 2012.

World Gastroenterology Organization Practice Guidelines (2007).Management of acute viral hepatitis.guidelines@worldgastroenterology.org

World Health Organization (2004). Hepatitis B vaccines. Weekly Epidemiological Record **79**: 255-263.

World Health Organization (2004).Weekly epidemiological record **79**(28):253–264

World Health Organization (2012). Hepatitis B fact sheet No. 204. Available from: http:/www.who.int/mediacentre/factsheets/fs204/en.

World Health Organization (2017). Weekly epidemiological record **92**(27):369–392. Available from: http://www.who.int/wer

World Health Organization. Emergencies preparedness, response: hepatitis: frequently asked questions. Available from: http://www.who.int/csr/disease/hepatitis/world_hepatitis_day/question_answer/en/

Xu WM., Cui CY., Wang L. (2004). Efficacy and safety of Lamivudine in late pregnancy for prevention of mother to child transmission of Hepatitis B; a multi-center randomized, double blind, placebo controlled trial. *J Hepto* **40**:272A

Xu DZ., Yan YP., Zou, ShS., Wang X. (2001). Role of placental tissues in intrauterine transmission of hepatitis B virus. *Am J Obstet Gynecol* **185**:981–987.

Yeshambel Belyhun, Melanie Maier, Andargachew Mulu, Ermias Diro and Uwe Gerd Liebert (2016).Hepatitis viruses in Ethiopia: a systematic review and meta-analysis. *BMC Infectious Diseases 16:761. DOI 10.1186/s12879-016-2090-1*

Yeshi Metaferia, Walelign Dessie, Ibrahim Ali, and Anteneh Amsalu (2016). Seroprevalence and associated risk factors of hepatitis B virus among pregnant women in southern Ethiopia: a hospital-based cross-sectional study: Epidemiology and Health. 38: Article ID: e2016027, 7 pages http://dx.doi.org/10.4178/epih.e201602

Yohannes Zenebe, Wondemagegn Mulu, Mulat Yimer and Bayeh Abera (2014). Sero-prevalence and risk factors of hepatitis B virus and human immunodefciency virus infection among pregnant women in Bahir Dar city, Northwest Ethiopia: a cross sectional study. BMC Infect Dis 14:118.

Yosef Habte, Berhanu Seyoum and Tadesse Alemayehu (2016). Hepatitis B Virus Infection and Associated Factors among Blood Donors at Dire Dawa, Eastern Ethiopia. *J Antivir Antiretrovir* **8**: 103-106.

Zhou JJ., Wu WS., Sun CM., Dai HQ., Zhou N., Liu CB. (2003). Long - term evaluation of immune efficacy among newborns 16 Years after HBV vaccination. *Chinese Journ Vacci and Immun*.**9**:129-133.

Zhu Q., Yu G., Yu H., Lu Q., Gu X., Dong Z. (2003). A randomized control trial on interruption of HBV transmission in uterus. *Chin Med J* (Engl)**116**:685-687

ANNEXES

Annex -1 Structured Questionnaire

Questionnaire for the Investigation of sereo-prevalence and Associated Risk Factors of HBV infections among pregnant women in Gandhi Memorial Hospitals in Addis Ababa, Ethiopia.

For data collectors: For each question, please put a cross inside one box .In case of any mistake; you can simply cross out the mistake and put a cross in the correct box.

Date: ……. Participant Code……

I. Socio demogrhaphic information

1. Age: A. 15-19 ☐ B.20-24 ☐ C.25-29 ☐ D. 30-34 ☐ E. 35-39 ☐ F.>40 ☐

2. Marital status: A. Single ☐ B. Married ☐ C. Separated ☐ D.Widowed ☐

3.Educational status

A. No formal education ☐ B. Primary School ☐

C. Secondary school ☐ D. College &above ☐

4. Occupation: A. Government employee ☐ B. Private Employee ☐ C.Unemployed ☐

D.Student ☐ E. Merchant ☐ F. House wife ☐

5.Residence A. Addis Ababa ☐ B.Outside Addis ☐

6. Monthly income in Eth. Birr A. <1000 ☐ B. 1000-2000 ☐ C. >2000 ☐

7. Do you have your own home? A.Yes ☐ B.No ☐

II. Risk factors for HBV

1 History of having multiple sexual partner in life A. Yes ☐ B. No ☐

2. History of blood transfusion A. Yes ☐ B. No ☐

3. If your answer to the above is yes, A. Donated ☐ B. Received ☐

4 .History of Tattooing A.Yes ☐ B. No ☐

5. History of abortion A. Yes ☐ B. No ☐

5.1 if your answer to the above is yes, where did you perform it.

A. at health station ☐ B. by private body ☐

6. History of FGM A. Yes ☐ B. No ☐

7. History of tooth extraction and implantation yes ☐ No ☐

8. History of surgery Yes ☐ No ☐

9. History of contact with jaundiced patients Yes ☐ No ☐

10. History of shaving by barbers Yes ☐ No ☐

11. History of noes piercing Yes ☐ No ☐

12 History of ear piercing A.Yes ☐ B. No ☐

13. Ethnic group A. Oromo ☐ B. Amhara ☐ C. Tigre ☐

D. Gurage ☐ E. Others ☐

14. Serology for HBsAg: A. Positive ☐ B. Negative ☐

ለመረጃ ስብሳቢዎች ፤ ጥያቄዉን ከጠየቃችሁ በኃላ መልሱን በተሰጠዉ ሳጥን ውስጥ ከተሰጡት አማራጮች አንዱን የክኤስ ወይም የራይት ምልክት ይጻፉ ፡፡

ቀን ..... መለያ ቁጥር.....

I. ጠቅላላ ጥያቄዎች

1.እድሜ: ሀ. 15-19 ☐ ለ.20-24 ☐ ሐ.25-29 ☐ መ. 30-34 ☐ ሠ. 35-39 ☐ ረ.>40 ☐

2. የጋብቻ ሁኔታ: ሀ.ያገባች ☐ ለ. ያላገባች ☐ ሐ. የፈታች ☐ መ. በሞትየተለየች ☐

3.የትምህርት ደረጃ ሀ. ያልተማረች ☐ ለ. 1-5☐ ሐ. 5-8 ☐ መ. 9-10 ☐

ሠ.11-12 ☐ ረ. ኮሌጅ እና ከዝያ በላይ ☐

4. የስራ ሁኔታ: ሀ. የመንግስት ☐ ለ. የግል ተቀጣሪ ☐ ሐ. ስራ የሌላት ☐

መ.ተማሪ ☐ ሠ. ነጋዴ ☐ ረ. የቤት እመቤት ☐

5.አድራሻ ሀ. አዲስ አበባ ☐ ለ. ከአዲስ አበባ ውጭ ☐

6. የወር ገቢ ሀ. <1000 ☐ ሐ. 1000-2000 ☐ መ. >2000 ☐

7. የግል መኖርያ ቤት ሀ.አለኝ ☐ ለ. የለኝም ☐

II. ለሄፓታይተስ ቢ ቫይረስ አጋላጭ ሁኔታዎች

1 ከአሁኑ የፍቅር ጓደኛዎ በፊት ከሌላ ሰው ጋር የፍቅር ግንኙነት ነበረዎት

ሀ. አዎ ☐ የለኝም ☐

2. ደም ለግሰው /ተለግሰው ያውቃሉ ሀ. አዎ ☐ ለ. አይደለም ☐

3. መልስዎ አዎ ከሆለ ሀ. ለገሼ አውቃለሁ ☐ ለ. ተለግሼ አውቃለሁ ☐

4 .ጥርስዎን /ሰውነትዎን ተነቅሰው ያውቃሉ ሀ. አዎ ☐ ለ. አላውቅም ☐

5. ጽንስ አቋርጠው/ አሰወርደው ያውቃሉ ሀ. አዎ ☐ B. አላውቅም ☐

5.1 መልስዎ አዎ ከሆነ, ዬት ነበር ያስወረዱት.

ሀ. በጤና ተቋም ☐ ለ. ከጤና ተቋም ውጪ ☐

6. የሴተ ልጅ ግርዛት ተፈጽሞብዎታል ሀ. አዎ ☐ B. አይደለም ☐

7. ጥርስ አስነቅለው/አስተክለው ያውቃሉ ሀ.አዎ ☐ ለ. አላውቅም ☐

8. ቀዶ ህክምና ተደርጎሎት ያውቃል ሀ.አዎ ☐ ለ.አያውቅም ☐

9. የወፍ በሽታ ካለበት ሰው ጋር ነክኪ ነኖሮት ያውቃል? ሀ.አዎ ☐ ለ.አያውቅም ☐

10. የጸጉር ቤት ቶንዶስ ተጠቅመው ያውቃሉ? ሀ. አዎ ☐ ለ.አላውቅም ☐

11. አፍንጫን የመበሳት ታሪክ ሀ. አለኝ ☐ ለ. የለኝም ☐

12 ጆሮን የመበሳት ታሪክ ሀ.አለኝ ☐ ለ.የለኝም ☐

13. ብሄር ሀ. ኦሮሞ ☐ ለ.አማራ ☐ ሐ. ትግሬ ☐ መ. ጉራጌ ☐ ሠ.ሌላ ☐

በላቦራቶሪ ባለሙያ ብቻ የሚሞላ

14. Serology for HBsAg: A. Positive ☐ B. Negative ☐

Annex- II. Information sheet of the study

Purpose: We are studying the prevalence and associated risk factors of HBV infections in pregnant women in Gandi Memorial Hospital in Addis Ababa. Hepatitis virus is the major cause of acute and chronic hepatitis and that patients with acute infection may not develop symptoms in most cases and serve as a source of infection. HBV infection is a contagious disease which may transmit vertically from the mother to the newborn or horizontally by means of blood products and body secretions. mother-to-child transmission is one of the most important modes of transmission, associated with many adverse effects. Early detection of the affected prevents vertical transmission and complications related to the infection of HBV. The objective of this study is to determine the prevalence of HBV infection in pregnant women and associated risk factors.

Safety: There is no safety problem related to sample collection, because the blood samples are collected using sterile materials which will be disposed after a single use.

Potential risk: there is no significant risk to you actually, because only a maximum of 2ml of venous blood is taken and this doesn't have any harm to your health except minor needle brick injury pain which lasts only for micro second. However if you have any discomfort you will be seen by physician.

Benefits: If the participant of the study is positive for HBV during investigation, opportunities for treatment will be arranged dealing with the Hospital care givers and she will be followed. If she is negative for HBV, she will be a counseling service for future care. If there is an access, vaccination for HBV will be given to prevent future HBV infection by dealing with the concerned body of the hospital.

Confidentiality: Any information that will be collected from participants of the study during this study will be kept confidential and only the principal investigator of the study will be able to link your identity with the code number.

Consent: Written consent will be obtained from the participant and data will be collected confidentially. No name of the respondents will be written on the questionnaire.

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

Annex-III. Consent form

I have been requested to participate in a research project that aims to determine the prevalence and associated risk factors of Hepatitis B virus infections among pregnant women in Gandi Memorial Hospital in Addis Ababa. I have been informed that all information I will be giving will be kept confidential. The aim and objectives of the study are sufficiently clear to me. I have not been pressurized to participate in any way. I understand that participation in this study is completely voluntary and that I may withdraw from it at any time without supplying reasons. I am fully aware that the results of this Study will be used for scientific purposes and may be published. I agree to this, provided that my privacy is guaranteed. I hereby give my consent for giving of blood sample and to fill the questionnaire. I confirm my agreement by putting my signature below.

Full Name: _____

Signature: _____

Date:_____

ቀን.....

መለያ ቁጥር.....

እኔ ስሜ ከዚህ በታች ተገለጸው ታካሚ በጋንዲ መታሰብያ ሆስፒታል በነፍሰጡር ሴቶች ላይ የሄፓታይቲስ ቢ እንፌክሽንን ስርጨት ለማሳየት በሚካሄድ ጥናት ላይ በቃለመጠይቅ እና የደም ናሙና በመስጠት እንድሳተፍ የተጠየቅሁ ሲሆን በዚህ ጥናት ላይ የምሰጠው እንፎርሜሽን እና የምርመራ ዉጤቱ ሚስጢራዊነት በአግባቡ የሚጠበቅ መሆኑ እና በዚህ ጥናት ላይ መሳተፍ በእኔ ሙሉ ፈቃደኝነት የሚወሰን መሆኑ በዚሁም መሰረት በማንኛውም ጊዜ ያለምንም ጥያቄ ከጥናቱ እራሴን ማውጣት እንደምችል በአግባቡ ተረድቼአለሁ፡፡

በተጨማሪም የዚህ ጥናት ዉጤት የግል ሚስጥርን በጠበቀ ሁኔታ ለሳይንሳዊ ጥናት ሊዉል እንደሚችል እንዲሁም በተለያዩ ሳይንሳዊ ፅሁፎች ላይ ታትሞ ሊወጣ እንደሚችል በአግባቡ ተረድቼ እንደዚሁም የጥናቱንም ዓላማ በአግባቡ በመገንዘብ ያለምንም ተፅዕኖ ቃለመጠየቆችን ለመሙላት እና የደም ናሙና ለመስጠት በሙሉ ፈቃደኝነት የተስማማሁ መሆኔን በፊርማዬ አረጋግጣለሁ

ሙሉ ስም .....

ፊርማ .....

Annex- IV. Ethical clearance

አዳማ ሳይንስና ቴክኖሎጂ ዩኒቨርስቲ
ADAMA SCIENCE AND TECHNOLOGY UNIVERSITY

✉1888 Phone: +251222110025 E-mail: geach.jon@gmail.com
Adama Science and Technology University

አዳማ - ኢትዮጵያ
Adama, Ethiopia

ቀን 18/07/09
ቁጥር:Bio/883/009/09

አፕላይድ ተፈጥሮሳይንስ ት/ቤት
አፕላይድ ባዮሎጂ መርሀ ግብር ኃላፊ

ለአዲስ አበባ ጤና ቢሮ
አዲስ አበባ

ጌታቸዉ ባንቲሁን

ጉዳዩ- የኢቲካል ክሊራንስ መጠየቅን መለከታል

ተማሪ ዘመኑ አበራ በአዳማ ሳይንስና ቴክኖሎጂ ዩኒቨርሲቲ በአፕላይድ ባዮሎጂ መርሃ ግብር በክረምት ፕሮግራም የሁለተኛ ዲግሪውን የመመረቂያ ፅሁፍ Assessement of Seropervalence and Associated Risk Factors of Hepatitis B surface Antigen among pregnant women in Gandi Memorial Hospital in Addis Ababa በሚል ርዕስ የፀደቀለት ሲሆን ፤ መረጃ ለመሰብሰብ ይረዳው ዘንድ የኢቲካል - ክሊራንስ እንዲሰጠው አስፈላጊውን ትብብር እንዲደረግለት በአክብሮት እንጠይቃለን ::

ለሰላምታ ጋር

ጌታቸው ባንቲሁን Getachew Bantihun Belay
አፕላይድ ባዮሎጂ መርሀ ግብር
Applied Biology Program
Chairperson

ተማሪ ዘመኑ አበራ

አዲስ አበባ ከተማ አስተዳደር ጤና ቢሮ
City Government of Addis Ababa Health Bureau

Ref. No AAHB 5869/22
Date 11/8/09

- Gahandi Memorial Hospital

Subject: Request to access health facilities to conduct approved research

This letter is to support **Zemenu Abera** to conduct research, which is entitled as **"Assessment on Seroprevalence and Associated Risk factors of Hepatitis B Surface Antigen Among Pregnant Women in Gahandi Memorial Hospital in Addis Ababa"** The study proposal was duly reviewed and approved by Addis Ababa Health Bureau ERC, and the principal investigator is informed with a copy of this letter to report us if any changes in the study procedures and submit an activity progress report to the Ethical Committee as required.

Therefore, we request your office and staffs to provide support to the Principal investigator.

With Regards

Kasayenew Amare

Ethical Clearance committee

The Addis Ababa City Administration Health Bureau

CC

- **Zemenu Abera**
- **Ethical Clearance Committee**
  Addis Ababa