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SERO - PREVALENCE AND ASSOCIATED RISK FACTORS OF HEPATITIS B VIRUS AMOUNG PREGANAT WOMEN IN HAWASSA UNIVERSITY TEACHING AND REFERRAL HOSPITAL.

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ABBREVIATIONS

ANC	Antenatal care
CDC	Center for Disease Control and Prevention
CI	Confidence intervals
DNA	Deoxyribonucleic acid
ELISA	Enzyme-linked immunosorbent assay
HBcAg	Hepatitis B core antigen
HBeAg	Hepatitis B e Antigen
HBIG	Hepatitis B Immunoglobulin
HBsAg	Hepatitis B surface Antigen
HIV	Human immunodeficiency virus
HRP	Horseradish peroxidase
HUTRH	Hawassa University Teaching and Referral Hospital
IOM	Institute of Medicine
IRB	Institutional review board
OD	Optical density
OR	Odds ratio
RPM	Revolution per minute
SNNPR	South Nation Nationalities Peoples of Representatives
TEB	Tetramethylbenzidine
WHO	World Health Organization

ABSTRACT

Background: Hepatitis B virus (HBV) is a public health problem worldwide. It is highly endemic in Asia and Sub-Saharan Africa. Even though there was an introduction of universal infants HBV immunization in 2007,distribution of HBV remains a public health problem in Ethiopia.

Objective: To determine the sero-prevalence of HBsAg among pregnant women and to identify potential risk factors associated with the infection.

Methods: A cross-sectional study was conducted from April-May, 2015 among pregnant women attending the antenatal clinic (ANC) of Hawassa University Teaching and Referral Hospital. After obtaining written and informed consent, blood sample was collected from 269 pregnant women using consecutive non- duplicative sampling method. Serum was separated from whole blood and tested for HBsAg using Bioline strip test and enzyme-linked immunosorbent assay (ELISA) method for further testing**.** Data was collected using pre-structured questionnaire and analyzed using SPSS version 21. Chi-square and bivariate logistic regression analysis was used to determine the association between explanatory variables and the outcome variables. The result was considered statistically significant at $p < 0.05$.

Result: In this study, 269 pregnant women were participated. The mean age was 26.0 years (standard deviation (SD), 4.5; range, 16 –39 years) and the majority of study participants live in urban 226(84%). The overall prevalence of sero-positive HBsAg among pregnant women was 21(7.8%). The HBV/HIV co-infection rate was 2/14(14.2%). Of the studied risk factors only educational status [($\chi 2$= 8.1; p- value=0.044)] had significant association with HBV infection. There was no statistically significant association between history of blood transfusion, multiple sexual practices, hospital admission, genital mutilation, surgical procedure, body tattooing history of abortion and HBV infection.

Conclusion: The highest prevalence of HBV infection was detected as compared to the previous studies in different region of Ethiopia. Hence, screening of pregnant women for HBV irrespective of basis of risk factor may reduce exposure to HBV infection.

Key words: Hepatitis B virus, Risk factors, Pregnant women, Hawassa

1. INTRODUCTION

1.1. Background

Hepatitis B virus (HBV) is an envelope virus with a viral genome of partially double stranded circular DNA which belongs to the family *Hepadnaviridae*[1, 2].

HBV causes acute and chronic infections of the liver. It is a major cause of chronic hepatitis, cirrhosis, and hepatocellular carcinoma. Due to its largely asymptomatic nature, viral hepatitis is a silent epidemic; most people are unaware of their infection [3]. Infections by HBV in pregnancy come with its attendant effect on both mother and child [4]. It has been reported that 10-20% of HBsAg positive pregnant women transmit the virus to their babies and women, who are positive for both HBsAg and HBeAg, have a chance of transmitting HBV to their newborns at birth nearly 100%. Up to 90% of the newborns born to these mothers go on to develop chronic hepatitis B if they do not receive hepatitis B immune globulin and hepatitis B vaccine at birth [5]. Although this means of transmission has not been reported to be teratogenic, a higher incidence of low birth weight, low intelligence quotient, liver cirrhosis and hepatocellular carcinoma in young adulthood may result [2].

HBV is not directly cytotoxic to hepatocytes but severity of hepatocellular injury is modulated by the strength of host immune responses [6, 7]. The dynamic balance between viral replication and host immune response plays a key role in the pathogenesis of liver disease from HBV infection [8]. There are three possible routes of transmission of HBV from infected mothers to infants: transplacental transmission of HBV in utero, natal transmission during delivery or postnatal transmission during care of infant or through breast milk. In patients with acute hepatitis B infection vertical transmission occurs in up to 10% of neonates when infection occurs in the first trimester and in 80 -90% of neonates when acute infection occurs in the third trimester [9]. Chronic HBV infection during pregnancy is usually mild but may flare after delivery or with discontinuing therapy [10]. The sero-markers and bio-markers associated with HBV infection include HBsAg, anti-HBs, HBeAg ,anti-HBc, IgM-anti- HBc and IgG-anti- HBc.

At least one serologic marker is present during the different phases of HBV infection. The presence of a confirmed HBsAg result is indicative of ongoing HBV infection, so all HBsAg-positive persons should be considered infectious. In newly infected persons, HBsAg is the only serologic marker detected during the first 3–5 weeks after infection, and it persists for variable periods at very low levels. The average time from exposure to detection of HBsAg is 30 days (range: 6–60 days). Chronic HBV infection occurs when HBsAg persists for > 6th months in the presence of HBeAg or anti-HBc or detection of IgG-anti- HBc, where as acute HBV infection occurs within 6th months of infection (detection of IgM-anti- HBc) [11].

Management of chronic HBV infection in pregnancy is mostly supportive with antiviral medications indicated in a small subset of HBV infected women with rapidly progressive chronic liver disease. Because of the high risk of developing chronic HBV among infant born to HBsAg positive mother, administration of Hepatitis B Immunoglobulin (HBIG) in combination with hepatitis B vaccines as post exposure prophylaxis is very important [12].

1.2. Statement of the problem

Hepatitis B virus (HBV) infection is a challenging global health problem [13]. According to recent WHO report, an estimated of more than 2 billion people have been infected with HBV. Of these, approximately 350 million are chronically infected and at risk of serious illness and death from cirrhosis and hepatocellular carcinoma (HCC), diseases that are estimated to cause 1million deaths each year worldwide [3]. In the continents of Africa and Asia, it remains a major cause of morbidity and mortality [13]. The WHO considers HBV second to tobacco among the carcinogens causing malignancy [14]. The prevalence of chronic HBV infection varies widely according to geographical area, and is closely interlinked with the predominant routes of HBV transmission [15]. Vertical transmission of HBV infection is thought to be a major route of transmission in low resource areas [16]. A recent study conducted in Ethiopia showed moderate endemicity (2-8%) of HBV among pregnant women [17, 18, 19, 20, 21].

HBV infection during pregnancy is closely related to high risks of maternal complications including: premature contractions, placenta praevia, preterm delivery, placental separation,

premature rupture of membranes, vaginal bleeding, preterm labor, gestational diabetes mellitus and mortality with a high rate of vertical transmission leading to fetal and neonatal hepatitis [22]. The fatality rate among persons with reported acute hepatitis B is 0.5%–1.5%, with highest rates in adults aged >60 years. Although the consequences of acute hepatitis B can be severe, the majority of serious sequelae associated with HBV disease occur in persons who are chronically infected. Primary infections also become chronic and more frequently seen in immunosuppressed persons (e.g., hemodialysis patients persons with human immunodeficiency virus (HIV) infection and pregnant women) [23]. Several prognostic factors, such as serum HBV DNA concentrations, HBeAg status, serum aminotransferases, and certain HBV genotypes, have been identified to predict long-term outcome such as cirrhosis and HCC [8]. However diagnosis of this biomarker is difficult in a resource limited setting like Ethiopia. So early screening using serological marker and preventing transmission is very essential. The epidemiology of the virus may vary from region to region and even with time. In spite of this, routine antenatal screening for HBV infection and vaccination is lacking in many Ethiopian health facilities. Therefore, assessing the prevalence of HBV infection in pregnant women becomes most important, because pregnant women can represent the majorities of the population in the communities and also pregnant women's are the interface for their sexual partners and infants. At the same time, individuals who have chronic infection serve as the major reservoir for continued HBV transmission [24]. Most people who were infected long ago with HBV are unaware of their chronic infection. They are at high risk of developing severe chronic liver disease and can unknowingly transmit the infection to their child. Individuals who have chronic infection will receive appropriate medical management and those who are not infected with HBV will be vaccinated [13, 23].

1.3. Significance of the study

As far as our knowledge is concerned there is no published data in the study area. Therefore, the aim of this study is to investigate the sero-prevalence and associated risk factors for markers of HBV (HBsAg) among pregnant women at Hawassa University Teaching and Referral Hospital (HUTRH) ANC clinic. Such data are fundamental for health planers and care givers for evidence-based intervention, creating awareness for the health professionals working in ANC clinic and baseline information for further large scale study.

2. LITERATURE REVIEW

2.1. Prevalence and Risk factors for Hepatitis B virus

HBV occurs worldwide and constitutes a serious public health problem. It is predominantly a problem in resource-limited countries and its prevalence varies markedly in different geographic areas of the world, as well as in different population subgroups [25]. It ranges over 10% in some Asian, Western Pacific and sub-Sahara African countries to under 0.5% in the United States and northern European countries. Overall, approximately 45% of the global populations live in areas of high chronic HBV prevalence. The prevalence of chronic HBV infection worldwide could be categorized as high (>8%), intermediate (2-8%) and low endemicity (<2%) [26].

It is estimated that 2 billion people worldwide have been affected, of which 350 million people have chronic infection, and 10% of these are in sub-Saharan Africa and East Asia [14]. These chronically infected ones may develop liver cirrhosis or HCC. Mother to child transmission is one of the major modes of spread of HBV [16]. The reported increase in HBV prevalence over time and the influence of HIV substantiated the need for updated data in order to adopt the WHO recommendations of screening pregnant women and offering at birth-dose of HBV vaccine to prevent perinatal transmission [14,27, 28]. HBV-infected pregnant women are at risk of infecting their babies with a consequence of developing fulminant HBV infection especially when pregnant women are HBsAg positive [29], and more so when HBeAg is also positive. For this purpose, data about prevalence rates are needed, especially in populations at risk for disease transmission, such as pregnant women.

A studies conducted among pregnant women in different part of the world showed that a HBsAg prevalence of 0.9% among 541 in Brazil [30], 6.71% among 6398 in China [31], 2.1% among 2654 in Northern Turkey[2], 1.6% among 755 in Saudi Arabia[32], 6.67% among 180 in Nigeria[4], 12.6% among 1368 in Ghana[33], 1.5% among 1,500 in Libya[12], 8% among 293 in Mali[34], 11.8% among 397 Uganda [35], 10.8% among 400 in Yemen[36], 5.6% among 423 in Sudanese [37].

A study from Ethiopia, overall seroprevalence of HBsAg in pregnant women attending Debre-Tabor Hospital, South Gondar was 5.3% [17], in rural Southern Ethiopia 6.1% [18], in Bahir Dar 3.8% [19], in Addis Ababa (3%) [20], and in Jimma 3.7% [21]. This shows an intermediate endemicity of HBV infection in Ethiopia according to WHO criteria [26]. In Jimma investigation, when age specific prevalence was considered, age group 16-22 had a higher prevalence (75.9%), and there was a tendency of declining in the prevalence in the remaining age groups, even though there was no statistical significant difference across various age groups [21]. This finding is in contrast to HBV infection prevalence, which normally shows a linear rise with age reported by other investigators in pregnant women and in other studies [30]. Such discrepancy could be due to the differences in study design, population and geographical area [30].

The differenent demographic characteristics of the study population such as; socio-cultural environment, tribal practices, traditional operation, sexual practices were considered in different studies [17,19 20, 21,30,31,32]. Risk factors, including, use of sharp materials, hypodermic needles and practice of tattoo for cosmetics were associated with HBV infection. Factors that were described to lead a relatively higher sero-prevalence in Kenya included low socio-economic status and female genital mutilation [38] .In Nigeria, high parity, polygamy, multiple sexual partners and previous history of sexually transmitted diseases have been shown to be among the significant risk factors for HBV infection in pregnant women [29].

In a study done at the Muhimbili National Hospital (MNH) in Tanzania, the sero-prevalence of HBV showed no association with marital status, previous history of jaundice, history of blood transfusion and age [39] .Studies conducted at São Luís, Maranhão in Brazil was observed that only family history of hepatitis and low level of education was positively associated. Family history could be explained by the possibility that some patients have family members with HBV infection, who could have transmitted the disease to them and then cleared the virus (remaining the markers of contact). Such occurrence is frequent after five years of age, which already clearly shown in some regions of the world, including Brazil [30].Another explanation could be the possibility of episodes of acute infection in sexual partners, who may have transmitted the disease, with subsequent progression to cure. The level of education, probably a surrogate of low socioeconomic status, reflects poor knowledge about prevention as well as poor access to health

services, which lead to greater susceptibility to infection. This study demonstrated the low prevalence of chronic HBV infection in pregnant women assisted in public maternity hospitals of São Luís. The presence of serological markers for HBV infection was associated with lower educational levels and family history of hepatitis. These results confirm that investments in education are important for the prevention of most diseases [30].

In a previous study conducted in Ethiopia, a number of risk factors such as dental procedure, caesarean section ,tattooing , needle stick injury, sharp injury, and occupation (among health professionals) [17] and absence of vaccination confirmed that HBV infection had statistically significant association. The socio-demographic status of the study population shows that a high proportion of HBsAg positivity was among the illiterate 11/18(61%) [17].

3. OBJECTIVE

3.1. General objective

- ✓ To assess the sero-prevalence and associated risk factor of HBV among pregnant women at Hawassa University Teaching and Referral Hospital ANC clinic from April –May, 2015.

3.2. Specific objective

- ✓ To determine the sero-prevalence of HBV in pregnant women at Hawassa University Teaching and Referral Hospital ANC clinic.

- ✓ To identify associated risk factor related to HBV among pregnant women at Hawassa University Teaching and Referral Hospital ANC clinic.

3.3. Hypothesis

- Prevalence of HBV among pregnant women in this study area is similar to other study areas in Ethiopia.

4. METHODS AND MATERIALS

4.1.Study Area and period

Study was conducted at HUTRH, which is located in Hawassa city administration, SNNPR, Ethiopia. Hawassa has a has total of an estimated population of 328,283 out of those 168,886 male and 159,393 female [40] and has more than 50 different ethnic groups. Each ethnic group has their own composition of tribes with distinctive language, custom, traditional beliefs and cultural diversity. HUTRH is a tertiary level teaching hospital that provides health service over 6 million inhabitants in southern Ethiopia. It has around 68 staff members including Laboratories Nurses/Midwifes and physicians. The hospital ANC clinic gives services for more 20 than pregnant women per day, Monday through Friday and it has 56 bed rooms to serve pregnant women. It is located 275 Km south from the capital city, Addis Ababa. The study was conducted from April - May 2015.

4.2.Study design

A cross sectional study was conducted among pregnant women attending HUTRH ANC clinic.

4.3.Population

4.3.1. Source population

The source population was all women visiting ANC clinic of HUTRH.

4.3.2. Study population

The study population was all pregnant women who signed and informed a consent form.

4.4.Eligibility

4.4.1. Inclusion criteria

- ✓ All pregnant women whose pregnancy is confirmed by clinical history and examination or an obstetric ultrasound scan and volunteer to participate in the study.

4.4.2. Exclusion criteria

- ✓ Pregnant women who are critically sick and unable answer the questionnaire.

4.5.Study Variables

4.5.1. Dependent variable: - Sero-prevalence of Hepatitis B virus

4.5.2. Independent Variables

Maternal age	Residence	Occupational status
Educational status	Hospital admission	Any surgical procedure
Place of previous birth	Gestational age	Body tattooing
Genital mutilation	History of blood transfusion	
History of multiple sexual practices		

4.6. Sample size and sampling technique

The sample size was calculated based on single sample size estimation. The prevalence rate (p= 6.1%) taken from a previous study conducted in a rural hospital in Southern Ethiopia [18] with the precision of 3% because when prevalence of the disease is going to be below 10% or more than 90% , d taken as a half of p [41] and non-duplicative consecutive convenient sampling technique was used. From this, a sample size was calculated as;

$$\mathbf{N= Z^2 \alpha/_2 p\,(1-p)} \qquad d^2$$

$$N= \frac{(1.96)^2 x\ 0.061\ (1-0.061)}{0.0009}$$

$$N = \frac{3.8416 x 0.057}{0.0009} \qquad N = 244.45 \sim 245$$

By assuming 10% non –response rate, the final sample size is calculated as:

$$Nf = \frac{nx10}{100} + 245$$

$$Nf = \frac{245 \times 10}{100} + 245$$

$$Nf= 269$$

Whereas **n** = required sample size

$\mathbf{Z\alpha/_2^2}$ = critical value for normal distribution at 95% confidence interval

Which equals to 1.96 (Z value at alpha=0.05).

P = proportion of prevalence HBV on pregnant women

$\mathbf{d^2}$ = marginal error= 3% and **Nf** = final sample size

4.7. Data collection method

4.7.1. Enrollment and data collection procedures

After obtaining an informed and written consent, a pre-tested structured questionnaire was delivered to eligible pregnant woman for interview to obtain socio-demographic information including maternal age, gestation age, occupation, residence, highest level of education and other information on risk factors for transmission of HBV, including a history of previous blood transfusions, Genital mutilation, place of previous birth, any surgical procedure, tattooing, and hospital admission by trained midwives nurse.

4.7.2. Specimen Collection and Processing

5 ml of venous blood was drawn under aseptic conditions in disposable vacuntainer tubes by experienced laboratory personnel. These tubes was labeled and processed at the time of collection. The blood samples taken from the participants was centrifuged at 3000 revolution per minute (RPM) for at least 10 minutes at room temperature. The rapid test was performed to deliver the result of the pregnant women at the time of screening. The leftover serum was separated and collected in eppendorf tubes, stored at -20°C at HUTRH and transported to regional blood bank for performing ELISA test.

4.7.3. Laboratory Testing Methods

Sample testing for HBsAg was done using ;

Bioline strip test - has sensitivity and specificity of greater than 99%. It is a qualitative, solid phase, two-site sandwich immunoassay for the detection of HBsAg in serum or plasma. The membrane is pre-coated with anti-HBsAg antibodies on the test band region and anti-mouse antibodies on the control band region. During testing, the serum sample reacts with the dye conjugate (mouse anti HBsAg antibody colloidal gold conjugate) that will be coated in the test strip. The mixture then by capillary action reacts with anti-HBsAg antibodies on the membrane and generates a red band. Presence of this red band indicates a positive result while its absence indicates a negative result.

ELISA kits, (DIALAB, Wiener Neudorf, Austria) which has a sensitivity of 100% and specificity of 99.87% [42] and uses antibody sandwich ELISA method in which polystyrene microwell strips are pre-coated with monoclonal antibodies specific to HBsAg. Patient's serum or plasma sample added to the microwells together with a second antibody conjugated with horseradish peroxidase (HRP) and formed in case of presence of HBsAg in the sample, is captured on the solid phase. The amount of color can be measured and is proportional to the amount of antigen in the sample. Wells containing samples negative for HBsAg remain colorless. Every procedure was followed according to manufacture instruction and HBsAg rapid test was performed first and then HBsAg ELISA. Positive results by HBsAg ELISA was repeated to report .

4.8.Quality Assurance

To make sure that the questionnaire was appropriate and understandable, it was pretested on 20 pregnant women at Adare hospital other than the actual study sites to avoid redundancy. The collected data were checked daily for consistency and accuracy. Standardized procedures were strictly followed during blood sample collection, storage and analytical process. Positive and negative controls were run alongside of the test.

4.9.Data analysis

Data was entered and analyzed using SPSS version 21. Chi-square and bivariate logistic regression analysis was used to determine the association between explanatory variables and the outcome variables. Odds ratio (OR) and their 95% confidence intervals (CI) calculated and the result was considered statistically significant at $p < 0.05$.

4.10. Ethical Consideration

Ethical approval of this study was reviewed and approved by the "Department of Research and Ethical Review Committee" of the Medical Laboratory Sciences, School of Allied Health Sciences, College of Health Sciences, and Addis Ababa University. The ethical letter was submitted to Hawassa University College of medicine and health science institutional review board (IRB) then support letter was obtained from Hospital administration. The information sheet and consent form, purpose and importance of the study explained to each study participants. To ensure confidentiality of participant's information, codes was used where by the name of the participant and any identifier of participants was not be written on the questionnaire. Participant was interviewed alone to keep the privacy. All participants not pay for test. Voluntary Participation clearly stated that they can choose to participate or not; and they can still receive all the services they usually do if they choose not to participate. Test results were given to the clinicians who are working on ANC clinic of the Hospital for further diagnosis and management.

5. RESULTS

5.1. Socio-demographic characteristics

In this study, 269 pregnant women were participated. The mean age was 26.0 years (standard deviation (SD), 4.5; range, 16 –39 years), and substantial number (39.4%) were in the age category 30–39 years. Two hundred twenty six (84.0%) were urban in residence. The majority of the study participants were housewives 106 (39.4%) followed by employed 98 (36.4%) and merchants 38 (14.1%). Around half (47.2%) of the women had tertiary educational level while 31(11.5%) were illiterate. With regard to place of giving birth and gestational age, 102(37.9%) were under 3rd tri-minister, 113(42.0%) gives delivery at hospital. There was no significant difference in the frequency of HBsAg for the study participant's age, residence, occupation, ethnicity, religion, gestational age, and place of previous delivery. However, the frequency of HBsAg detection was significant for the level of education (Table 1).

Table 1: Socio-demographic characteristics of pregnant women attending antenatal care at HUTRH, April-May 2015.

Variable		Total Number (%)
Age(in years)	16 -20	43(16.0)
	21- 25	84(31.2)
	26- 30	106(39.4)
	31-35	28(10.4)
	36-40	8(3.0)
Residence	Urban	226(84.0)
	Rural	43(16.0)
Educational status	Illiterate	31(11.5)
	Primary	42(15.6.1)
	Secondary	69(25.7)
	Tertiary	127(47.2)
Occupation	Employed	99(36.8)

	House wife	106(39.4)
	Daily laborer	6(2.2)
	Merchant	38(14.1)
	Student	20(7.4)
Ethnicity	Sidama	66(24.4)
	Amahara	64(23.8)
	Wolayta	22(8.2)
	Guragi	30(11.2)
	Oromo	61(22.7)
	Hadya	13(4.8)
	Others	12(4.5)
Gestational age	1st trimester	87(32.3)
	2nd trimester	80(29.7)
	3rd trimester	102(37.9)
Place of previous delivery	No birth	109(40.5)
	Home	47(17.5)
	Hospital	113(42.0)

Prevalence of HBV infection

From the 269 study participants, the prevalence of HBsAg (using the ELISA kit) was 21(7.8%). Of these, 17(6.3%) were HBsAg positive using rapid test (Table 2). Even though all positive results by HBsAg rapid test also positive by HBsAg ELISA there were a discordant results. i.e. 4 additional positive tests by HBsAg ELISA but these positive samples were repeated and also these results were positives. This shows high sensitivity of ELISA.

Table 2: The sero-prevalence of HBV using rapid test and ELISA method among pregnant women (n=269) at HUTRH, April – May 2015.

	HBV status N (%)	
Tests	Positive	Negative
HBsAg ELISA	21(7.8)	248(92.2)
HBsAg Rapid test	17(6.3)	252(93.7)

5.2.Risk factors for hepatitis B virus infection

The factors associated with exposure to HBsAg were also determined by comparing the proportion of HBsAg detection for study participants as shown in Table 3. None of the expected risk factors (History of blood transfusion, multiple sexual practices, hospital admission, genital mutilation, surgical procedure, body tattooing and history of abortion) had been found to be associated with HBsAg sero-positivity. Almost all the study participants reported exposure to at least one HBV infection risk factor.

Of the 269 pregnant women tested for HIV antibody, 14(5.2%) were positive. Out of these, 2 (14.2%) of the study participants who were HIV positive were also positive to HBsAg (using ELISA). However only 1(7.1%) of the study participants who were HIV positive were also positive to HBsAg (using rapid test). There was no statistically significant association between HIV infection status and hepatitis B virus prevalence, (OR, 2.07; 95%CI, 0.431 - 9.933). The HIV/HBV co-infection rate out of the total was 0.7% (2/269). None of the women were an aware of HBV status and immunized against HBV.

Table 3: Possible risk factors and prevalence of HBsAg among pregnant women (n=269) attending antenatal care at HUTRH, April - May 2015**.**

Variable		HBV status of mothers				
		Total N (%)	Negative N (%)	Positive N (%)	COR (95% CI)	(p- value)
Multiplesexual practices	Yes	39(14.5)	37(94.9)	2(5.1)	1(.134-2.686)	0.090
	No	230(85.5)	211(91.7)	19(8.3)	1	
Hospital admission	Yes	91(33.8)	83(91.2)	8(8.8)	1.2((.488-3.068)	0.667
	No	178(66.2)	165(92.7)	13(7.3)	1	
Genital mutilation	Yes	202(75.1)	183(90.6)	19(9.4)	3.4(.765-14.886)	0.108
	No	67(24.9)	65(97.0)	2(3.0)	1	
Surgical procedure	Yes	57(21.2)	51(89.5)	6(10.5)	1.5 (.571- 4.181)	0.392
	No	212(78.,8)	197(92.9)	15(7.1)	1	
Body tattooing	Yes	49(18.2)	46(93.9)	3(6.1)	1(.207-2.589)	0.628
	No	220(81.8)	202(91.8)	18(8.2)	1	
History of abortion	Yes	74(27.5)	67(90.5)	7(9.5)	1.4(.523-3.491)	0.535
	No	195(72.5)	181(92.8)	14(7.2)	**1**	
	Neg	255(94.8)	236(92.5)	19(7.5)	**1**	
HIV/AIDS status	Pos	14(5.2)	12(85.7)	2(14.3)	2.070(0.431-9.933)	0.363

6. DISSCUSION

In this study, the overall seroprevalence of HBsAg in pregnant women was 7.8%. This shows almost high endemicity of HBV infection according to WHO criteria [14]. This is the highest prevalence as compared to the previous studies in different region of Ethiopia among pregnant women which report, 6.1% in rural hospital in Southern Ethiopia[18], 3% in Addis Ababa; central Ethiopia [20], 3.7% in Jimma; South west Ethiopia [21], 5.3% and 3.8% in Debre-Tabor Hospital[17], and Bahir Dar [19]; North West Ethiopia respectively. Similar findings were reported in other developing countries like Sudan (5.6%) [37], Nigeria (4.3% - 8.3%) [4, 43, 44, 45], Sierra Leone (6.2%) [46] and Mali (8.0%) [34]. However, a higher prevalence was found among a similar study population in Hong Kong (10%) [47]. The difference in prevalence might be due to the difference in hepatitis epidemiology in the general population, study design, sample size and traditional practice. In developed nations where regular screening and vaccination of HBV is provided for pregnant women, low prevalence rate(< 2%) was reported in USA, except Asian Americans (0.14%-0.97%) [47], in Mexico (1.65%) [48] and Saudi Arabia (1.6%) [32]. Similar practices in developing countries may reduce the mother to child transmission and public health significance of hepatitis B virus infection.

Regarding socio-demographic status of the study participants, educational status had significant association with HBV infection ($\chi 2$= 8.1; p- value=0.044) showing the highest proportion among pregnant women who are illiterate, which might be due to low level of awareness about transmission of HBV. This is in agreement with a report from Nigeria [4]. In this study, no statistical significant difference was observed across various age groups. This is agreed with research conducted in Addis Abeba [20], Bahir Dar [19],Jimma [21], Debretabor [17]. However, study from Zaria [49] and Nigeria [50] reported the highest prevalence of HBV infection among pregnant women of 20 to 24 years of age that might by the relationship between HBV infection and high risk sexual practices in this age group but this is contrasted with our study because the highest prevalence of HBsAg was found under age group of 36-40. At least one HBsAg positive result was observed in each age group and in every education category. However, only daily laborer and student had more HBsAg positive results (16.7%) and (15.4%) respectively and might be due to lack of work safety in daily laborer, high sexual activity in students.

Although no statistically significant difference with residence ,pregnant women who have the highest prevalence (11.6%) of HBsAg were live in rural area. This may be due to lack of awareness and way of transmission about HBV.The HBsAg positivity rate among those tested varies widely by gestational age with highest in first-trimester (11.5%) followed by second trimester (7.5%) third triminster (4.9%).In the present investigation, when previous place of delivery prevalence was considered, pregnant women who delivered at home have a highest prevalence (12.8%) than at hospital (8.0%) and those who had no previous of delivery (5.5%). This might be justified by unsafe delivering system at home (contamination of materials during traditional birth).

Unlike the previous reports that showed previous history of blood transfusion, body tattooing and surgical application was one of the risk factor for HBV transmission [17, 19], none of the expected risk factors (blood transfusion, genital mutilation, multiple sexual practices, surgical procedures, hospital admission, tattooing and history of abortion) were associated with sero-positivity for HBsAg in this study (p-value >0.05). Although there was no statistical significant difference, the odds of having HBsAg were more than three times with those with history of genital mutilation (circumcision) than with other risks factors. This might be due to use of unsterilized instruments and contamination which was in agreement with other study [49]. Among the possible risk factors for HBV infection assessed, the highest possible risk factor was surgical procedures (10.5%) , the lowest was history of multiple sexual practice (5.1%) and there was no HBsAg detection in pregnant women who had blood transfusion. This may indicate that there is strengthen screening of HBsAg during blood donation.

In contrast to our study, HBsAg positivity was significantly higher in pregnant women who had previous history of abortion [21]. This may be due to implementation of policies aimed at reducing the incidence of unsafe abortions and promotion of barrier contraception in the country may assist in reducing the incidence. In this study, the overall prevalence of HIV infection was 5.2% ,lower than a study in Bahir dar (6.6%) [19]. The frequency of HBV and HIV co-infection was 14.3%. This is higher than the study done in Southern Ethiopia (0.6%) [18] and Nigeria (4.2 - 9.5%) [46, 50]. However, lower than study conducted in Bahir Dar (19.0%) [19].

Limitation of the study

The limitation of the study was small sample size and due to resource constraints and lack of laboratory setup, neither markers of HBV like HBeAg, HBV-DNA, Anti-HBs nor Anti-HBc were detected.

Conclusion

Almost high prevalence of hepatitis B infection was detected among pregnant women attending ANC of Hawassa university teaching and referral hospital. None of the risk factors had statistically significant association with HBV infection except for educational status. Screening of pregnant women for HBV irrespective of basis of risk factor and intensified prevention targeting this group may reduce mother to child transmission of HBV infection.

Recommendation

- Since the prevalence of HBV infection is alarming; ensuring routine antenatal screening, especially in rural areas is important.
- It is better for hospital laboratories to use high sensitive methods ,such as ELISA.
- Further large scale studies should be done to ensure the independent .predictor of HBV infection.

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Annex 1: **Principle and procedure**

Principles of Bioline HBsAg test strip

Bioline HBsAg One Test: -The Bioline HBsAg One Test is a qualitative, solid phase, two-site sandwich immunoassay for the detection of HBsAg in serum or plasma. The membrane is pre-coated with anti-HBsAg antibodies on the test band region and anti-mouse antibodies on the control band region. During testing, the serum sample reacts with the dye conjugate (mouse antiHBsAg antibody colloidal gold conjugate) that will be coated in the test strip. The mixture then by capillary action reacts with anti-HBsAg antibodies on the membrane and generates a red band. Presence of this red band indicates a positive result while its absence indicates a negative result. Regardless of the presence of HBsAg, as the mixture continues to migrate across the membrane to the immobilized goat anti-mouse region a red band at the control band region will always appear. The presence of this red band serves as verification for sufficient sample volume and proper flow and as a control for the reagents.

Procedure

1. The Bioline HBsAg test strip will be removed from foil pouch.
2. The test strip in the serum samples will be immersed with printed sample pointing toward the serum or plasma.
3. Then waiting for the red bands to appear. The test will read after approximately 5 minutes. Results after 30 minutes will not be interpreted.

Interpretation of the test

Positive - Two distinct red bands appear, one in test region and another in the control region.

Negative - A single red band appears in the control region. No apparent red or pink band appears in the test region.

Invalid - Control band fails to appear which means improper testing procedure or deterioration of reagents probably.

Principles of ELISA kit

HBsAg ELISA directed against a different epitope of HBsAg. During incubation, the specific immuocompex kit uses antibody sandwich ELISA method in which polystyrene microwell strips are pre-coated with monoclonal antibodies specific to HBsAg. Patient's serum or plasma sample added to the microwells together with a second antibody conjugated with horseradish peroxidase (HRP) and formed in case of presence of HBsAg in the sample, is captured on the solid phase. After washing to remove sample serum proteins and unbound HRP-conjugate, chromogen solutions containing tetramethylbenzidine (TMB) and urea peroxide are added to the wells. In presence of the antibody-antigen-antibody (HPR) "sandwich" immunocoplex, the colorless chromogens are hydolyzed by the bound HRP -Conjugate to a blue colored product. The blue color turns yellow after stooping the reaction with sulfuric acid. The amount of color can be measured and is proportional to the amount of antigen in the sample. Wells containing samples negative for HBsAg remain colorless [42].

Procedure

1. Pipette 100 micro liter of Specimen diluent to all wells of the microtiter plate except well 1A and pipette 50 micro liter of controls or serum samples into the appropriate wells.
2. Apply cover seal. Incubate at 37o C ± 1o C for 1-hour ± 5 minutes.
3. Place the microtiter plate on the AutoWash and wash all the wells five times with Wash Buffer (1X).
4. Add 100 micro liter of Antibody Conjugate to all wells except 1A.
5. Apply cover seal. Incubate at 37o C ± 1o C for 1-hour ± 5 minutes.
6. Prepare sufficient Substrate Solution to fill the control and test wells. Allow time for the OPD tablets to dissolve completely.
7. Place the microtiter plate on the AutoWash and wash all the wells five times with Wash Buffer (1X).
8. Add 100 micro liter of Substrate Solution to all the wells including 1A.
9. Apply cover seal. Incubate at room temperature for 30 minutes ± 1 minute in the dark.
10. Add 50 micro liter of 4N sulfuric acid (H2SO4) to all wells including 1A.
11. Read the reaction at 450 nm.
12. Retest positive samples in duplicate using this procedure.

Reading, Interpretation and cut off value.

Each microplate must be considered separately when calculating and interpreting results of the assay, regardless of the number of plates concurrently processed. The results are calculated by relating each sample optical density (OD) value to the Cut -off value (C.O.) of the plate. If the Cut-off reading is based on single filter paper reader, the results must be calculated by subtracting the blank well OD value from the print report values of samples and controls. In case the reading is based on dual filter plate reader, do not subtract the blank well OD from the print report values of samples and controls.

Calculation of Cut-off value: Cut-off (C.O.) = Nc*2.1

Where, Nc -The mean absorbance value for three negative controls.

- Negative Results (S/C.O.<1): samples giving an absorbance less than the Cut-off value are considered negative, which indicates that no hepatitis B surface antigen has been detected with this HBsAg ELISA kit, therefore the patient is probably not infected with HBV.
- Positive Results: (S/C.O.>1): samples giving an absorbance greater than or equal to the Cut-off value are considered initially reactive , which indicates that hepatitis B surface antigen has probably been detected with this HBsAg ELISA kit. Any initially reactive samples retested in duplicates. Repeatedly reactive samples could be considered positive for HBsAg, therefore the patient is probably infected with HBV and the blood unit should not be transfused.
- Borderline: Samples with absorbance to Cut-off ratio between 0.9 and 1.00 are considered borderline samples and retesting is recommended. Repeatedly positive samples can be considered positive for HBsAg.

❖ Important point ;If the mean OD value of the negative control is lower than 0.05, take it as 0.05.If higher than 0.05, see the Quality Control Range .

Annex 2: Information sheet and consent form

Information sheet

Purpose

We are conducting a research to assess seroprevalence of HBV and associated risk factors among pregnant women. Your feedback on this research is important and will help to prevent the occurrence of HBV in pregnant women.

Participation

We are asking you and others to voluntarily participate in this study. What is expected from everyone is to respond some question which take about ten minutes and give 5 ml of venous blood and blood samples are collected using sterile and disposable equipments.

Risks

While you are participating, you are likely to have some risks. The risks associated with this study could be some discomforts and in a rare occasion a hematoma may be developed when we collect 5ml of venous blood from you. However, these things do not produce serious pain and if in case any problem arises during and following sample collection, we shall offer you necessary medical interventions until you fully recover.

Benefits

If you are positive for HBV during investigation, opportunities for management will be arranged and you will be followed. If you are negative for HBV, you will recommended for vaccination to prevent future HBV infection by dealing with the concerned body if possible.

Confidentiality

All the data obtained will be kept strictly confidential by using only code numbers which is filled by the investigators and locking the data.

Right to refuse

Since participation in this study is entirely voluntarily, you can refuse to participate in this study at any time. Your refusal will not affect your job.

If you have any question concerning the study you can ask with the following address

Principal investigator: Yeshi Metaferia

Address: School of allied health science, department of Medical Laboratory Sciences ,Addis Ababa University.

Tel: 251 0112 75 11 70 Addis Ababa, Ethiopia

Consent form

I, the undersigned, confirm that, as I give consent to participate in the study with a clear understanding of the objectives and conditions of the study and with recognition of my right to withdraw from the study if I change my mind.

I……do hear by give consent to Mr./Miss…….to include me in the proposed research. I have been given the necessary information about the research. I have also been assured that I can withdraw my consent at any time without penalty or loss of benefits. The proposal has been explained to me in the language I understand.

Code of the participant: _____

Participant's signature: _____

Name of data collector: _____

Witness: _____Date:_____

May I begin the interview now?

Yes [continues interviewing] ☐ No [interviewer: end interview] ☐

Name of interviewer_____

Start time_____ End time_____

I certify that I filled this questionnaire in accordance with the training I was given and instruction started in it. I have confirmed that information in it is correct.

Signed_____ Date_____

Annex 3: Questionnaire

A questionnaire prepared to assess the sero-prevalence of Hepatitis B Virus and Associated Risk factors among pregnant women at Hawassa university teaching and Referral Hospital.

PART I: Socio-demographic and socioeconomic characteristics

Direction**:** Fill or use √ mark on box for response categories

Code ----------

S. No	Questions	Response of categories	Remark
101	Can you tell me your age?	_____year	
102	Where you live?	1. Urban ☐ 2. Rural ☐	
103	What is your educational status?	1. Illiterate ☐ 2. 1-8 ☐ 3. 9-12 ☐ 4. TVET diploma ☐ 5. University degree & above ☐	
104	What is your occupational status?	1. Employed ☐ 2. House wife/home activities ☐ 3. Daily laborer ☐ 4. Merchant ☐ 5. Student ☐ 7. Other Specify_____	
105	Ethnicity	1.Sidama ☐ 2.Amahara ☐ 3. Wollaita ☐ 4.Gurage ☐ 5.Oromo ☐ 6.Hadiya ☐	

		7.Others_____	
106	How much your gestational age?	1st trimester ☐	
		2nd trimester ☐	
		3rd trimester ☐	
107	Is there any abortion?	1. yes ☐ 2. No ☐	
108	Where is Place of previous birth?	1. No birth ☐	
		2. Home ☐	
		3. Hospital ☐	
109	Do you Screened for HBV before now ?	1. yes ☐ 2. No ☐	
110	Do you vaccinated for HBV ?	1. Yes ☐ 2. No ☐ ☐	

Part II: Route associated risk factors and behavioral activities

S. No	Questions	Response of categories	Remark
201	Is there any history of b/d transfusion?	1. Yes ☐ 2. No ☐	
202	Have you ever admitted in hospital?	1. Yes ☐ 2. No ☐	
203	Do you expose any surgical procedure?	1. Yes ☐ 2. No ☐	
204	Did have multiple heterosexual activities in your life?	1. Yes ☐ 2. No ☐	
205	Have you had a tattoo done in your life?	1. Yes ☐ 2. No ☐	
206	Is there any genital mutilation?	1. Yes ☐ 2. No ☐	

This is the end of the questionnaire. Thank you very much for taking time to answer these questions. We appreciate your help.

Annex 4. Questionnaire in Amharic version

ለጥናቱ መረጀና መግለጫ ቅጽ

የጥናቱ ኣላማ

የዚህ ጥናት አላማ መሰረት ያደረገው ሄፐታይተስ "ቢ" ተብሎ የሚጠራውን የጉበት ህመም አምጪ ቫይረስና ከሱ ጋር የተያያዙ ተጋላጪ መንስኤዎችን በነፍሰ-ጡር እናቶች ላይ ለማጥናት ነው፡፡ የእርሶ በዚህ ጥናት ውስጥ መሳተፍ ነፍሰ-ጡር እናቶች በሄፐታይተስ "ቢ" እንዳይጠቁ ለመከላከል ይረዳል፡፡

በጥናቱ ስለመሳተፍ

በዚህ ጥናት መሳተፍ በሙሉ ፈቃደኝነት ላይ የተመሠረተ ነው፡፡ ስለሆነም በመጀመሪያ በጥናቱ እንዲሣተፉ ፈቃደኝነተዎን በትህትና እንጠይቃለን፡፡በዚህ ጥናት ለመሳተፍ ከፈቀዱ ለ አስር ደቂቃ ያህል ለጥያቄዎች ምላሽ ይሰጡናል፡፡

በጥናቱ በመሳተፍ የሚገኝ ጥቅም

የደመዎ ናሙና በላብራቶሪ ሲመረመር ሄፐታይተስ "ቢ" ከተገኘ የህኪም ክትትልና አስፈላጊውን ምክር ይሠጠዎታል፡፡ ደሙም ነፃ ከሆነ ደግሞ ከመስሪያ ቤቱ ጋር በመነጋገር ክትባት እንዲሰጥዎት ጥረት ይደረጋል፡፡

ምስጢርን ስለመጠበቅ

በጥናቱ ውስጥ የተሰበሰቡ ማናቸውም ግላዊ መረጃዎች ሚስጥራዊነታቸው የተጠበቀ ይሆናል፡፡ ከማንነትዎ ጋር በቀጥታ ተያያዥነት ያላቸው መረጃዎች በሙሉ በዋና ተመራማሪው ሚስጥራዊ በሆነ የመረጃ ጥንቅር ዘዴ ከተቀየሩ በኋላ ብቻ ለምርምር ሂደቱ የሚውሉ ይሆናሉ፡፡

ከጥናቱ ስለመውጣትና ስለማቋረጥ

ይህ ጥናት በፈቃደኝነት ላይ የተመሰረተ እንደመሆኑ መጠን በማናቸውም ወቅት በፈቃደዎ ከጥናቱ መውጣት ይችላሉ፡፡ ከጥናቱ ቢወጡም እንኳ በስራዎት ላይ ምንም አይነት ችግር አያመጣም ፡፡

ከጥናቱ ጋር በተያያዘ ማናቸውም ጥያቄ ቢኖርዎ በሚከተለው አድራሻ ጥያቄዎን ማቅረብ ይችላሉ፡፡

ዋና ተመራማሪ፡- የሺ መታፈረያ

አድራሻ፡ አዲስ አበባ ዩኒቨርስቲ ጤና ትምህርት ክፍል ሜዲካል ላቦራቶሪ ሳይንስ

ስልክ Tel: 251 0112 75 11 70 **አዲስ አበባ ኢትዮጵያ**

ስለስምምነቱ ማረጋገጫ ፊርማ

እኔ ስሜ ከታች የተገለፀው የጥናቱ ተሳታፊ ለመሆን ስወስን የጥናቱን አላማዎች አሰራሮችና ቅድመ ሁኔታዎች በግልጽ በመረዳትና ከጥናቱ ተሳታፊነት ፈቃደኝነቴን በማንኛውም ደረጃ የማንሳት መብቴን በማረጋገጥ ነው፡፡

እኔ---------- በጥናቱ ተሳታፊ መሆኔን በፊርማዬ እያረጋገጥሁ ይህንን ስወስን በጥናቱ ሳቢያ ሊከሰቱ የሚችሉ አደጋዎች በሚገባ የተረዳሁና ከጥናቱ በማንኛውም ደረጃ እራሴን ለመሰረዝ ብወስን ቅጣት እንደሌላውወይንም ተገቢ የሆነ ጥቅም እንደማይነፈገኝ በማመን ነው፡፡ እነዚህ መረጃዎች ሁሉ በሚገባ በምረዳው ቋንቋ የተገለጸልኝ መሆኑን በፊርማዬ አረጋግጣለሁ፡፡

የተሳታፊው መለያ ቁጥር ---------- ፊርማ-------

የተመራማሪው ሙሉ ስም፣ ወ/ሮ----------ፊርማ -----

የምስክር ሙሉ ስም ----------ፊርማ----------

አሁን ጥያቄዎቹን መጠየቅ እችላለሁ ?

አዎ---------- ወደ ጥያቄ አይቻልም---------- ያበቃል

የጠያቂ ስም _____

መጠይቁ የተጀመረበት ሰዓት____ ደቂቃ __

ያለቀበት ሰዓት_____ ደቂቃ_____

የጠያቂው ቃል ፡-ይህንን መጠየቂያ በላዩ ላይ በተፃፈው መመሪያና ጥያቄ እንዲሁም በተሰጠኝ ሥልጠና መሠረት ሞልቻለሁ :: በላዩም ላይ የሰፈረው ትክክልኛ መሆኑን አረጋግጣለሁ::

ፊርማ _____ ቀን_____

መጠይቅ ክፍል 1 ፡- የቤቴሰብ ማህበራዊ ገጽታ

መመሪያ: የሚሰጠው ምላሽ በጽሁፍ መሙላት/ በሳጥኑ ዉስጥ √ምልክት መጠቀም

መለያ ቀጥር----------

ተ.ቁ	ጥያቄ	በየክፍሉ የተሰጠ መልስ	ምርመራ
101	እድሜዎት ስንት ነው?	_____ዓመት	
102	የሚኖሩት የት ነው?	1. ከተማ ☐ 2. ገጠር ☐	
103	የት/ት ሁኔታ?	1. ያልተማረ ☐ 2. 1-8 ☐ 3. 9-12 ☐ 4. ቴክኒክናሙያድኘሎማ ☐ 5. የዩኒቨርሲቲ ድግሪና ከዚያ በላይ ☐	
104	ሥራዎ ምንድ ነው?	1. ተቀጣሪ ☐ 2. የቤት እመቤት ☐ 3. የቀን ሰራተኛ ☐ 4. ነጋዴ ☐ 5.ተማሪ ☐ 6.ሌላ ከሆነ ይግለጽ _____ ☐	
105	ብሔርዎ ምንድነው?	1.ሲዳማ ☐ 2.አማራ ☐ 3. ወላይታ ☐ 4.ጉራጌ ☐ 5.ኦሮሞ ☐ 6.ሌላከሆይጻፉ_____	
106	እርግዝናዎ ምን ያህል ጊዜ ሆኖታል?	1. የመጀመሪያ ሶስት ወር ☐ 2. የሁለተኛ ሶስት ወር ☐ 3. የመጨረሻዎቹ ሶስት ወር ☐	

107	ውርጃ አጋጥሞዎት ያውቃል?	1. አዎ ☐ 2. አላዉቅም ☐	
108	ከዚህ በፊት የትነዉ የወለዱት?	1. አልወለድኩም ☐ 2. ቤት ☐ 3. ህክምና ቦታ ☐	
109	ሄፓታይቴስ ቢ ቫይረስ ተመርምረው ያውቃሉ?	1. አዎ ☐ 2. አላዉቅም ☐	
110	የሄፓታይቴስ ቢ ቫይረስ ክትበት ያውቃሉ?	1. አዎ ☐ 2. አላዉቅም ☐	

መጠይቅ ክፍል 2; ሄፓታይቴስ ቢ ቫይረስና ተጋላጪ መንስኤዎች

ተ.ቁ	ጥያቄ	መልስ	ምርመራ
201	ደምና የደም ዉጤቶችን ወስደው ያዉቃሉ?	1.አዎ ☐ 2.አላዉቅም ☐	
202	ቀደ-ጥገና አጋጥሞዎት ያውቃል?	1. አዎ ☐ 2. አያቅም ☐	
203	ህክምና ቦታ ተኝተው ያዉቃሉ?	1. አዎ ☐ 2. አላዉቅም ☐	
204	በህይዎትሆ ከብዙ ሰው ጋር ጥንቃቄ የጎደለው ግብረ-ሥጋ ግኑኝነት አድርገዋል?	1. አዎ ☐ 2. አላደረኩም ☐	

205	ንቅሳት ተነቅሰው ያውቃለ?	1. አዎ ☐ 2. አላውቅም ☐	
206	ተገርዝዋል?	1. አዎ ☐ 2. አልተገረዝኩም ☐	

አመሰግናለሁ!!

DECLARATION

I, the undersigned, declare that this proposal is my original work, has not been presented for a degree in Addis Ababa University or any other universities. I also declare that all sources of materials used for the proposal have been duly acknowledged.

Name of the principal investigator YESHI METAFERIA (BSC)

Signature _____

Name of advisor **IBRAHIM ALI**(PHD)

Signature _____

Name of advisor **WALELIGN DESSE (**BSc, MSc)

Signature _____

Name of advisor **ANTENEH AMSALU** (BSC, MSc)

Signature _____