ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES

DEPARTMENT OF MEDICAL LABORATORY SCIENCES

# Sero-prevalence of HBsAgamong Pregnant Women and Rate of Vertical Transmission in Selected Health Facilities of Addis Ababa, Ethiopia

By: AbayneshTeklu

Advisors: - KassuDesta (MSc, PhD candidate)

Aster Tsegaye(PhD)

A thesis submitted to the Department of Medical Laboratory Sciences, College of Health Sciences, Addis Ababa University, in partial fulfillment of Master of Science Degree in Clinical Laboratory Sciences (Diagnostic and Public Health Microbiology).

July, 2021

Addis Ababa, Ethiopia

## Addis Ababa University

## School of Graduate Studies

This is to certify that this thesis prepared by AbayneshTeklu, entitled: **Sero-prevalence of HBs Ag among pregnant women and rate of vertical transmission in selected health facilities of Addis Ababa, Ethiopia** and submitted in partial fulfillment of the requirements for Master of Science degree in Clinical Laboratory Sciences (Diagnostic and public health microbiology track) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

**Signed by the Examining Committee:**

**Examiner_____ Signature _____Date _____**

**Examiner_____ Signature _____Date _____**

**Advisor_____ Signature _____Date _____**

**Advisor_____ Signature _____Date _____**

_____

Chairman of the Department or Graduate Program Coordinator

## Acknowledgement

I would like to acknowledge Aster Tsegaye (PhD)and KassuDesta (MSc, PhD candidate),my Advisors for theirexpertiseadvice. Thisthesis could not have been donewithout the invaluable help and knowledge sharing.

I also would like to thank and acknowledge thestudy participants for their participation and permission for their sample included for this study.

I gratefully acknowledged Addis Ababa University, College of Health Science, Department of Medical laboratory Sciences for providing this opportunity to perform my thesis work.

I also thank the Myungsung Christian Medical Center Medical director, laboratory staff for their support and giving me this opportunity to test the examination.

Finally special thanks goes to my family and friends those who supported me throughout my research work.

# Table of Contents


Acknowledgement ..... ii

Table of Contents ..... iii

Abbreviations ..... vi

Abstract ..... vii

1. Introduction ..... 1

1.1. Background ..... 1

1.2. Statement of the problem ..... 3

1.3. Significance of the study ..... 4

2. Literature Review ..... 5

3. Objectives ..... 8

3.1. General objective ..... 8

3.2. Specific objectives ..... 8

4. Hypothesis ..... 8

5. Materials and Methods ..... 9

5.1. Study area ..... 9

5.2. Study design and period ..... 9

5.3. Population ..... 9

5.3.1. Source population ..... 9

5.3.2. Study population ..... 10

5.4. Inclusion and exclusion criteria ..... 10

5.4.1. Inclusion criteria ..... 10

5.4.2. Exclusion criteria ..... 10

5.5. Study variable ..... 10

5.5.1. Dependent Variable ..... 10

5.5.2. Independent variable ..... 10

5.6. Sample size detrmination ..... 11

5.7. Measurement and data collection ..... 11

5.7.1. Laboratory Analysis ..... 12

5.8. Quality Assurance ..... 13

5.8.1. Pre-analytical ..... 13

5.8.2. Analytical ..... 13
5.8.3. Post-analytical ..... 13
5.9. Data analysis and interpretation ..... 13
5.10. Ethical considerations ..... 14
5.11. Dissemination of the result ..... 14
5.12. Operational Definitions ..... 14
6. Results ..... 15
6.1. Socio-demographic characteristics of the study participants ..... 15
6.2. Clinical characteristics of the study participants ..... 17
6.3. Seroprevalence of HBSAg in mothers and vertical transmission rate ..... 18
6.4. Risk factors for HBSAg ..... 20
7. Discussion ..... 21
8. Limitation and strength of this study ..... 23
8.1. Limitation ..... 23
8.2. Strength ..... 23
9. Conclusion and Recommendation ..... 24
9.1. Conclusion ..... 24
9.2. Recommendation ..... 24
10. References ..... 25
11. Annex ..... 29
Annex I English Versions of Participant Information sheet and consent form ..... 29
Annex II Amharic version of Participant Information sheet and consent form ..... 32
Annex III Standard Operating Procedure (SOP) ..... 35
Annex IV Data collection Log Sheet ..... 47
Declaration ..... 49

## List of Tables

Table 1.Socio demographic characteristic of pregnant women attending antenatal Care from two general Hospital in Addis Ababa, January– April, 2018……..16

Table 2.Antenatal care history of pregnant women from two general Hospitals in Addis Ababa, January– April, 2018……...17

Table 3.Prevalence of HBV in pregnant women from two general Hospitals in Addis Ababa, January– April, 2018……19

## Abbreviations

| | |
|---|---|
| ANC | Antenatal Care |
| CL | Confidence Limits |
| CO | Cutoff Value |
| DNA | DeoxyriboNucleic Acid |
| EIA | Enzyme Immunoassay |
| ELISA | Enzyme Linked Immunosorbent Assay |
| HBsAg | Hepatitis B Surface Antigen |
| HBV | Hepatitis B Virus |
| HRP | Horse Radish Peroxides |
| MTCT | Mother to Child Transmission |
| NPV | Negative Predictive Value |
| OD | Optical Density |
| PPV | Positive Predictive Value |
| RDT | Rapid diagnostic test |
| TMB | Tetramethylbenzidine |
| TTI- | Transfusion Transmittable Infection |
| UP - | Urea Peroxidase |

## Abstract

**Background**: Pregnant women who are carriers of the HBV pose a significant risk to their health andnewborn babies. Majority of countries in developing world have not yet adopted universal screening for pregnant women.There are limited studies on maternal and their newborns HBV status in Ethiopia.

**Objective**: - To determine the sero-prevalence of HBsAg among pregnant woman and rate of vertical transmission in selected health facilities of Addis Ababa, Ethiopia

**Methods**: A prospective cross sectional studywasconductedat two hospitals from January– April, 2018 G/C. Sociodemographic data were collected using pretested structured questionnaire. Serawere collected to determine the HBSAg usingImmunochromatographic test kit andconfirmation by ELISA. Data entry and analyseswere done using SPSS 20 statistical software. Descriptive statistics were used to summarize data. Proportions and the corresponding 95% CI were presented. Mean and standard deviation wereused for numerical variables. Frequencies wereused for categorical variables. Bivariate analysis was carried out to see association between dependent and independent variables. P value of < 0.05 was considered significant associations.

**Results:**A total of 420 samples from mothers and cord blood from newborns of positive mothers were collected. Of these 404(96.2%) lived in rural part of the country, 87 % (357)and 58.6 % (246) were married and have primary level education, respectively.Government workers accounted 20.7 % (87) of the study participants and 74.8% did not havehistory of blood transfusion. In this study 3 % (13) of pregnant women were vaccinated and 14,5 % (61) were in their first time pregnancy. Among 420 participants,2.38 % (10/420) were positive for HBV and no mother to child transmissionwas observed.

**Conclusion**:Although the low prevalence ratein this study concurs with what has been categorized as intermediate endemicity and no vertical transmission is noted,every pregnant woman should be testedfor HBV and check mother to child transmission.



**Key words**: - HBsAg test, Vertical transmission of HBsAg, prevalence of HBsAg, pregnancy

# 1. Introduction

## 1.1. Background

Hepatitis B virus (HBV) is a DNA virus belonging to Hepadnaviridae family identified in 1965. The term hepatitis means inflammation of the liver caused by different medications, chemical toxins, alcohol, autoimmune diseases and viruses. In the case of hepatitis B, the disease is caused by the HBV and is the most serious type of viral infections [1].

Globally HBV is a public health problem. It accounts for over 370 million chronic infections out of which 65 million individuals reside in Sub- Saharan Africa. Approximately 2 million deaths globally are attributed to HBV infections annually [2, 3]. Hepatitis B virus causes both acute and chronic infectionthat can range from asymptomatic infection or mild disease to severe or fulminatehepatitis. Hepatitis B is the most common serious liver infection in the world that attacks liver cells and can lead to liver failure, cirrhosis (scarring) or cancer of the liver later in life. HBV is transmitted through contact with infected blood and bodily fluids [4]. It spreads predominantly by percutaneous or mucosal exposure to infected blood and various body fluids, including saliva and menstrual, vaginal and seminal fluids. Pregnant women infected with hepatitis B virus can transmit the infection to their fetuses and newborns [5].

Vertical transmission of HBV is primarily includes from Mother to child transmission (MTCT) accounting for approximately half of the transmission routes of chronic HBV infections. Prevention of MTCT is an essential step in reducing the global burden of chronic HBV. Transmission at birth accounts for most of MTCT, and providing immuno-prophylaxis to newborns is an excellent way to block natal transmission. [6]. Prenatal transmission is the major route of HBV transmission in many parts of the world, and an important factor in maintaining the reservoir of the infection in some regions, particularly in China and South-East Asia [7,8].

Diagnosisof hepatitis Busing either serum or plasma is performed by different methods [9,10].Serological assays are typically used as the first line of the testing strategy to screen for exposure to a virus because of their relatively low cost.Rapid diagnostic tests (RDTs) are single-use disposable assays that are provided in simple-to-use formats that generally require no

additional reagents except those supplied in the test kit. They are read visually and can give a simple qualitative result in under 30 minutes. Due to their simplicity, cost and rapid turnaround time, they can be performed by trained lay providers or health-care workers, without the need for vein puncture for specimen collection.Most laboratory-based serological immunoassays such as enzyme immunoassay (EIAs), chemiluminescenceimmunoassays (CLIAs) and electrochemiluminescence (ECL)detect antibodies, antigens or a combination of both and differ only in the mode of detection of immune complexes formed. A cut-off value, usually determined by the manufacturer of the assay, specifies the point at which the results are considered to be reactive, and therefore, EIA results are generally reported as optical density divided by the assay cut-off (OD/CO) values [10,11].

Nucleic acid testing technologies scan detect the presence of viral nucleic acid – DNA or RNA – through targeting a specific segment of the virus, which is then amplified. The amplification step enables the detection of low levels of the virus in the original specimen, which might not otherwise have been detectable [11].

Preventing prenatal HBV transmission is an integral part of a strategy to eliminate hepatitis B.Hepatitis B virus infection in a pregnant woman poses a serious risk to her baby at birth. For example an estimated 40% of infants born to HBV-infected mothers in the United States weredeveloping chronic HBV infection in the absence of postexposureimmuno-prophylaxis. Moreover, approximately 25% of themwereeventually die from chronic liver disease.Prenatal HBV transmission can be prevented by identifying HBV-infected (i.e., hepatitis B surface antigen [HBsAg]-positive) pregnant women and providing hepatitis B immune globulin and hepatitis B vaccine to their infants within 12 hours of birth [12].

There is limited information regarding the prevalence of HBV among pregnant women and their newborn in Ethiopia. Hence, this study tried to generate additional data in selected hospitals of Addis Ababa, Ethiopia

## 1.2.Statement of the problem

Viral hepatitis is a systemic disease primarilyinvolving the liver. The complex antigenfound on the surface of HBV is called HBsAg. Highest prevalence of hepatitis B infection has been reported in Africa and East Asia [12].

HBV is highly contagious and relatively easy to betransmitted from one infected individual to another byblood to blood contact, during birth, or during delivery [13, 14].The new born is affected when the virus transmission occurs. Despite this risk of mother to child transmission, knowledge about HBV among pregnant women is poor and need to be improved to prevent MTCT of HBV [15].

The management of pregnant women who are HBV carriers remains poor [16].In most health institutions rapid kit is used to screen for HBV infection; so it needs a confirmatory test for the positive results. In Ethiopia,although published data on HBV related information are available in different parts of the country, HBV prevalence and mother to child transmission related burden is very scarce [17] and this work tried to generate data pertaining to prevalence among pregnant women and weather their conceived baby has the virus or not as measured by cord blood.

## 1.3.Significance of the study

The result of this study will generate valuable data on the prevalence of HBV infection in the pregnant women and the vertical transmission to newborns which in turn is useful to design intervention. The finding can also enforce policy makersto take appropriate measures and to improve the antenatal services in general.

Finally, the finding could also provide information for other researchers who are interested to undertake related study in future they can use it as references.

## 2. Literature Review

According to Center for Disease Control and Prevention (CDC), Hepatitis B virus (HBV) infection in a pregnant woman poses a serious risk to her infant at birth. Without postexposureimmuno-prophylaxis, approximately 40% of infants born to HBV-infected mothers in the United States weredeveloping chronic HBV infection, approximately one-fourth of whomwereeventually dying from chronic liver disease. Prenatal HBV transmission can be prevented by identifying HBV-infected (i.e., hepatitis B surface antigen [HBsAg]-positive) pregnant women and providing hepatitis B immune globulin and hepatitis B vaccine to their infants within 12 hours of birth[18].

In a study conducted in South India by Rajendiran S *et_al*, a total of 1282 pregnant women in the age group of 20-35 years whoattended the antenatal outpatient department in the studyperiod were included. Out of these 1282 cases 13antenatal women were found to be sero-positive forHBsAg giving a prevalence rate of 1.01%.The highest prevalence was observed in the age group26-30 years (46.1%),followed by the age group 31-35years (30.8%),age group 20-25years(23.1%). The authors concluded all pregnant women irrespective of risk factors and increasing awarenessabout HBV infection had low prevalence and riskof HBV infections. However, if the pregnant women are notdiagnosed and managed properly, the future burden of the disease for society and health care resources were veryhigh[19].

In Nigeria of the total of 180 Pregnant women screened for HBsAg 6.67% (12/180) were sero-positive. the highest of infection was noted among pregnant women aged 40-44 the study revealed that marital status, gestational age, parity, education, history of blood transfusion and surgery were not statistically significantly associatedrisk factors ($p>0.05$) . They concluded that there is high prevalence of HBsAg in the study and the administration of prophylaxis for babies of HBsAg positive motherscould greatly assist in reducing maternal transmission of HBV [20].

A study conducted in Sudan in among pregnant women at Khartoum Teaching Hospital, Sudan by Abuelgasim MH*et_al*, investigated 160 Sudanese women for the presence of HBsAg. They also evaluatedthe associated risk factors for its acquisition. Prevalence of HBV infection was 7.5 %. The mean age of studied population was 28 years. The mean of the parity was 3.34; most

of the studied populations were in the third trimester. The majority were in higher secondary school level. Only 2 (1.2%) participants were employed. The authors concluded that prevalence of hepatitis B pregnant women in Sudanese and many risk factors (history of surgery, history of jaundice in their husband, blood transfusion history, work in medical field) correlated with HBV infection [21].

A study conducted in Southwest Ethiopia Jimmatown at Jimma specialized hospital and its four training health center (Jimma health center. (H.C), Agaro H.C, Asendabo H.C, ShebeH.C) by Awole M et_al. and investigated493 sera from pregnant women for HBsAg, of them 18 (3.7%) were positive, ranging from 1.4 % to 6.4% foreach health center and Jimma University specialized hospital. It was highest in Jimma and lowest in Shebe. In conclusion, except abortion, all the other known risk factorsincluded in the study were not associated with HepatitisB virus infection. The study also suggested determiningthe extent of prenatal transmission of HBV,and the prevalence of HBeAg among the HBsAg carriermothers should be determined in a further study [22].

A study was conducted by Kirba S *et_al*,to determine sero-prevalence of HBsAg among pregnant woman and rate of vertical transmission in three teaching hospitals in Addis Ababa. A total of 530 pregnant women were recruited in the study.Among those 24(4.5%) were positive for HBsAg of whom only 2(0.377 %%) had sero-positivity for HBeAg and none had acute clinical hepatitis. About 75% of mothers with markers for HBV were delivered by vaginal delivery and the rest by cesarean section. In conclusion the prevalence of HBV among pregnant mothers in Addis Ababa is of intermediate endemicity.The proportions of mothers who have bothHBeAg and HBsAgare small (8.3%). There was no prenatal transmission in this study and the prenatal outcome of pregnant women with HBsAg seems to be not adversely affected [23].

On the other hand, a study by Tegegn*et_al*, which was conducted in two health facilities in Addis Ababa, revealed an equal rate of positivity in cord bloods, almost equal to those infected mothers. In this study which recruited 265 delivering women with the mean age of 25.8 years, 8 (3.0%) of mothers were positive for Hepatitis B Virus surface antigen, whereas 6 (2.3%) of cord bloods were positives with 75% concordance rate of exposed infants with sero-positive mothers. Of the

total mothers assessed for possible risk factors, while 161 (60.8%) had multiple exposure factors such as ear pricing, history of tribal marks, abortion, multiple-sexual partner and history of surgical procedures experienced from high to low frequency[17].

Despite the risk of pregnancy outcome a few researches were done to see the prevalence and the vertical transmission of HBV from pregnant women in Ethiopia. The two published studies in Ethiopia for example showed inconsistent findings regarding vertical transmission. Thus, regular monitoring of vertical transmission is of paramount importance such that interventions measures to be considered.

# 3. Objectives

## 3.1. General objective

To assess the sero-prevalence of HBsAg among pregnant woman and rate of vertical transmission in selected hospitals of Addis Ababa, usingRapid and ELISAtechniques

## 3.2. Specific objectives

- To determine the prevalence of HBsAgamong pregnant women.
- To determine theproportion of vertical transmission of HBV from pregnant women to offspring as measured by the presence of HBSAg marker from cord blood.
- To assess factors associated with HBV seropositivity

# 4. Hypothesis

There is no difference in the prevalence of HBV among mothers and their new borns.

# 5. Materials and Methods

## 5.1. Study area

This study was conducted in two hospitals Yekatit 12 and RasDestahospitals. Yekatit 12 hospital was established in 1915 by Emperor Hileselassie 1 with the name of BethsidaTefferiMekonen hospital. The hospital started service by one physician and 25 nurses. It was also where the 1st nurses school started in Ethiopia(Red Cross nursing school). The hospital gives medical service in internal medicine, General surgery , Gynecology and obstetrics and others . In the hospital the monthly delivery case ranges from 450-500 and they have 10 delivery coach and 37 beds[24].

RasDesta Hospital is located in Addis Ababa, Ethiopia. The hospital gives different medical service including Gynecology, internal medicine, pediatrics and others; this hospital is also giving delivery service which was started in October 2009 E.C and on average the hospital gives a delivery services for 130 pregnant women monthly. It has five catchment health centers that refer to this hospital. The hospital has two gynecologist and 15 midwifes[17].

## 5.2. Study design and period

A hospital based cross sectional studywas conducted in two hospitals in Addis Ababa starting from January – April, 2018 G.C

## 5.3. Population

### 5.3.1. Source population

The source populations were all pregnant women who visitedthe two hospitals for antenatal care anddelivery.

### 5.3.2. Study population

The study population was thosepregnant women deliveringin the hospital and their newborns which fulfill the eligibility criteria.

## 5.4. Inclusion and exclusion criteria

### 5.4.1. Inclusion criteria

- Age 18 years and above
- All volunteering pregnant women attending ANC for Delivery.
- All pregnant mothers who came for delivery without follow up

### 5.4.2. Exclusion criteria

- Those pregnant mothers attending ANC below 28 weeks of Gestation

## 5.5. Study variable

### 5.5.1. Dependent Variable

- HBsAg Statusof mothers and their newborn

### 5.5.2.Independent variable

- Age
- Gestational age at delivery(Term/preterm)
- Mode of delivery
- Birth weight/height
- Knowledge, attitude, practice of mothers in relation to HBV
- education status
- marital status

### 5.6. Sample size detrmination

Sample size determination were done with using Single proportion formula calculated the sample size.

Samplesize

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

Were n= sample size

$z^{\alpha}/_{2}$=1.96 (standard normal probability for 95% CI ), P=50% (to get maximum sample size), d=5% error

n=384

10% contingency=422

Thus a minimum of 422 pregnant mothers and their newborn

### 5.7. Measurement and data collection

After obtaining informed consent routine HBsAg examination wereperformed from pregnant women and cord blood samples[25] from HBsAg positive mother during delivery over a period of 4months from January – April, 2018 G.C. Pregnant mothers presenting at ANC and delivery units of the two hospitals wereselected after getting an informed consent.Sampleswere collected based on the standard operating procedures (SOPs). The tests were carried out within 2 hours from the time of collectionusing Rapid test kit and aliquot were transferred and stored in deep freezer then analyzed using the ELISA.

### 5.7.1. Laboratory Analysis

#### 5.7.1.1. Specimen collection and processing

- ✓ Sample type: - Venus blood and cord blood collected in EDTA tube
- ✓ Amount required:-4ml
- ✓ Plasma sample used for test
- ✓ Transport and storage:- (2-8$^0$C)
- ✓ Stability:- 7 days after collection at 2-8$^0$C and-21$^0$C for months

#### 5.7.1.2. Rapid HBsAg test kit Method principle

Asatestsampleflowsthroughthemembraneassemblyofthetestdevice,thecolored monoclonalanti-HBsAg-colloidalgoldconjugatecomplexeswiththeHBsAginthesample.Thiscomplexmovesfurther on themembranetothetestregionwhere it is immobilizedby another monoclonalanti-HBsAgantiserumcoatedonthemembrane. Thisleadstoformationofapink-purplecoloredbandwhichconfirms appositive testresult. Absenceofthis coloredbandinthetestregionindicatesanegativetestresult.Theunreactedconjugateandunboundcomplexifany movefurtheronthemembraneandaresubsequentlyimmobilizedbytheanti-rabbitantiserumcoatedonthemembrane at the controlregion,formingapink-purpleband.Thiscontrolbandservestousethetestkit working properly [26]. The detail standard operating procedure is found in the annex.

#### 5.7.1.3. ELISA Method principle

This HBsAg ELISA kit uses antibody sandwich ELISA method in which polystyrene microwell strips are pre-coated with monoclonal antibodies specific to HBsAg. Plasma sample is added to the micro well together with a second antibody conjugated with horse radish peroxides (HRP) and directed against a different epitope of HBsAg. During incubation, the specific immune complex 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 containingTetramethylbenzidine (TMB) and urea peroxides are added to the wells. In presence

of the antibody- Antigen antibody (HRP) "sandwich" immune complex, the colorless chromogensare hydrolyzed by the bound HRP- conjugate to a blue colored product. The blue color turns yellow after stopping the reaction with sulfuric acid. The amount of color can be measured and is proportional to the amount of antigen in the sample. Wells containing samples negative for HBsAgremain colorless [27]. The detail standard operating procedure is found in annex.From all HBSAg positive mothers, cord blood was taken and analyzed for the presence of HBSAg using both rapid and ELISA methods.

## 5.8. Quality Assurance

### 5.8.1.Pre-analytical

From sample collection,handling processing and storage the quality wasensured by strictly following standard operating procedure. That is starting with identification of the client, explaining the test for clients andpreparing them for sample collection; then matching the sample collection log sheet and label code properly in test tube and finally collectingappropriate volume of blood with proper sample container.

### 5.8.2.Analytical

When performing the test of HBsAg in Rapid test kit and ELISA methods, the analysis procedures were strictly followed as per the manufacture's instruction and standard operating procedure. Internal Quality control was performed for both before analysis of the patient sample and properly aliquot was stored in refrigerator and the temperature was strictly monitored daily

### 5.8.3.Post-analytical

The patient information and the data were checked and record appropriately on the registration book. Samples were disposed properly following SOP after final analysis is completed.

## 5.9. Data analysis and interpretation

The data werecollected, entered and analyzed using SPSS 20 statistical software. Descriptive statistics wasused to summarize data. Proportions and the corresponding 95% CI werepresented. Mean and standard deviation used for numerical variables. Frequency was used for categorical variables, bivariate analysis wasdone for each variable in the study and P value of $< 0.05$ was used to consider statistical significance.

## 5.10. Ethical considerations

Before starting the research work, ethical clearance was obtained from the Addis Ababa University, College of Health Sciences, Department of Medical Laboratory Sciences Department Research and Ethical Review committee (DRERC). Formal letters of cooperation were sent to the two hospitals. The study aim, benefit, riskand right for withdrawal anytime from the study were explained to the study participants and informed consentswere obtained. Samples were coded and confidentiality of patient data maintained throughout the study.Results of those positive mothers and their newborn were communicated to the attending physicians.

## 5.11. Dissemination of the result

Study findings of this study willbe presented and submitted to Addis Ababa University, College of Health Sciences, and Department of Medical Laboratory Sciences to serve as a reference material in the library. The studyfinding will be disseminated and presented at local and international workshops and seminars. Manuscript will be submitted to peer reviewed journals for possiblepublication.

## 5.12. Operational Definitions

**Vertical Transmission:** the transmission HBsAg virus from mother to offspring prenatal infection

**Gray zone**: The difference between the cutoff value and 10% of it

## 6. Results

### 6.1.Socio-demographic characteristics of the study participants

A total of 420 pregnant women were involved in this study. The mean age was 30years.The lowest age was 18 and maximum age was 40 years.Nearly half of the pregnant women 206(49%) were within theage group between 26-35years.

About404(96.2%)pregnant women live in urban area or Addis Ababa and the rest 16(3.8%) live in rural area of the outskirt of Addis Ababa.

Majority of pregnant women were married 403(96%), 248(59%) had primary level education and 29.8 % (125 ) were having work in non-government organization.Majority, 83.6 % (351) of pregnant women were orthodox Christians. Table 1 summarizes the socio-demographic characteristics of the pregnant women in this study.

Table 1 Socio demographic characteristic of pregnant women attending antenatal Care from two general Hospitals in Addis Ababa, January– April, 2018

| **Variables** | **Number/Percent** |
|---|---|
| **Age (Years)** | |
| 18-25 | 181(43.1) |
| 26-35 | 206(49.0) |
| 36-40 | 33(7.9) |
| **Residence** | |
| Urban | 404(96.2) |
| Rural | 16(3.8) |
| **Marital status** | |
| Single | 15(3.6) |
| Married | 403(96) |
| Divorced | 2(0.5) |
| **Education** | |
| Primary | 248(59) |
| Secondary | 102(24.3) |
| Diploma | 45(10.7) |
| Degree | 24(5.7) |
| Above Degree | 1(0.2) |
| **Occupation** | |
| Government | 85(20.2) |
| Non-Government | 125(29.8) |
| Others | 210(50.0) |

### 6.2. Clinical characteristics of the study participants

Of the 420 pregnant women, 25.2 (106) of them had history of abortion, 2.4 % had history of blood transfusion, only 13 (3.1%) were HBV vaccinated, 30.5% of them did not know ways of HBV transmission. Importantly 30.5 % (128) pregnant women did not know how HBV is transmitted (Table 2).

Table2.Antenatal care history of pregnant women those attending two general Hospitals in Addis Ababa, January– April, 2018

| Variable | Number/Percent |
|---|---|
| **Abortion** | |
| Yes | 106(25.2) |
| No | 314(74.8) |
| **Blood transfusion** | |
| Yes | 10(2.4) |
| No | 410(97.6) |
| **HBV Vaccinated** | |
| Yes | 13(3.1) |
| No | 407(96.9) |
| **First time Pregnancy** | |
| Yes | 60(14.3) |
| No | 360(85.7) |
| **Knowledge of HBV Transmission** | |
| Sexually | 171(40.7) |
| Unsafe Needle | 36(8.6) |
| MTC | 6(1.4) |
| All | 79(18.8) |
| None | 128(30.5) |
| **Gestational age** | |
| >38 | 60(14.3) |
| 38-40 | 230(54.8) |
| >40 | 102(24.3) |
| Unknown | 28(6.8)% |

### 6.3. Seroprevalence of HBSAg in mothers and vertical transmission rate

Prevalence of HBV among pregnant women and occurrence of mother to child transmission rate was determined.Overall, 10 (out of 420pregnant women screened were HBV seropositive using both rapid immune chromatographic method and ELISA tests. This makes the prevalence of HBSAg2.38%. Fifty % of seropositivity was found among pregnant women in the age group of 18- 25 years. All seropositive women were living in the urban part of Addis Ababa, they were all married, not vaccinated and present among pregnant women with not first time pregnancy. Moreover, 7/10 (70%) were attaining primary education, 9/10 (90%) had no history of blood transfusion, 7/10 (70%) had history of abortion, and 6/10 (60%) were knowledgeable of ways of transmission, indicating that the HBV sero-positivity among the studied pregnant women was irrespective of risk factors (Table 3).

All cord blood samples whose mothers' sera were positive for HBSAg were found to be negative for HBSAg using both the rapid and ELISA methods possibly indicating the absence of Risk of vertical transmission.

Table3.Prevalence of HBV in pregnant women from two general Hospitals in Addis Ababa, January– April, 2018

| Variable | HBsAg rapid | | HBsAg ELISA | |
|---|---|---|---|---|
| | Negative | Positive | Negative | Positive |
| **Age** | | | | |
| 18-25 | 176(42.9) | 5(50) | 176(42.9) | 5(50) |
| 26-35 | 203(49.5) | 3(30) | 203(49.5) | 3(30) |
| 36-40 | 31(7.5) | 2(20) | 31(7.5) | 2(20) |
| **Address** | | | | |
| Urban | 394(96) | 10(100) | 394(96) | 10(100) |
| Rural | 16(3.9) | 0(0) | 16(3.9) | 0(0) |
| **Marital status** | | | | |
| Single | 15(3.6)% | 0 | 15(3.6)% | 0 |
| Married | 393(95.8)% | 10(100)% | 393(95.8)% | 10(100)% |
| Divorced | 2(0.48)% | 0 | 2(0.48)% | 0 |
| **Education** | | | | |
| Primary | 241(58.7)% | 7(70)% | 241(58.7)% | 7(70)% |
| Secondary | 99(24.1)% | 3(30)% | 99(24.1)% | 3(30)% |
| Diploma | 45(10.9)% | 0 | 45(10.9)% | 0 |
| Degree | 24(5.8)% | 0 | 24(5.8)% | 0 |
| Above Degree | 1(0.24)% | 0 | 1(0.24)% | 0 |
| **Occupation** | | | | |
| Government | 84(20.5)% | 1(10)% | 84(20.5)% | 1(10)% |
| Nongovernment | 119(29)% | 6(60)% | 119(29)% | 6(60)% |
| Others | 207(50.5)% | 3(30)% | 207(50.5)% | 3(30)% |
| **Abortion** | | | | |
| Yes | 103(25.1)% | 3(30)% | 103(25.1)% | 3(30)% |
| No | 307(74.8)% | 7(70)% | 307(74.8)% | 7(70)% |
| **Blood transfusion** | | | | |
| Yes | 9(2.19)% | 1(10)% | 9(2.19)% | 1(10)% |
| No | 401(97.8)% | 9(90)% | 401(97.8)% | 9(90)% |
| **HBV Vaccinated** | | | | |
| Yes | 13(3.17)% | 0 | 13(3.17)% | 0 |
| No | 397(96.8)% | 10(100)% | 397(96.8)% | 10(100)% |
| **Is Your first time pregnancy** | | | | |
| Yes | 60(14.6)% | 0 | 60(14.6)% | 0 |
| No | 350(85.3)% | 10(100)% | 350(85.3)% | 10(100)% |
| **Knowledge of HBV transmission** | | | | |
| Sexually | 170(41.5)% | 1(10)% | 170(41.5)% | 1(10)% |
| Unsafe needle | 34(8.3)% | 2(20)% | 34(8.3)% | 2(20)% |
| MTC | 6(1.5)% | 0 | 6(1.5)% | 0 |
| All | 73(17.8)% | 6(60)% | 73(17.8)% | 6(60)% |
| None | 127(30.9)% | 1(10)% | 127(30.9)% | 1(10)% |
| **Gestational age** | | | | |
| >=38 | 60(14.6)% | 1(10)% | 60(14.6)% | 1(10)% |
| 38-40 | 230(56)% | 7(70)% | 230(56)% | 7(70)% |
| <41 | 102(2.9)% | 1(10)% | 102(2.9)% | 1(10)% |
| Unknown | 28(6.8)% | 0 | 28(6.8)% | 0 |

### 6.4. Risk factors for HBSAg

Analysis of the known risk factors like blood transfusion, history of abortion and other factors showed that none of them were found to be associated with HBsAgseropositivity. Data are not shown as only 10 mothers had positiveHBSAg results. The numbers of positive mothers per category were small to give meaningful logistic regression analysis.

## 7. Discussion

The current study aimed to assess the sero-prevalence of HBsAg among pregnant woman and rate of vertical transmission in two hospitals of Addis Ababa. HBV rapid test method is widely used for screening propose in the clinical practice as indicator of patient infected with this virus or not and used confirmatory test like ELISA[18,25,28,29]. This study used rapid testkit by chromatography principle and ELISA method by AFIAS-6 analyzer... According to CDC Hepatitis B virus (HBV) infection in a pregnant woman poses a serious risk to her infant at birth[18]. Without post exposure immuno-prophylaxis, approximately 40% of infants born to HBV-infected mothers can be infected. Prenatal HBV transmission can be prevented by identifying HBV-infected women [20,30]

In this study of the 420 pregnant women tested for HBsAg, 10 (2.38%) were HBV seropositive by both rapid test and ELISA methods. The current finding is higher compared to a study from south India by Rajendiran S *et_al*who investigated a total of 1282 pregnant womenand identified 13 sero-positive for HBsAg giving a prevalence rate of 1.01% [19]. The highest prevalence was observed in the age group 18-25 years followed by 26-35 years as opposed to the study from South India where the highest sero-positivity rate was seen in the age group 31-35 followed by 26-30 years [19].On the other hand, the prevalence study conducted in Nigeria by Pennap GR et al which demonstratedHBsAgsero-positivity rate in 6.67% (12/180) pregnant women. The highest rate of infection was noted among pregnant women aged 40-44 [20] as opposed to the current study which revealed highest rate in the young women.A similar study conducted byAbuelgasim MH et_alin a Teaching Hospital, in Sudan, found a higher rate compared to the current study in which 7.5% of the 160 pregnant women positive for HBsAg. The majority of their study participants were in higher secondary school level while in the current study the majority was in primary school [21]. Though differences in the study settings could explain the differences between the current and the other studies which reported a relatively higher rates of sero-positivity, the time difference could also party explain it [19-21] since in recent years the attention given for HBV screening among ANC attendees is increasing. The finding that 70% of the study participants in the current study were knowledgeable that HBV is transmitted through sexually, use of unsafe needle, and from mother to child vertically supports that health awareness during ANC visits might have played a role. Thecurrent study finding is also lower than the sero-positivity rate of 3.7% (18/49) reported by Awole M et_al.from Jimma, Ethiopia,In their

study except abortion, all the other known risk factors included in the study were not associated with Hepatitis B virus infection[22].

In the current study, none of the newborns from seropositive mothers were HBsAg positive when tested by both the rapid test and ELISA. This finding is concordant with the report of Kirba et al who reported no prenatal transmission in mothers from three teaching hospitals in Addis Ababa. But the positivity rate in the pregnant mothers was higher compared to the current study finding in which 24/530(4.5%) were positive for HBsAg [23]. In both studies the prenatal outcome of pregnant women with HBsAg seems to be not adversely affected. It is a good agreement with our study that there is no transmission by sero positive mothers to the childeven if the sample size is different [23]. Unlike these two studies, the study by Tegegne et al which investigated 265 pregnant women of whom 8 (3.0%) of mothers were positive for Hepatitis B Virus surface antigen, 6 (2.3%) of cord bloods were positive. This gives 75% concordance rate of exposed infants with their sero-positive mothers [17].

In sum, HBV was a global public health problem both globally, regionally and in our country both in the general population and pregnant women [31-41]. The current study demonstrated what has been described as intermediate endemicity of the virus as determined by HBsAg as in previous studies in Addis Ababa [23].

# 8. Limitation and strengthof this study

## 8.1. Limitation

ELISA were used to confirm positive sera only as there might be difference in HBSAg results if rapid test negative mothers were tested. Likewise, cord blood samples only from HBSAg seropositive mothers were tested by both rapid and ELISA. Babies from negative mothers were not tested.

## 8.2. Strength

The study used ELISA to confirm positive results from both the mothers and their babies.

# 9. Conclusion and Recommendation

## 9.1. Conclusion

The HBV is widely request laboratory test especially in the antenatal care for the prevention of HBV transmission and mother to child transmission.But the current finding indicated among those positive mothers (10/420) there is no risk of transmission during birth and this result was confirmed with both rapid and ELISA methods from the mother and from neonate from cord blood.

Among the study participant prevalence of HBsAg positive is 2.38% which is of intermediate endemicity.

## 9.2. Recommendation

Based on our findings, though the seroprevalence of HBSAg among pregnant women is 2.38% which is an intermediate endemicity, continuous screening of pregnant women and their baby is very important. Moreover, educating mothers about the transmission and prevention of HBV is crucial specially during their prenatal, antenatal and during postnatal time.

## 10.References


1. Toulouse B. Hepatitis B. NATAP. Available from: http://www.hepcadvocacy.org/factsheets/Hepatitis B.pdf. Accessed on October 2017.
2. World Health Organization. Viral hepatitis: Report by the Secretariat. Geneva: WHO. 2009.
3. Hwang EW, Cheung R. Global epidemiology of hepatitis B virus (HBV) infection. N Am J Med Sci. 2011; 4(1):7-13.
4. Hepatitis B Guidelines for pregnant women. http:// www.hepb.org. Accessed October 2017.
5. Yakasai IA, Ayyuba R, Abubakar IS, Ibrahim SA. Sero-prevalence of Hepatitis B Virus infection and its Risk factors among pregnant Women Attending Antenatal clinic At Aminu Kano Teaching Hospital,Kano,Nigeria.J Basic Clin Rep Sc 2012;1:1- 2.DOI: 10.4103/2278-960X.104297.
6. Navabakhsh B, Mehrabi1 N, Estakhri1 A, Mohamadnejad M, Poustchi1 H.Hepatitis B Virus Infection during Pregnancy: Transmission and Prevention. Middle East J Dig Dis. 2011;3(2):92-102.
7. Yogarajah H, Lestari W, Yasa S. Laboratory Diagnosis of Hepatitis B. Available at https://ojs.unud.ac.id. Accessed on October 2017..
8. Yi P, Chen R, Huang Y, Zhou RR, Fan XG. Management of mother-to-child transmission of hepatitis B virus: propositions and challenges. J ClinVirol. 2016; 77:32–39.
9. Sarin SK, Kumar M, Lau GK, Abbas Z, Chan HL, Chen CJ et al. Asian-Pacific clinical practice guidelines on the management of hepatitis B. Hepatol Int. 2016;10(1):1–98.
10. Krajden M, McNabb G, Petric M. The laboratory diagnosis of hepatitis B virus. Can J Infect Dis Med Microbiol2005; 16(2):65-72.
11. World Health Organization. WHO-guidelines-HBVHCV-testing-Feb 2017. Accessed October 2017.
12. World Health Organization (2009). Hepatitis B Fact sheet N0 204. Accessed October 2017. Available; http://www.who.int/csr/disease/hepatitis. Accessed on October 2017.

13. World Health Organization. Hepatitis C: Factsheet No. 164. 2015. http://www.who.int/topics/hepatitis/factsheets/en/. Accessed 10 October 2017.
14. World Health Organization. Hepatitis B: Factsheet No. 204. 2015. http://www.who.int/mediacentre/factsheets/fs164/en/. Accessed 10 October 2017.
15. Han Z, YinY, Zhang Y, Ehrhardt S, Thio CL, Nelson KE et_al. Knowledge of attitudes towards hepatitis B and its transmission from mother to child among pregnant women in Guangdong province,China. PLOS One2017;12(6):doi: 10.1371/journal.pone.0178671.
16. Giles ML, Grace R, Tai A, Michalak K, Walker SP. Prevention of Mother to child transmission of hepatitis B Virus(HBV) during pregnancy and the puerperium: current standards of care. AustN Z J ObstetGynaecol2013; 53: 231-235.Aust N Z J
17. Tegegne D, Desta K Tegbaru B, TilahunT.sero-prevalence and transmission of Hepatitis B virus among delivering women and their new born in selected health facilities Ethiopia. BMCRes Notes 2014; 7:239.
18. Prenatal transmission of HBV report. Available from https://www.cdc.gov/hepatitis/hbv/prenatalxmtn.htm. Accessed on November 28, 2017)
19. Rajendiran S Gopalan U, Jayakumar K. Sero-prevalence of Hepatitis B infection among pregnant women south Inida.Int J Re Co ObstetGynecol. 2017;6(1):249-251
20. Pennap GR, Osanga ET, Ubam A. Sero-prevalence of Hepatitis B surface antigen among pregnant women attending antenatal clinic in federal Medical center keffi, Nigeria. Re J of Med Sci 2011;5(2):80-82.
21. Abuelgasim MH, Barak MBK. Prevalence of Hepatitis B infection among pregnant women at Khartoum teaching hospital, Sudan. J US-China MediSci2015;12:58-63.
22. Awole M, Gebre SS. Sero-prevalence of HBsAge and its risk factors among pregnant women in Jimma, Southwest Ethiopia.Ethiop .J .Health Dev. 2005;19(1)
23. Kirba S, Negash S, Yusuf L, G/Hiwot Y. Sero-prevalence of HBsAg among pregnant woman and rate of vertical transmission in three teaching hospitals in Addis Ababa. Thesis. Available from http://etd.aau.edu.et/handle/123456789/1068?show=full Accessed on October 7, 2017.Partner profile. Available From www.inctr.org.(Accessedon December 2 2017).
24. Robert Q, Coombs MD. Umbilical Cord Blood Collection standard operating procedure.2013; 1:0-18.

25. On site rapid test of HBsAg test .Available from www.ctkbiotech.com. Accessed on November 8 2017
26. National blood bank HBsAg ELISA Screening Protocol. FRM SOP LAB 2012;1-8.
27. CusterB,SullivanSD,Hazlet TK.Iloeje U, Veenstra DL, Kowdley K. Global epidemiology of hepatitis B virus. J ClinGastroenterol. 2004;38 (1), 158–168.
28. DeveciO.Investigation of intrauterine transmission of HBV to childrens from HBsAg positive womans.JMID. 2011;1(1)14-16.doi: 10.5799/ahinjs.02.2011.01.000.
29. El-Magrahe H, Furarah AR, El-Figih K, El-Urshfany S, Ghenghesh KS. Maternal and neonatal seroprevalence of Hepatitis B surface antigen (HBsAg) in Tripoli, Libya. J Infect DevCtries. 2010;4(3):168-170.
30. Burnett R, Francois G, Kew MC, Leroux-Roels G, Meheus A, Hoosen A A, et al. 2005.Hepatitis B virus and human immunodeficiency virus co-infection in sub-Saharan Africa: a call for further action. Liver Int. 2005; 25(2):201-213.
31. Walle F, Asrat D, Alem A, Tadesse E, Desta K. Prevalence of hepatitis B surface antigen among pregnant women attending antenatal care service at Debre-Tabor Hospital, Northwest Ethiopia. Ethiop J Health Sci 2008:17:13-20.
32. Kefene H, Rapicetta M, Rossi GB, Bisanti L, Bekura D, Morace G, ,et al. Ethiopian national hepatitis B study.J Med Virol, 24(1):75-84.et al. Ethiopian National Hepatitis B Study. J Med Virol. 1988 Jan;24(1):75-84. doi: 10.1002/jmv.1890240110. PMID: 3339335.
33. El-Magrahe H, Furarah AR, El-Figih K, El-Urshfany S, Ghenghesh KS. Maternal and neonatal seroprevalence of Hepatitis B surface antigen (HBsAg) in Tripoli, Libya. J Infect DevCtries. 2010;4(3):168-170.
34. Hoffmann CJ, Charalabous S, Martin DJ, Innes C, Churchyard GJ,Chaisson RE et al.Hepatitis B Virus infection and response to Antiretroviral Therapy (ART) in a South African ART Program.Clin Infect Dis 2008;47:1479–1485.
35. Tsega E. 1991.Viral hepatitis and chronic liver disease in Ethiopia, epidemiological and clinical aspects, PhD Thesis. University of Lund, Malmo, Sweden.13-598.
36. Pirillo MF, Bassani L, Germinario EA, Mancini MG, Vyankandondera J, Okong P, et al. Seroprevalence of hepatitis B and C viruses among HIV-infected pregnant women in Uganda and Rwanda. J Med Virol. 2007;79(12):1797-1801.

37. Tiyou C. Prevalence of HBV in pregnancy and vertical transmission rate in Africa, systematic review,WHO 2007.
38. Surya IG, Kornia K, Suwardewa TG, Mulyanto, Tsuda F, Mishiro S. Serological markers of hepatitis B, C, and E viruses and human immunodeficiency virus type-1 infections in pregnant women in Bali, Indonesia. J Med Virol. 200;75(4):499-503.
39. .Ramos JM, Toro C, Reyes F, Amor A, Gutiérrez F. Seroprevalence of HIV-1, HBV, HTLV-1 and Treponemapallidum among pregnant women in a rural hospital in Southern Ethiopia. J ClinVirol 2011;51: 83-85
40. Kiire CF. The epidemiology and prophylaxis of hepatitis B in sub-Saharan Africa: a view from tropical and subtropical Africa. Gut 1996; 38 Suppl 2:S5-S12.
41. Kang HS, Song BC, Ji CX, KimSY, KimSY, Kim SK. 2004. “Serologic Markers of Hepatitis B Virus in Pregnant Women in Jejo Island.” Korean J. Hepatol. 2004;10 (3): 191-196.

## 11.Annex

## Annex I English Versions of Participant Information sheet and consent form

Participant Information sheet

Addis Ababa University, College of Health Sciences,

Department of Medical Laboratory Sciences

You are invited to participate in a study to be conducted by MSc student AbayneshTekluat Addis Ababa University, College of Health Sciences, Department of Medical Laboratory Science. Please read the following statements and ask any unclear points before you agree to participate.

**Introduction**

The topic of this study is Sero-prevalence of HBsAg among pregnant woman and the assessment of rate of vertical transmission inselected health facilities of Addis Ababa, Ethiopia.Participation in this study is exclusively voluntarily. If you are not interested to participate, there will be no consequences. If you decide to participate, you have to sign on the consent form.

**What is expected from me as participant of the study?**

As a participant of this study, no additional blood sample will be taken from you. HBsAg screening is part of the standard care for pregnant women; thus about 3 ml blood will be collected. In addition, during delivery, about 3 ml cord blood will becollected by experienced professionals.

**Potential benefits to participant and/or to the society**

If you or your newborn is positive for HBV, the result will be communicated to your attending physician. Finding will help the hospital management for evidence based planning.. Hence, you are indirectly benefiting other patients and the society

**Compensation for participation**

You will notreceive any payment for your participation in this research study.

**Confidentiality**

On the request paper your name or your identities will nottbe mentioned. Samples and information given by the participants will serve only for this research not for any other purpose.

**Person to contact**

Please direct any questions you may encounter during this study to the principal investigator.

AbayneshTeklu

Department of Medical Laboratory Sciences, College of Health Sciences

Addis Ababa University

Cell phone: +2519 11 391016

Email: abayeteklu@gmail.com

Consent form

This page contains an agreement signature to participate in the study entitled “Sero-prevalence of HBsAg among pregnant woman and assessment of vertical transmission in selected health facilities of, Addis Ababa, Ethiopia" So please read the following points and sign your signature at the end in the space provided.

I understand the objective of the study in “Sero-prevalence of HBsAg among pregnant woman and the assessment of vertical transmission and comparison of Rapid Test kit with ELISA, Addis Ababa, Ethiopia.”

1. I know that the left over sample (blood) that I gave is going to be used for this study only.
2. I understand that, all the information and the results are confidential.
3. I understand that I will not get any money for my participation.
4. All the information is explained by phlebotomist and Principal investigator.
5. I understand that my participation is voluntary and can withdraw anytime from the studyand this will not affect the service I am getting from the hospital.

Therefore, with full understanding of the situations I agree to give blood for laboratory analysis.

Signature of the participant: _____

Address of the participant: _____

Date: _____

## Annex II Amharic version of Participant Information sheet and consent form

በአዲስአበባዩኒቨርሲቲ፤ የጠ/ሳይንስኮሌጅየ ህክምና ላቦራቶሪትምህርትክፍልበሀለተኛዲግሪተማሪየ መሚቂያጥ ናትላይእንዲሰተፉተጋብዘዋል፡ ፡ እባክዎበዚህጥናትላይከመሳተፍዎበፊትከዚህቀጥለየ ሚ ኘዉንምንባብበጥሞኛያ ንብዛይመፈለሱ፤ ግልፅያልሆነ ነገርካጋጠመዎትይጠይቁ፡ ፡

**መግቢያ**

**የ ጥናቱርዕስየ ዚህጥናትርእስ**

“በጥቁርአንበሳሆስፒታልእናበጋንዲሆስፒታልየ ሄፓታይተስቫይረስበእርጉዝእናቶችላይእንዲህምባዉሊደወቅትከ እናትወደልጅያለዉየ መላለፊያመንገ ደለማጥናትይሆናል።እርስዎበዚህጥናትላይየ ሚሮትሳትፍበርሶመለሙ ለብበጎ ፈቃደኝነ ትላይየ ተመሠረተነ ዉ ፡ በዚህጥናትዉስጥላለመሳተፍከወሰኑእርሶምባዚህየ ህክምና ቦታዉስጥየ ሚ ሰጠዎትአገ ልግሎትአይቋዋረጥም ፡ በጥናቱለመሳተፍእርሶየ ሚሞክሆነ የ ስምሜ ትቅጸላይበጸጠፍወይምጣዉሬ ርሞትንሚቀመጥይጠበቅቦታል፡ ፡

**የ ጥናቱተሳታፊበመሆኔየ ሚበቅረኝምንደኑ ዉ**

የ ጥናቱተሳታፊበመሆንዎምንምነይነ ትተፅዕኖየ ደምፍእንዲሰጠአይጠየ ቁም።እርስዎለምርመራበሚጠቅደምና ቱየ ሚሂደይሆናልእንጀአዲስናፍእንዲሰጠአይጠየ ቁም ፡

**በዚህጥናትመሳተፍየ ሚያስገኛቸውጥቅሞች**

በጥናቱዉጤመሳረትየ ላብራቶሪዉጤቶቹንለሚዳትሚተካከያይደረግበታል፡ ፡ ስለዚህምባሚኖቸመቀያየ ርምክን ያትየ ሚመጣዉጤመላያየ ትያስቀራል፡ ፡ በጥናቱበመሳተፍዎእርሶምሆኑየ እርሶልጅ/ልጅቸበተዘዋዋሪመንገ ደለ ሊሎችህመሞችበሽምህብረተሰቡይጠቅማሉትነ ው።

**በዚህጥናትበመሳተፍየ ሚከፈልክፈያ**

በዚህጥናትስለተሳተፉእርሶምሆኑየ እርሶልጅ/ልጅቸምንምነይነ ትክፍያአይከፈልም

**የ ተሳታፊዎቸሚጠርስለመብቅ**

በማየ ቂያወቀረቅትላይየ እርሶስምየይሙንነ ትኣይገ ለጸም።በተሳታፊዎቸየ ሚጥናመፍለዚህጥናትጥቅምባቸየ ሚ ያገ ለግልይሆናል።

ጥያቄካለዎትይህንጥናትበተመለከተወይምካዘሀጋረበተዛመደመልከስለሚጋጥመውን ተናችግሮችወይምጥያቄካለት በሚከተለዉአድራሻይጠቁሙ፡

አባይነ ሽተክሉ

የሕክምና ላብራቶሪሳይንስትምህርትክፍል፤ የጤና ሳይንስኮሌጅ፤ አዲስአበባዩኒቨርሲቲ

ሞባይል: +2519 11 391016

ኢ-ሜይል: abayeteklu@gmail.com

የስምምነ ትመጠየ ቅያቅጽ

የጥናቱተሳታፊመለያቁጥር፡ _____

የዘህጥናትርእስ“በጥቁርአንበሳሆስፒታልእናበጋንዲሆስፒታልየ ሄፓታይተስቫይረስበእርጉዝእናቶችላይእንዲሁ ምጣዉሊደውቅትከእናትወደልጅያለውየ መተላለፊያመንገ ድለማጥናትይሆናል።እባክዎትንከዘህበታቸየ ተዘረዘሩነ ጥቦችበጥሞያንብበእናበሚፈሻቸበተሰጠውክፍትቦታይፊርሙ::

1.

የዘህጥናትርእስ“በጥቁርአንበሳሆስፒታልእናበጋንዲሆስፒታልየ ሄፓታይተስቫይረስበእርጉዝእናቶችላይእንዲሁ ምጣዉሊደውቅትከእናትወደልጅያለውየ መተላለፊያመንገ ድለማጥናትይሆናል።የ ሚሄደውንጥናትዓላማዙረድቻለሁ ::

2. እኔየ ምነጠውናመጥለዘህጥናትበቻእንደሚፈልአወቂያለሁ፡፡

3. ለጥናቱየ ምነጠውናመጥእንዲህምጣጠበሚጥርእንደሚገዙተረድቻለሁ፡፡

4. በጥናቱበመተፊእኔየ ሚፈለንክፍያእንደሌለአወቂያለሁ፡፡

5. ሀለምየ ሚስፊልገ ወንነ ገርበተሞመሪወይበራሪልናል።

6.

በዘህጥናትላይየ ሞረኝተሳትፎመጨበመጨበበጎ ፈቃደኝነ ትላይየ ተመሠረተመሆኑን፣ በማናዋምጊዜመቂረጥእንደም ችልናበዘህጥናትዉጥበመተፍእኔ ምወይምእኔበዘህየ ህክምናቦታዉጥየ ሚጠኝንአገ ልግሎትእንደሚቋረጥተረ ድቻለሁ ፡

ስለዚህከላይየተጠቀሱትንነ ጥቦችበሚዳትናመፍ(ደምን ለመቀጠልተስማምቻለሁ።

የተሳታፊፈርማ _____

ቀን፡ _____

## Annex III StandardOperatingProcedure (SOP)

Specimen collection, handling and Transport

**Purpose**

This procedure provides instructions on specific procedures for blood specimen collection from adults with proper specimen identification and handling and transportation.

**Abbreviations**

- HBsAg= Hepatitis B surface antigen

**Materials**

- **Supplies**
  - ✓ Gloves
  - ✓ Spirit swabs
  - ✓ Tourniquet
  - ✓ Collection needles compatible with the Vacutainers
  - ✓ EDTA Vacutainer tubes
  - ✓ A tube rack which fits the Vacutainer tubes
  - ✓ Gauze Sponges
  - ✓ Band-aid
  - ✓ Sharps disposing Container
  - ✓ Cool packs (not frozen)

**Equipment**

- ✓ Vaccine carrier cool box or
- ✓ Cold box/or sample packaging container/box

**Sample**

- ✓ Sample type: - Whole blood collected in EDTA tube
- ✓ Amount required:-4ml form this
- ✓ Transport and storage:- (2-8$^0$C)
- ✓ Stability:- 7 days after collection at 2-8$^0$C and-21$^0$Cmonths

**Limitation**:-Heamolysed and clotted samples interfere with test results.

**Sample retention:** Samples are retain on deepfreeze .

**Special Safety Precautions**:-Refer to the Laboratory Safety Manual for standard safety procedures

- ✓ Follow infection prevention principle during sample collection
- ✓ Wear gloves for handling blood or serum
- ✓ Change gloves when they become contaminated
- ✓ Wash hands after handling specimens
- ✓ Wear protective clothing
- ✓ Decontaminate working area with 5% bleach solution

Any forms that are sent in contact with the specimens (i.e. wrapped around the specimen container) should be discarded after the proper information is obtained. They are not to be filed.

**Procedure**

- ✓ Label tubes with the client's name/identification number. (Labeling can also be done immediately after the specimen is obtained).
- ✓ Explain the blood drawing procedure to the client and reassure him/her.
- ✓ Wear the rubber gloves and make the patient a comfortable position
- ✓ Prepare the vacutainer tube, citratetubeand needle
- ✓ Tie the tourniquet around the arm of the patient just above the bend in the elbow. The tourniquet should be positioned 7.5cm to 10cm above the puncture site.
- ✓ Tell the patient to clench his/her fist
- ✓ Using the tip of the index finger examine the phlebotomy site , feel the vein, and decide exactly where to place the puncture

- ✓ Disinfect the phlebotomy site by swabbing the skin in small outward circles with alcohol swab or cotton wool soaked inisopropyl alcohol. Do not touch the prepared puncture site with your fingers after disinfecting the skin.
- ✓ Insert the needle directly into the vein and withdraw peripheral blood of approximately 4ml in the syringe and dispense in EDTAtube
- ✓ Tell the patient to open his/her clenched fist
- ✓ Release the tourniquet
- ✓ Withdraw the needle from the vein and cover the puncture site cotton swab and hold (or have the subject hold) pressure at the puncture site for 3 minutes or until adequate haemostasis is visible.
- ✓ Properly discard the used materials in a safe container and tell the subject to do so if handled the cotton swabs to stop the bleeding

**Sample Handling and Storage**

- ✓ Mix the blood properly by inverting the tube 6-8 times immediately after collection
- ✓ Label the tube with patient's identification, date and time of collection as well as the name of the collecting personnel.
- ✓ Double check the tube labels for accuracy with the sample request form
- ✓ Sample should reach the laboratory within 30min of phlebotomy.
- ✓ Do not refrigerate or freeze the specimens.
- ✓ Centrifuge and perform the test
- ✓ The samples should be rejected if they are clotted, hemolysed or frozen

**Sample transportation**

- ✓ Make sure that the lab request & specimen transports forms are filled properly.
- ✓ Make certain that the test tube labeled properly and clearly.
- ✓ Ensure that the outside of the specimen container is clean and uncontaminated.
- ✓ Check if the test tube (container) closed tightly so that its contents do not leak during transportation.
- ✓ Place test tube into test tube rack aseptically in appropriate manner.
- ✓ Pace tube rack in the transportation bag close tightly
- Deliver to main laboratory without shaking and mixing

**Clinical Utility**

serological analysis gives useful information for the patients and the sample must be handling carefully as infectious material

**HBsAg Test Kit**

**Purpose**

This procedure provides instructions how to perform HBsAg test whichisa rapid,qualitative,twositesandwichimmunoassayforthedetectionofHepatitis Bsurfaceantigen, amarkerforHepatitisBinfections,inserum/plasmaspecimen.

**Principle**

Asatestsampleflowsthroughthe membrane assemblyofthetestdevice, the colored monoclonalanti-HBsAg-colloidalgoldconjugatecomplexeswiththeHBsAg inthesample.Thiscomplexmovesfurther onthemembranetothetestregionwhereitisimmobilizedbyanothermonoclonalanti-HBsAgantiserumcoatedonthe membraneleadingtoformationofa pink-purplecoloredbandwhichconfirmsapositivetestresult. Absenceofthis coloredbandinthetestregionindicatesa negative testresult.Theunreactedconjugateandunboundcomplexifany movefurtheronthemembraneandaresubsequentlyimmobilizedbytheanti-rabbitantiserumcoatedonthemembrane atthecontrolregion,formingapink-purpleband.Thiscontrolbandservestovalidat the testresults.

**Clinical utility**

DetectionofHBVusingHBsAgasamarkertoscreenblood donorsis essential toreduce theriskoftransmissionofHepatitisBbybloodtransfusion.HBsAgdetectionisalsousefulforscreeninghighriskgroupsforHBV.

**Reagents stability and storage:**

Thesealedpouchesinthetestkitmaybestoredbetween4-$30^0$Ctilltheduration oftheshelflifeasindicatedonthepouch.**DONOTFREEZE!**

**Supplies**

- Disposableplasticdropper
- Disposable Gloves
- Packageinsert

**Equipment**

- Timer
- Micropipettes

**Limitations:**

Donotusehemolyzed,turbidorcontaminatedsamples.Turbidsamplesshouldbecentrifugedandclear supernatantmustbeusedfortesting

**Sample retention:** Samples are discarded after examination.

**Social safety precautions**

- Wear gloves, eye protection, and protective clothing at all times in the laboratory**.**
- Any chemical spilled on your skin or your clothing, should be washed immediately and thoroughly.
- Refer to safety manual for additional safety precaution information.

**Procedure**

- ✓ Bringthesealedpouchtoroomtemperature,openthepouchandremovethedevice.*Onceopened, thedevicemust beusedimmediately.*
- ✓ Dispense two drops (50µl) of serum/plasma specimen into the sample well 'S' using the dropper provided
- ✓ At theendoffifteenminutesreadtheresultsasfollows:

**NEGATIVE: Only**onepink-purplebandappearsatthecontrolregion'C'

**POSITIVE**: Inadditiontothecontrolband, apink-purplebandalsoappearsatthetestregion'T'.

**NOTE:**

Thetestshouldbeconsideredinvalidifneitherthetestbandnorthecontrolbandappears.*Repeatthetestwithanew device*

**Result Interpretation**

- **NEGATIVE**:Onlyonepink-purplebandappearsatthecontrolregion'C'
- **POSITIVE**:Inadditiontothecontrolband,apink-purplebandalsoappearsatthetestregion'T'

**Limitation**

Interferenceduetoheterophileantibodies,Rheumatoidfactorsandothernonanalytesubstancesinpatient'sserum, capableofbindingantibodiesmultivalentandprovidingerroneousanalytedetection in immunoassays,hasbeenreportedinvariousstudies

**HBsAg ELISA TEST USING DIALAB KIT**

**Materials and Equipment required**

- ✓ Refrigerator
- ✓ Distilled water
- ✓ Micro plate (ELISA) reader
- ✓ ELISA kits
- ✓ ELISA washer
- ✓ TTI forms
- ✓ Computer
- ✓ Pipette tips
- ✓ Micropipette (single & multi
- ✓ channel)
- ✓ Waste container
- ✓ Beaker
- ✓ Timer
- ✓ Printer
- ✓ Micropipettes rack
- ✓ Incubator
- ✓ Paper towel
- ✓ Trough
- ✓ Test tubes
- ✓ Marker
- ✓ Centrifuge
- ✓ Test tube rack
- ✓ pipette rack
- ✓ Paper
- ✓ Scissors
- ✓ Pen (Blue & Red)
- ✓ tips rack

**Safety:**

Use Personal protective equipment and consider all materials used in the assay as potentially Infectious. TMB substrate should not come in to contact with metal or bench surface.

**Principle and Procedural Steps:**

**ELISA tests are carried out as per the instructions given in the package insert of the kit.**

**Principle:**

This HBsAg ELISA kit uses antibody sandwich ELISA method in which polystyrene micro well strips are pre-coated with monoclonal antibodies specific to HBsAg. Serum or plasma sample is added to the micro well together with a second antibody conjugated with horse radish peroxides (HRP) and directed against a different epitope of HBsAg. During incubation, the specific immune complex formed in case of presence HBsAg in the sample is captured on the solid phase. After washing to remove sample serum proteins and unbound HRP conjugate, chromogen solutions containingTetramethylbenzidine (TMB) and urea peroxides are added to the wells. In presence of the antibody- Antigen antibody (HRP) "sandwich" immune complex, the colorless chromogens are hydrolyzed by thebound HRP- conjugate to a blue colored product. The blue color turns yellow after stopping the reactionwith sulfuric acid. The amount of color can be measured and is proportional to the amount of antigen inthe sample. Wells containing samples negative for HBsAg remain colorless.

**Procedural steps:**

- ✓ Bring all reagents to room temperature (18-24oc) at least for 30 minute before the assay.
- ✓ Receive serum/plasma from ABO serology unit
- ✓ Inspect samples for appropriate labeling and volume.
- ✓ Sequence the samples according to the pack number order
- ✓ Write test sample numbers on HBsAg screening Protocol
- ✓ prepare washing buffer solution (look kit insert for preparation)

- ✓ Perform the tests according to kit insert
- ✓ Add 50µl negative control to A1, B1 and C1
- ✓ Add 50µl positive controls to D1 and E1
- ✓ Add 50µl donor serum/plasma to the rest wells starting from F1
- ✓ Add 50µl enzyme conjugate to all wells including controls
- ✓ Seal and Incubate at 37$^{o}$c for 30minutes
- ✓ Wash one cycle and tap on absorbent paper and repeat 5 times for a total of 6 wash
- ✓ Tap the plate on the paper towel to remove any remaining fluid
- ✓ Add 50µl chromogenA first
- ✓ Add 50µl chromogenB
- ✓ Seal and Incubate at 37$^{o}$c for 10minutes in Dark
- ✓ Turn the reader "on" to stabilize the lamp
- ✓ Add 50µl stopping solution
- ✓ Load the plate on the reader and select HBsAg for DIALAB program from "SESSION/MENU"
- ✓ Read the absorbance at 450nm and (630nm reference wave lengths).
- ✓ Read the test by pressing start key
- ✓ Print test results
- ✓ Evaluate and validate the results
- ✓ Make sure that the printout is read with appropriate program
- ✓ Inspect the reading result with color developed on the plate.
- ✓ Make sure that mean absorbance of negative control must be < 0.1 and the mean
- ✓ absorbance of positive control must be > 0.6
- ✓ Reject and rerun if the test is invalid
- ✓ If the test is valid Calculate cutoff results according to the criteria provided in the Kit
    - o Insert. Cutoff value = NCx * 2.1
- ✓ Use 0.05 instead of the actual mean In case the mean of negative control replicates is <
    - o 0.05,
- ✓ Take Gray zone 10% to the Negative direction of cutoff valueGray zone = (NCx * 2.1)*0.9
- ✓ Interpret Sample with OD greater or equal to gray zone is reactive
- ✓ Interpret Sample with OD less than gray zone is nonreactive

- ✓ Encircle new reactive pack numbers with red pen on protocol
- ✓ Record reactive pack numbers on the space provided on protocol
- ✓ Collect your reactive bags
- ✓ Register your reactive pack numbers on
- ✓ Accept for validity of the test by laboratory case team coordinator
- ✓ Approve the validity of the test by quality officer
- ✓ Put the reactive Blood and Blood product in quarantine until their test result is confirmed
- ✓ File result print outs & respective protocol
- ✓ Prepare untested samples for the next test including reactive samples and their respective segments
- ✓ Re-test reactive Donations both from test tube and respective bag segment on the next test.
- ✓ Underline twice with red pen repeated reactive pack numbers on
- ✓ Record repeat reactive pack numbers on the space provided on
- ✓ Discard all repeat reactive blood and Blood products after acceptance and approval
- ✓ Recheck the test results prior to releasing blood and blood products
- ✓ Release non reactive blood and Blood product for distribution on safe blood product release form
- ✓ Archive your entire sample and store at least for five years
- ✓ If test tube and segment have discordant result asses the problem
- ✓ Check that correct test tube and segment is tested
- ✓ Rerun the test from the same pack number if not tested from the correct sample
- ✓ repeat all batches from test tube if the discrepancy is from test tube
- ✓ Repeat all batches from segment if the discrepancy is from bag segment
- ✓ Test repeat reactive samples with rapid test and report the results for post donation counselingpurpose

Cord Blood Collection

Aim

Cord Blood Collection using Butterfly Needle and Vacutainer Tubes:

- ✓ After the delivery of the infant, double clamp the umbilical cord and cut the umbilical cord as usual.
- ✓ Apply the first clamp near the placenta.
- ✓ Apply the second clamp to the cord on the baby side.



- ✓ Cleanse a 4”- 6” area of the umbilical cord with alcohol followed by betadine to remove maternal blood and contaminants (before the delivery of the placenta, if possible).
- ✓ Using the butterfly needle and vacutainer tubes:




- ✓ Collect cord blood in a SST or NON (red top) tube(s) for serum storage.
  NOTE: collect serum before EDTA or any other anticoagulant containing tube
- ✓ Collect cord blood in EDTA tube(s) for plasma and PBMC storage.
- ✓ Mix tube by inverting 8-10 times.
- ✓ Process specimens within one hour of collection.
- ✓ The Umbilicup Umbilical Cord Blood Collection System is packaged sterilely and holds approximately 100mL. It includes at its **bottom a needle**, which is enclosed to prevent needle sticks.

- ✓ Use standard vacutainer-type tubes with rubber caps when collecting the sample with the Umbilicup.
- ✓ Open the package using sterile technique. Remove the Umbilicup from the sterile packaging and place on a sterile field near the mother. WORK QUICKLY as blood clots rapidly
- ✓ After the delivery of the infant and placenta, double clamp the umbilical cord and cut the umbilical cord as usual. When placing the clamps on, apply the first clamp near the placenta and apply the second clamp on the baby side.
- ✓ Wipe the umbilical cord with alcohol followed by betadine to remove maternal blood and contaminants (before the delivery of the placenta, if possible).
- ✓ Remove the lid of the Umbilicup; position the Umbilicup under the cut end of the umbilical cord.
- ✓ Slowly release the clamp, allowing as much umbilical cord blood as possible to drip into the Umbilicup. The user may also milk the umbilical cord to obtain a larger volume of blood, as needed.
- ✓ Place the cap on top of the Umbilicup.
- ✓ Using SST tubes
- ✓ Collect cord blood in SST tubes for blood and serum collection.
- ✓ Insert an empty SST or red top tube into the needle sleeve at the bottom of the Umbilicup. Collect blood in the vacutainer tube(s).
- ✓ Place the SST tube in the refrigerator
- ✓ Using EDTA Tubes
- ✓ Collect cord blood in EDTA tube(s) for plasma and PBMC storage
- ✓ Mix EDTA tube by inverting 8-10 times.
- ✓ Keep EDTA tube at room temperature until delivery to the lab
- ✓ Ensure all samples are labeled with patient's PID and suffix "CRD". All sample labels should contain the time and date of collection as well as the time and date of delivery
- ✓ The cord blood can also be collected using your clinic or hospital's collectionprocedures as long as the safety procedures are followed and contamination of cord blood is avoided[27].

## Annex IV Data collection Log Sheet

Addis Ababa University, College of Health Sciences,

Department of Medical Laboratory Sciences

Data collection Log Sheet

| Number | Date | Code no | Age | Sex | Gestational age | Rapid test kit result | ELISA result | Remark |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |

## Questionnaire (መጠይቅ)

Addis Ababa University, College of Health Sciences,

በአዲስአበባዩኒቨርሲቲ፤ የጤናሳይንስኮሌጅ

Department of Medical Laboratory Sciences

የህክምናላቦራቶሪት/ክፍል

1. Code No(መለያቁጥር)_____
2. Age(እድሜ)_____
3. Living Address:(የመኖሪያአድራሻ)

   Urban(ከተማ) [ ] l (ገጠር) [ ]
4. Marital status(የጋብቻሁኔታ)

   Single(ያላገባ) Marri[ ]ባ)Divorced (የተፋታ[ ] [ ]
5. Educational status (የትምህርትደረጃ)

   Primary School (የመጀመሪያደረጃ) [ ] Secondary school(ሀለተኛደረጃ) [ ]

   diploma(ዲፕሎማ) degre[ ]) Above degree(ከ[ ]ላይ) [ ]
6. occupation (የስራሁኔታ)

   Government(የምንግስት) [ ]vernment(የመንግስትያልሆነ )others(ሌለ) [ ] [ ]
7. religion (ሃይማኖት)

   Orthodox(ኦርቶዶክስ) [ ]rotestant (ፕሮቴስታንት)Muslim([ ] catholic(ካቶሊክ) [ ]

   Others(ሌለ) [ ] [ ]
8. Abortion(ወርያአደርግወያወቃል)

   yes(አዎ)n[ ] [ ]
9. Blood transfusion (ደምማሰጥሽያወቃል)

   yesአዎno[ ] [ ]
10. are you take HBV vaccine (የሄፓታይተስቫይረስክትባትወስደዋል)

    yesአዎno[ ] [ ]
11. Is this first time pregnancyየመጀመሪያእርግዝናዎነው

yesአዎno☐ ☐

12. What is HBV mode of transmission by(የ ሄፔታይተስቫይረስበምንይተላለፋል)

Bysex(በግብረስጋግንኙ ት)☐needle(በተከለሞፊ) ☐

Mother to child (ከናትወደልጅ)AI☐ none (በ☐ ☐

## Declaration

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

Name: **AbayneshTeklu( BSc, MSc candidate)**

Signature _____

Place: Addis Ababa University, Department of Medical Laboratory Sciences, Ethiopia

Date of submission _____

This thesis has been submitted with our approval as University Advisors.

Name:KassuDesta PhD Candidate, Associate Professor

Signature _____

Place: Addis Ababa University, Department of Medical Laboratory Sciences, Ethiopia.

Name: Dr. Aster Tsegaye, PhD, Associate Professor

Signature _____

Place: Addis Ababa University, Department of Medical Laboratory Sciences, Ethiopia.