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Seroprevalence of HBV, HIV and Associated Factors among Pregnant Women Attending Selected Health Centers at Yeka Sub City, Addis Ababa, Ethiopia

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This is to certify that the thesis prepared by Yibeltal Getaneh entitled: ***Seroprevalence of HBV, HIV and Associated Factors among Pregnant Women Attending Selected Health Centers at Yeka Sub City, 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) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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Abbreviations

AAU	Addis Ababa University.
AIDS	Acquired Immunodeficiency Syndrome
ANC	Antenatal Care
AOR	Adjusted Odd Ratio
COR	Crude Odd Ratio
ELISA	Enzyme Linked Immunosorbent Assay
EPI	Expanded Program on Immunization
HBsAg	Hepatitis B Surface Antigen
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
HRP	Horseradish Peroxidase
HSV	Herpes Simplex Virus
MDGs	Millennium Development Goals
MTCT	Mother to Child Transmission
PICT	Provider Initiated Counseling and Testing
PMTCT	Prevention of Mother to Child Transmission
SOP	Standard Operating Procedures
SPSS	Statistical Package for the Social Sciences
WHO	World Health Organization

Abstract

Background: Hepatitis virus causes highly contagious viral infection, which kills millions globally than any other diseases. Among those hepatic viruses, Hepatitis B virus plays pivotal role in causing hepatic failure. Morbidity and mortality increases when it combines with Human immunodeficiency virus which negatively impacts the natural history of Hepatitis B virus, increasing the risks for cirrhosis or hepatocellular carcinoma. Infection with Hepatitis B virus and Human immunodeficiency virus leads to increased morbidity and mortality as compared to hepatitis B virus infections and independent Human immunodeficiency virus

Objective: To assess seroprevalence and associated factors of HBV, HIV infections among pregnant women attending ANC at selected Health Centers of Yeka Sub City, Addis Ababa, Ethiopia

Methods: A health facility based cross sectional study design was conducted among 400 pregnant women attending four randomly selected Health centers of Yeka Sub City, Addis Ababa, Ethiopia between February and March 2018. Convenient sampling technique was used to enroll the pregnant women. A pretested structured questionnaire was used to collect socio-demographic characteristics and possible associated factors. HBsAg was determined using rapid screening test. Positive samples were confirmed by enzyme linked immunosorbent assay. In addition to this, antibodies to HIV-1/2 were tested based on the national testing algorithm of Ethiopia. Descriptive statistics and logistic regression analysis were done and a P value less than 0.05 was considered statistically significant. Data was analyzed using SPSS version 22.

Result: A total of 400 pregnant women, of whom 4.8% of them were unmarried, enrolled in this study. Seroprevalence of 2.5% (10/400) and 0.5% (2/400) were found for HBsAg and HIV, respectively with co-infection rate of 0.25%. None of the socio-demographic and potential risk factors showed significant association, except history of surgery which showed significant association with HBV infection with an AOR=5.56, 95% CI, 1.14-27.27.

Conclusion: the prevalence of current study implicates a strong need to work on the prevention of HBV and HIV transmission to the community. All pregnant women need to access screening for HBV, and HIV in their antenatal care to prevent HBV and HIV transmission.

Key words: HBV, HIV, Pregnant Women

1. Introduction

1.1Background

According to WHO 2017 global hepatitis report, an estimated 257 million people were living with hepatitis B virus infection (defined as hepatitis B surface antigen positive) (1). In 2015, the global prevalence of HBV infection in the general population was 3.5%. Prevalence was the highest in the African region (6.1%). Assuming that women of reproductive age constitute 25.3% of the world's population (United Nations data), adults chronically infected may include 65 million women of childbearing age who can potentially transmit HBV to their babies (2). Perinatal infection is the predominant mode of transmission. Approximately 10%-20% of neonates born to hepatitis B surface antigen (HBsAg)-positive mothers and 90% of those born to both HBsAg- and hepatitis B e antigen (HBeAg)-positive mothers will become infected with HBV (3).

Viral hepatitis during pregnancy is associated with a high risk of maternal complications, has a high rate of vertical transmission causing fetal and neonatal hepatitis and has been reported as a leading cause of maternal mortality (4). In hepatitis B infection, most of the vertical transmission (85%) occurs in the peripartum period by ingestion of infected maternal fluid and only 15% transplacentally.10% of infants born to women with acute HBV infection during the first trimester of pregnancy are HBsAg-positive at birth and 80 to 90% of neonates become HBsAg positive without prophylactic therapy if acute maternal infection develops during the third trimester of pregnancy (5).

HIV and HBV are common public health problems recognized worldwide. According to WHO 2017, about 2.7 million of the 36.7 million living with HIV are also infected with HBV (2). Globally, there were still more than 1.4 million (1.3 million– 1.6 million) pregnant women with HIV in 2013 (all whom needed interventions for PMTCT of HIV) in low- and middle-income countries (6). HIV is the leading cause of death in women of reproductive age globally. Since nearly all HIV infections in children are acquired from their mothers, the global epidemiology of HIV in children reflects that of HIV in women. It has been estimated that, in 2008, 1.4 million HIV-infected women gave birth in low- and middle-income countries and that there were 430 000 new pediatric infections (7).

Co-infection rate of hepatitis B virus and HIV is common which leads to increased morbidity and mortality as compared to HIV or HBV mono-infections (8). The prevalence of HIV,HBV co-infection is reported as high as 10-20% in countries where HBV infection is either endemic or intermediate to high (8). Moreover, in countries where the viruses are highly endemic, the rate can be as high as 20-25% (8).

There are three mechanisms of HBV trans-mission from HBsAg positive mothers: (i) trans-placental intra-uterine transmission; (ii) transmission during delivery by contact with maternal infected fluids in the birth canal; and (iii) postnatal transmission from mothers to infants during child care or through breastfeeding (9).They are also mainly transmitted through blood transfusion, sexual contact, or contact with contaminated surgical instruments or body fluids of infected persons(10). Sub-Saharan Africa has a high endemicity, and more than 50 million people are believed to be chronic carriers of the HBV (9). Two thirds of all HIV/AIDS infected persons and 75% of all HIV infected women live in Sub-Saharan Africa (11).

Few previously conducted studies and clinical reports in Ethiopia indicated that the burden of liver disease is posing great health problems. Of those research findings, a nationwide research report has shown that the prevalence of HBV found to be 5% among pregnant women(12). The overall of prevalence of HIV among pregnant women attending antenatal care (ANC) was 5.3%. Moreover, the prevalence was higher in pregnant women from urban 9.5% than rural areas 2.2% (8).

As part of reducing maternal and child mortality to achieve one of the millennium development goals (MDGs), Ethiopia rolled out childhood immunization against HBV in 2007 (13). The vaccine is delivered in a pentavalent form as part of expanded program on immunization (EPI) of newborns. In spite of this, routine antenatal screening for HBV infection and vaccination is lacking in many Ethiopian health facilities. Newborns are vaccinated without a prior screening of mothers for underlying hepatitis infection. This study aims to assess the current Seroprevalence of HBV, HIV and associated factors among Pregnant Women attending selected health centers in Yeka sub city, Addis Ababa, Ethiopia.

1.2 Statement of the problem

Hepatitis B and Human immunodeficiency viruses exert a high toll worldwide. Both can lead to chronic disease, cancer, and death, and neither can be eradicated with the use of current therapies (14). Hepatitis B transmission is similar to that of HIV; therefore, co-infection with HIV and hepatitis B is not unusual (15). The risk of perinatal transmission of HBV is 70% – 90% for infants born to mothers who are HBsAg and HBeAg positive, but it decreases to 5% – 20% for infants born to anti-HBe positive mothers (16). However, previous studies reported the co-infection rate of 22.6% (17).

Hepatitis B virus and HIV are posing a paramount health impact especially in pregnant women and their neonates. The economic impact of these infections is immense since liver cancer has a high fatality rate in Africa and usually affects economically productive age groups. (17).

In Ethiopia as part of other sub-Saharan Africa countries, the prevalence of HIV and HBV is high and posing a great public health problem. Apart from its significant prevalence, liver disease contributes approximately 12 % of the hospital admissions and 31 % of the mortality in medical wards of Ethiopian Hospitals (18). A research conducted in Tirunesh Beijing General Hospital, Addis Ababa ,Ethiopia by Desalegn *et al*, in [2014] to determine Hepatitis B and human immunodeficiency virus co-infection among 215 pregnant women revealed a prevalence rate of HBV 13 (6%) and HIV 4.2% (18).

Both Hepatitis B virus and Human immunodeficiency virus are important public health problem. HBV and HIV cause hepatic cancer and death. Understanding the knowledge and practice about HIV/HBV infection helps in formulating a strategy for prevention and treatment of HIV and HBV. Continuous monitoring of the magnitude of HBV, HIV among pregnant women is important as treating those HBV,HIV infected mothers before delivery prevent transmission around birth (2) However, there are no many researches done at primary health care level where it is important to define the seroprevalence of HBV, HIV infection among pregnant women.

1.3 Significance of the study

Hepatitis B virus and HIV are the main health impacts worldwide which causes paramount risk for the transmission of the diseases especially in pregnant women. The current study was significant to

- Policy makers to get more evidences to support their decision in the effort to meeting MDGs related to maternal and child health
- Pregnant women to help them get appropriate treatment and prevent transmission to their newborns.
- Federal ministry of health, Addis Ababa health bureau and different health institutions for appropriate planning and design intervention strategies based on the magnitude and identified associated factors.
- Different researchers could get updated information.
- Non-governmental organizations which participate in combating HIV and HBV among pregnant women.
- Health centers which participates in this study for planning and designing appropriate intervention.
- Health professionals' improve managing the pregnant mothers and their new born.

2. Literature review

In 2015, the global prevalence of HBV infection in the general population was 3.5%. Prevalence was the highest in the African region (6.1%). Assuming that women of reproductive age constitute 25.3% of the world's population (United Nations data), adults chronically infected may include 65 million women of childbearing age who can potentially transmit HBV to their babies (2).

HBV and HIV are among the leading causes of infectious disease deaths worldwide. Hepatitis B virus co-infection with HIV is becoming a major challenge in developing countries (19). The two viruses are highly endemic in sub-Saharan Africa. Pregnant women who are co-infected with HBV and HIV are highly viremic for HBV and may be at a high risk of transmitting HBV to their infants (20). Variation in the Sero epidemiology of HBV and HIV in different countries has varied results in prevalence (21). For example, in sub-Saharan Africa, heterosexual exposures are responsible for most HIV infections (4). Sub-Saharan Africa accounts for most (65%) HIV infections worldwide and has a high prevalence of chronic HBV infection because of perinatal and early childhood transmission patterns (22).

2.1 Magnitude of HBV infection

In a study conducted in Greece by Papaevangelou *et al*, in [2006] on a screening program for HBV infection in a total of 3,760 delivering pregnant women, shows that prenatal screening for HBsAg was documented in 91.3%. HBsAg prevalence was 2.89%. Higher prevalence of HBV-infection was noted in immigrant women 9.8%. Other risk factors associated with maternal HBsAg positivity included young maternal age and absence of prenatal testing. No prenatal or perinatal HBsAg testing was performed in 3.2% women. Delivering in public hospital and illiteracy were identifiable risk factors for never being tested (16).

In Iran, a study done on the prevalence of HBV infection among pregnant women in 2015 on a total of 24853 women shows that Prevalence of HBV infection among pregnant women was estimated as 1.2%. Among different risk factors assessed, only familial history in four studies out of five relevant evidences was statistically significant (23).

Other study done in Jazan region, kingdom of Saudi Arabia, by Ibrahim B *et al*, in 2012 on the prevalence and risk factors of HBV among pregnant women shows an overall HBV prevalence

of 4.1%. The study identified past history of hospitalization and jaundices are important risk factors for transmission of the infection (24)

In a cross sectional study conducted in Southwest Nigeria by Chinenye Gloria A.*et al*, in 2013 prevalence, socio-demographic features and risk factors of Hepatitis B virus infection among 180 pregnant women were determined. Seroprevalence of HBsAg was 8.3% out of which 26.7% were positive for HBeAg, 53.3% had HBeAb, 20% had neither HBeAg nor HBeAb. The highest HBV infection rate occurred in 25-29 age groups. Multiple sexual partners (OR= 3.987) and early age at sexual debut (OR- 11.996) were independent risk factors for HBV infection (25).

A study conducted on the seroprevalence of HBsAg and factors associated in 289 pregnant women in Dawuro zone, SNNPR, Southwest Ethiopia by Chernet *et al,* in 2015 shows an overall prevalence of 3.5%. Multiple sexual partner (AOR = 6.923), and abortion history (AOR = 4.975), were significantly associated with hepatitis B virus surface antigen (HBsAg) infection (26).

In a hospital-based cross-sectional study conducted in Southern Ethiopia, by Metaferia Y.*et al*, in 2015, among the 269 pregnant women investigated the overall seroprevalence of HBsAg was 7.8% and the prevalence of HIV infection was 5.2%, of whom two participants (14.2%) were also positive for HBsAg. Pregnant women with no formal education (odds ratio, 3.68) were more likely to be infected with HBV than those who had completed at least secondary school. Although HBsAg was detected more often in pregnant women who had multiple exposure factors (8.8%) than in pregnant women who had not experienced possible risk factors (4%,), this difference was not statistically significant (OR, 2.33) (27).

A cross sectional study was conducted in selected health facilities of Addis Ababa Ethiopia by Tegegne et al, (2012) to determine seroprevalence and transmission of Hepatitis B virus among delivering women and their new born. From the total of 265, 3.0% of mothers were positive for HBsAg, whereas 2.3% of cord bloods were positives with 75% concordance rate of exposed infants with seropositive mothers. However, only one maternal positive case was observed for Hepatitis BeAg test. Only 11% of the mothers know their Hepatitis B Virus status. Of the total mothers assessed for possible risk factors, 26% had only one type, while 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. The remaining 13.2% of the participants had not experienced possible risk factors (28).

2.2 Magnitude of HIV infection

In a cross-sectional study conducted in Brazil by Angelica E *et al*, in 2010 to determine the prevalence of HIV among young pregnant women, aged 15-24 years of age a total of 2071 pregnant women were involved. Of these women who were attending in Brazilian public hospitals the overall prevalence of HIV infection was 0.7%. This shows that higher prevalence than women of all ages in Brazil (29).

National hospital based study conducted in Brazil, by Dominguez *et al*, on a total of 23,894 participants in 2011-2012 prenatal testing rates and prevalence of HIV during pregnancy was determined. The study shows that among participating women, the coverage of testing for HIV infection was 81.7% among those who presented with prenatal card. The prevalence of HIV infection among pregnant women was 0.4%. There was an increasing of HIV prevalence with increasing of maternal age and decreasing of schooling level. The study did not include miscarriage women (30).

A cross sectional study conducted on the utilization of HIV testing services among pregnant mothers in low income primary care settings in northern Ethiopia by Alemu *et al*, (2013) in East Gojjam, Ethiopia, a total of 416 pregnant women were studied. Of them, the proportion of mothers who tested for HIV was 277(67%). Among mothers who were not tested for HIV, lack of HIV risk perception (49%) was a major self-reported barrier for HIV testing. Pregnant women who had comprehensive knowledge about MTCT had an Adjusted Odd Ratio (AOR) of 3.73, having comprehensive knowledge on prevention of mother to child transmission (PMTCT) of HIV an AOR of 2.56 , and a favorable attitude towards persons living with HIV an AOR of 2.42 were more likely to be tested for HIV (31).

2.3 Magnitude of HIV-HBV coinfection

A research conducted in India by Vandana B *et al*, in 2015 on the Sero prevalence of HBV in 1600 pregnant women and its co-infection with HCV and HIV shows that the overall prevalence of HBV positivity was 2.37%. Out of 38 HBV positive samples, 4 (0.1%) were identified as HCV positive and 1 (0.03%) was HIV positive (4).

A study conducted in India by Jindal N *et al*. in 2012 to determine the prevalence of sexually transmitted infections (HIV, HBV, HSV and syphilis) on 500 asymptomatic pregnant women shows an overall prevalence of 4.8%. The highest prevalence was HBV (2.4%) followed by HSV-2 (2%) and HIV (0.4%). No woman was tested positive for syphilis and there were no multiple infections. All the infections were more common in illiterate, multigravida, monogamous women of low socio-economic status. High-risk sexual behavior of the husbands, history of sexually transmitted infections in husbands, and blood transfusions were the other factors associated with the prevalence of these infections (32).

A study conducted in Bamenda northwest region, Cameroon by Lem Edith A. *et al*, in 2015 to determine seroprevalence of HIV and HBV co infection among 301 pregnant women. The study reveals that the prevalence of HIV and HBV co-infection 5 (1.7%) was significant compared to mono infections of HIV (6.6%) and HBV (6%). A significant difference was observed when good knowledge of HIV and HBV were compared (94.0% vs. 11.3%) and also when good practices towards HIV and HBV (97.0% vs. 15.3%) were compared (6.0%) (33).

In Ekiti, South West Nigeria, a study was conducted by Thompson Joseph A.*et al*, in 2014 to determine HIV and HBV among 1433 pregnant women. In this study prevalence of HIV, HBV was 5.16% and 3.63% respectively, while 0.70% were co-infected with HIV and HBV with highest prevalence in the age group 31-40 years. This study showed high prevalence of both HIV and HBV infections with HIV higher than HBV (10).

In a cross sectional study conducted at Temeke municipal health facilities, Dar es Salaam, by Joel M.*et al*, in 2014, seroprevalence of hepatitis B virus and human immunodeficiency virus infections were determined among 249 pregnant women attending antenatal clinic. The overall prevalence of HBsAg and HIV were 8.03% and 17.2%, respectively. HBV/HIV co-infection rate was 2.8%. HBsAg positive rate was significantly high in women who were HIV positive (34).

In a community based study conducted in the democratic republic of Congo by J.M. Kabinda *et al,* in 2013, seroprevalence of Hepatitis B, Hepatitis C and HIV was studied in 581 Pregnant Women. The study shows that prevalence among pregnant women was 4.1% for HIV, 5.9% for the HBsAg and 4.1% for hepatitis C. The risk factors for HIV were, blood transfusion history in women and for HBsAg the main risk factor was the tattoo history and no statistically significant factor was found for hepatitis C (35).

In across sectional survey conducted on 13,121 pregnant women in Rwanda, Mwumvaneza M, *et al,* in 2017 aimed to study the prevalence of HBV and HIV coinfection among pregnant women. The prevalence of HBsAg was 3.7% and the proportion of HIV-infection among HBsAg-positive pregnant women was 4.1%. The prevalence of HBV-HIV co-infection was higher among women aged 15-24 years compared to those women aged 25–49 years (AOR = 6.9) (36).

A cross-sectional study conducted in Bahir Dar Northwest Ethiopia by Zenebe *et al,* [2013] on seroprevalence and risk factors of HBV and HIV infection among 318 pregnant women shows an overall prevalence of 6.6% HIV and 3.8% HBV. HIV/HBV co-infection rate was 19.0%.While previous history of blood transfusion (AOR = 3.7) body tattooing (AOR= 5.7), history of surgery (AOR = 11.1) and unsafe injection (AOR = 5.6) were significantly associated with HBV infection. Previous history of piercing with sharp materials (AOR= 3.0) and history of abortion (AOR = 6.6) were also statistically significantly associated with HIV infection (8).

A hospital based research conducted in Tirunesh Beijing General Hospital, Addis Ababa, Ethiopia by Desalegn *et al,* (2014) on a total of 215 pregnant women shows that the overall prevalence of HBV infection was 13 (6 %). Among the total, 9 (4.2 %) of the positive cases were detected among primary school completed. Multivariate analyses indicated that history of abortion ($p = 0.003$), history of surgery ($p = 0.022$), and tattooing ($p = 0.033$) were significantly associated with HBV infection. A total of 9 (4.2 %) women were found to be HIV seropositive, of whom 2 (22.2 %) were co-infected with HBV (18).

In general, the above studies done across the world can be ample evidence that still HBV and HIV are causing major public health problem worldwide, especially in pregnant women of African countries where their transmission is commonly high. Updated prevalence data will assist in making evidence based decision; hence, the current study tried to fill gaps of

information in primary health care level by studying pregnant women attending selected health centers of Yeka sub city in Addis Ababa.

3. Conceptual frame work

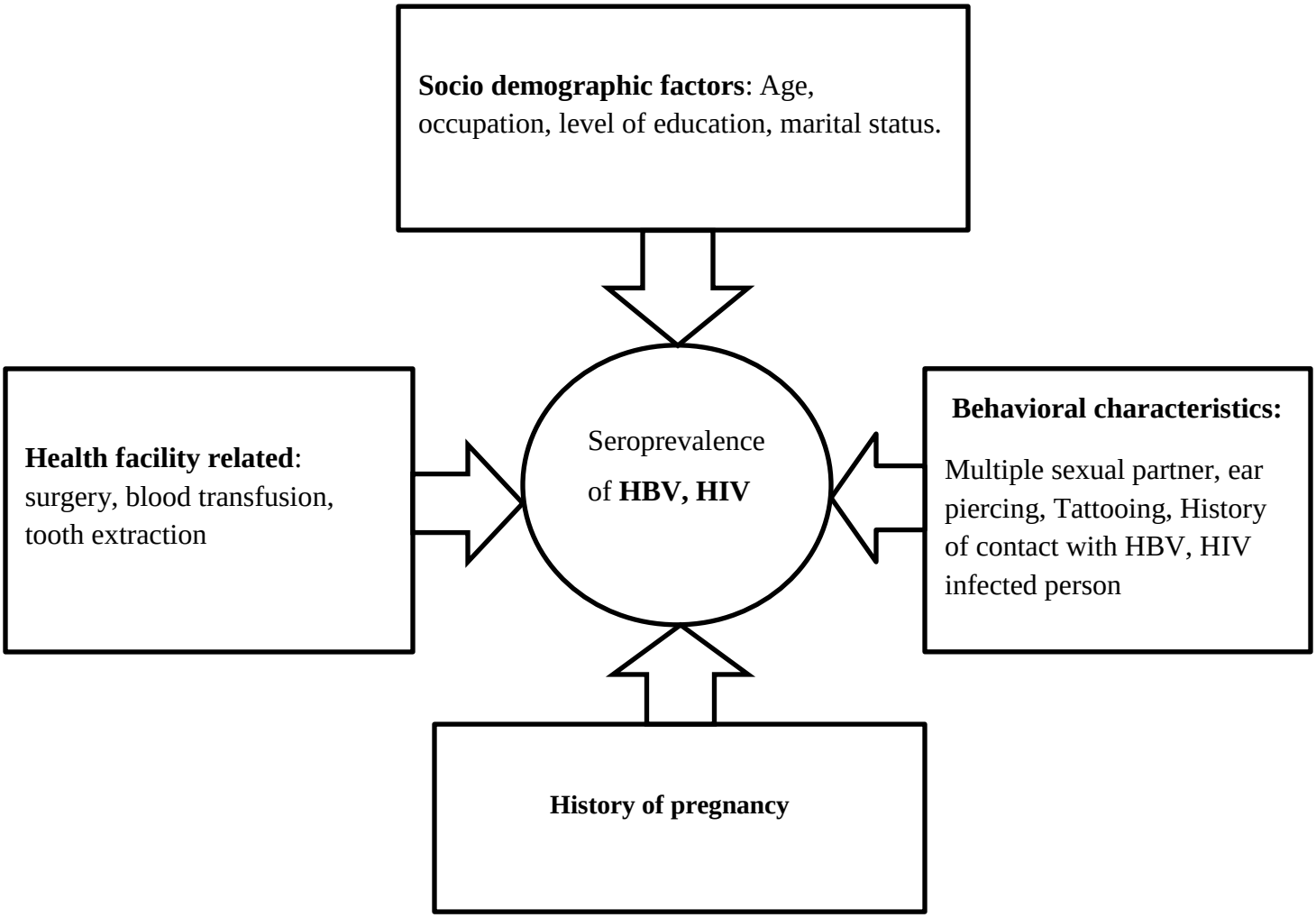

Figure 1**:** Conceptual framework for this study, Addis Ababa, Ethiopia, 2018.

4. Objectives

4.1. General objective

- To assess seroprevalence and associated factors of HBV, HIV infections among pregnant women attending selected Health Centers of Yeka Sub City, Addis Ababa, Ethiopia

4.2. Specific objectives

- To determine seroprevalence of HBV among pregnant women.
- To determine seroprevalence of HIV among pregnant women.
- To determine HBV/HIV co-infection rate among pregnant women
- To identify associated factors with HBV and HIV infections

5. Hypotheses

Null hypothesis

$H_o=H_1$

- There was a difference in the prevalence of HBV, HIV infections among pregnant women with that of previous studies in Addis Ababa.

6. Materials and methods

6.1. Study area

Yeka is one of the ten sub cities in Addis Ababa city administration. It is situated in northern part of Addis Ababa, bounded from south by Bole sub city, from west by Lideta sub city and from north and east by Oromia region. At present, the sub city is divided into thirteen Woredas and hundred twenty four sub Woredas. According to 2007 census, the total population of this sub city is 346,486. Among these 143,243 and 203,243 are male and female respectively. Based on the sub city health department, the sub city has a total of 133,766 child bearing age women whose age is between 18 to 49 years. Among those bearing age brackets 10,345 had first ANC visit in the year 2017 G.C. There are fourteen health centers and among these, six health centers give ART service and 4 health centers give abortion service. In addition to this, almost all routine laboratory services are performed.

There are also seventy five different level private clinics which deliver routine health services to the community. The study was conducted in four randomly selected health facilities (Woreda 13, Woreda7, Woreda 11, and Woreda 5) which have a total client flow of 147, 253, per year. An average 36,813 outpatient flow in each health facility among this, 3,432 are antenatal care followers. An average 858 pregnant women come from January to February in Yeka sub city, Addis Ababa, Ethiopia.

6.2. Study design and period

- A cross sectional study was conducted from February to March, 2018 in Yeka Sub City, Addis Ababa, Ethiopia.

6.3. Population

6.3.1. Source of population

- All pregnant women visiting the selected health centers during the study period.

6.3.2. Study population

- Pregnant women visiting selected health centers and fulfilling the eligibility criteria during the study period were the study population.

6.4. Inclusion and exclusion criteria

6.4.1. Inclusion criteria.

- All pregnant women of 18 years old and above who were referred to the laboratory for Serological investigations and willing to give informed written consent.

6.4.2. Exclusion criteria

- Critically ill participants were excluded from the study population.

6.5. Study variables

6.5.1. Dependent variables

- Seroprevalence of HBV, HIV

6.5.2. Independent variables

Socio-demographic variables like**:**

- Age
- Occupation
- Level of education,
- Marital status

Other variables like:

- Multiple sexual partners,
- Ear piercing,
- Tattooing,
- History of contact with infected person,
- Blood transfusion,
- Tooth extraction,
- Surgery
- History of pregnancy.

6.6. Measurement and Data collection

6.6.1. Sample size determination

The sample size was calculated based on single proportional formula. The value of P was taken as 6%(0.06) from the previous study on Hepatitis B virus and Human Immuno deficiency virus co-infection among pregnant women in Ethiopia (18).

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

- n=sample size
- Z= at 95% confidence interval which is 1.96.
- P= prevalence
- d =degree of error which is 5 %(0.05).

Previous study	HIV	HBV
Prevalence	4.2%	6%
Sample size	62	87

$$n = \frac{(1.96)^2 0.06 \times 0.94}{(0.05)^2} = 87$$

- Including 10% non-responding rate the sample size becomes 96.

Hence, from each health facilities 100 study participants were taken (Woreda 13, Woreda7, Woreda 11, and Woreda 5). A total of 400 study participants were taken.

6.6.2. Sampling method

Convenient sampling technique was used for all pregnant women whose age is 18 years and above, who came to get ANC service. During the study period, pregnant women who were attending ANC service in those selected health centers were invited to be participant in the study.

6.6.3. Laboratory analysis

6.6.3.1. Data and sample collection

The clinical diagnosis was done by a clinician and a written informed consent was obtained from participants using a clinician during the study period. All study participants were informed about the study by both verbal and written form to be sure that they had all the information. This included aims of the study, blood collection procedure, assurance of test results as well as confidentiality of any information given. The pre designed interview questionnaire had socio-demographic characteristics and risk factors. The data was collected by a trained clinician after having a half day training that was provided by the principal investigator. And participants who gave a written informed consent were sent to the laboratory to give blood to detect antibody for HIV and antigen for HBV. Five milliliter of venous blood was drawn by a sterile needle from each participant by the laboratory technologist or technician. Yellow top serum separator tube was used to collect blood. The specimens were labeled properly using participants' code .Standard operating procedures for sample collection was used properly. The blood was allowed to clot and serum was separated by centrifugation at room temperature at 3000 rpm for 10-15 min and was stored in the freezer at -20 ^{o}c until being tested.

6.6.3.2 HBsAg test

Genedia® HBsAg rapid device (Green Cross Medical Science, Korea)

Principle

The GENEDIA® HBsAg Rapid Device is made up of test strip and plastic case. The test strip is composed of nitro cellulose membrane, dried gold particle pad, absorbent pad and sample pad. The nitrocellulose membrane is immobilized with goat anti-HBs on the test band region and goat anti mouse IgG on the control band region. During the assay, the serum flows laterally through an absorbent pad and a gold conjugate pad where it mixes with the color reagent .If the serum contains HBsAg, the colloidal gold antibody conjugate binds to the antigen ,forming an antigen-antibody colloidal gold complex. The complexes migrate chromatographically through the nitrocellulose strip by the capillary action. The serum and anti HBs colloidal gold complexes

move through the immobilized goat anti-HBs capture band region and then on to control band region. For a positive result, a colored band (pink or red) with the complexes will form in the test band region on the membrane. Absence of this colored band in the test band region suggests a negative result. To serve as a procedural control, a colored band (pink or red) at control region appears in the control band region (37).

In brief , after taking test device out of the pouch, it was be placed in a dry flat surface, labeled with name and identification number, 100 ul specimen was dispensed into the well of the test device and the result was read after waiting for 30+/-2 minutes. Two pink or red bands appearing in both regions showed a positive result while a Negative result was indicated by A pink or red band appearing only in the control region. The test was invalid if there was no colored band appears in both regions or in case color development in the test region only.

6.6.3.3 HIV test

HIV testing was carried out following the national testing algorithm.

WANTAI rapid test (Beijing Wantai Biological Pharmacy Enterprise Co., Ltd. China**)**

Principle

Wantai rapid test for antibody to human immunodeficiency virus (HIV) (Colloidal Gold Device) employs chromatographic lateral flow device in a cassette format. Colloidal gold conjugated recombinant antigens corresponding to HIV-1(gp120,gp41) and HIV-2 (gp-36) are dry-immobilized at the end of nitrocellulose membrane strip.HIV1+2 antigens are bound at the test zone (T) and antibodies are bound at the control zone (C).when the specimen is added, it migrates by capillary diffusion rehydrating the gold conjugate. If present in specimen, HIV1/2 antibodies will bind with the gold conjugated antigens forming particles. These particles will continue to migrate along the strip until the test zone (T) where they are captured by the HIV1+2 antigens generating a visible red line. If there is no HIV-1or 2 antibodies in specimen, no red line is formed in the test zone (T).The gold conjugate will continue to migrate alone until it is captured in the control zone(C) by the antibodies aggregating in a red line, which indicates the validity of the test(38).

In brief, after placing the cassette on flat surface, labeling with name and ID, and allowing the test cassette to reach room temperature, 80uL (or two drops using the provided pipette) of serum was added slowly into the specimen window(s). Immediately, one drop diluent buffer was added into the specimen window. The result was read from 10 minutes after specimen and buffer loading, to maximum of 30 minutes.

Result interpretation

Reactive results: One red line appears 10 to 30 minutes next to the Test Zone (T) which indicates that antibodies to HIV 1+2 have been detected through using this test.

Non-reactive results: No red lines appear within 30 minutes next to the test zone (t) which indicates that no antibodies to HIV 1+2 have been detected with this test. However, this does not exclude the possibility from infection with HIV.

Quality control: One red line will always appear next to control zone (c) indicating that the validity of the test.

Invalid result

- If no red line appears the test is invalid ,discard the test and repeat with new specimen and new cassette.at the control line 'C' within the stipulated time then the result is invalid.

UNI-GOLD^{TM} HIV (Trinity Biotech Plc., Ireland)

Principle

Uni-Gold^{TM} HIV is a rapid immunoassay based on the immunochromatographic sandwich principle. Recombinant proteins representing the Immuno dominant regions of the envelope proteins of HIV -1 and HIV-2, glycoprotein gp41, gp120 (HIV-1) and glycoprotein gp36 (HIV-2) respectively, are immobilized at the test region of the nitrocellulose strip. These proteins are also linked to colloidal gold and impregnated below the test region of the device. A narrow band of the nitrocellulose membrane is also sensitized as a control region (39).

In brief, the device was placed on a flat surface, labeled with patient name and ID, and the sample pad was filled with two drops of serum by holding the pipet, which was included in the

kit, vertically. After allowing the sample to fully absorb and ensuring that air bubbles are not introduced into the sample port, two drops of wash solution was added to the sample port by holding the dropper bottle of wash solution in a vertical position and above the sample port. The test results were read after 10 minutes but no later than 12 minutes incubation time.

Result interpretation

Reactive test result

Two pink/red lines of any intensity of 'C and 'T' in the device window.

Non-reactive test result

A pink/red line of any intensity 'C'in the device window.

Invalid result

No pink/red line appears in the device window adjacent to control line irrespective of whether or not a pink/red line appears in the device window adjacent to test line.

VIKIA®HIV ½(Biomerieux SA**,** France)

Principle

VIKIA®HIV ½ is an immunochromatographic test for the qualitative detection of antibodies to HIV-1 and HIV-2.The test consists of a plastic containing

1. a chromatography membrane to which are fixed:

- In the test region (T):synthetic peptides specific for HIV-1 (gp41 of group M and group O),and HIV-2 (gp36)
- In the control region (c): two color indicators.

2. A test strip impregnated with a conjugate consisting of a mixture of synthetic peptides specific for HIV-1 group M (gp41 of group M and group O), and HIV-2 (gp36), coupled to blue-dyed polystyrene microspheres. The sample is added to the sample well and migrates by capillary along the membrane. If the sample contains anti-HIV antibodies they form an antigen-antibody complex with the peptides, specific to this virus, present on the blue dyed polystyrene microspheres. The antigen-antibody complexes migrate and bind to the synthetic peptides

immobilized on the nitrocellulose membrane. This is revealed by a blue line in the test region (T) (40). After removing the test device from the sealed pouch it will be labeled with appropriate patient name or identification number. Using the plastic dropper 75ul of serum will be mixed with one drop (40 ul) of buffer without trapping bubbles in the sample well.

Result interpretation

Positive: the line in the control region changes from blue to pink/red and a blue line appear in the test region.

Negative: the line in the control region changes from blue to pink/red and no line appear in the test region.

Invalid results

- The line in the control region does not change color.
- The line in the control region does not change color and a blue line appears in the test region**.**

Currently the national testing algorithm of HIV follows with PICT (provider initiated counseling and testing) and VCT. Hence, assuring the voluntariness of respondents and counseling will be carried out by the nurse or midwives and testing is performed in the laboratory. Consequently, testing follows the national testing algorithm of HIV where sera is first tested for the qualitative detection of HIV ½ antibodies using a solid phase colloidal gold immunochromatographic technique **Wantai Rapid Test**. Nonreactive samples will be reported as HIV negative whereas reactive samples will be tested using a more specific secondary test **Trinity Biotech HIV1/2 Uni-Gold™** test kit. Any discordant results will be tested using a **VIKIA®HIV ½, a tie breaker**. Finally the reactive samples will be reported as HIV –positive and non-reactive results reported as negative. To assure confidentiality, test results will never be given to pregnant women rather for the requesting physicians or nurse

6.6.3.4. Safety precautions

Adherence of universal safety precaution for handling infectious materials that are referred in the national health and safety guideline was made during the study.

- All materials of human origin were considered as capable of transmitting infection.
- Glove and lab coat were used by the operator.
- The operator was advised to cover any abrasions or skin break on hand with adhesive tape.
- Recapping was not used to avoid needle stick injury.
- In case of needle stick injury squeezing the wound while flushing the bleeding with running water was advisable.
- All used sharp objects was placed in a puncture resistant container (safety box)

6.7. Data quality assurance of the study

The questionnaire preparation was checked by the advisor and pre-tested before the detailed work starts. Data and sample collectors were trained prior to data and sample collection time. In addition, there was follow up by the principal investigator. Study participants were interviewed with questionnaire given secret code and the data stored in a secure area where no one reaches and blood was collected with the same secret code. Standard operating procedures were followed during blood sample collection processing and analysis of data. Sera was screened for viral HBsAg according to the manufacturer's instruction and HIV rapid test(according to the Ethiopian national testing algorithm).The performances of GENEDIA® HBsAg has been determined and was recorded to have a sensitivity of 100% and specificity of 99%. The sensitivity and specificity for (Wantai) is 100%, 99.20%. Uni-gold (Uni-GoldTM HIV) is 99.9%, 99.9 and VIKIA (VIKIA®HIV ½)100%, 100% respectively. According to the manufacturer´s instructions, positive samples should be retested in duplicate before the final interpretation. As indicated in the package inserts, positive and negative controls was run as the test runs.

- **Pre-analytical phase**

Blood sample was collected aseptically from pregnant women in a properly labeled tube with the patient unique identification number. The samples was centrifuged and the serum is separated so as to process and positive samples was appropriately stored at optimum temperature (-20^{o}c) until it was confirmed by ELISA.

- **Analytical phase**

To assure the control performance of our procedure, we used in house control for both negative and positive control samples, in addition to controls provided from the manufacturer .The reagents and the test method were assessed with known positive and negative control materials so as to evaluate the storage conditions and performance capability of the method. Moreover; all manufacturers' instructions that were necessary were strictly followed up. Positive samples were confirmed by specific test procedures and were analyzed separately, as well as during conformation of HBsAg three negative and two positive controls were used simultaneously with the sample. Here, the standard laboratory procedures that address the quality of the test were also strictly followed.

❖ **Post-analytical phase**

The results were recorded with the unique patient identification number and errors of data entry are avoided through repeated checking.

6.8 Data analysis and interpretation

Collected data was entered into EpiInfo 5.3.1 software and double checked before analysis and reported to SPSS version 22 for analysis. The descriptive statistics mean, percentage, frequency were calculated and Chi-square test was used to establish association between serological results and different associated factors considered in the study. It was used to compare categorical data, and to evaluate the difference in prevalence between groups in the bivariate logistic analysis as well as the statistical significance between relevant variables. The result at p-value < 0.05 considered as statistically significant. The strength of the association was measured using an odd ratio and interpreted using the 95% confidence interval. Finally, the results were presented on Graphs and Tables.

6.9. Operational definitions

Hepatitis B surface antigen (HBsAg): is a marker present in people who have current infection of Hepatitis B Virus in both acute and chronic infection.

Seropositive: an individual's status of having a positive reaction when using serological test.

Seronegative: an individual's status of having negative reaction when using serological test.

6.10. Ethical Considerations

The study was approved by "Department of Research and Ethical Review Committee" of the Department of Medical Laboratory Science; after approval, a written letter was obtained from the department before the actual work starts. In addition ethical clearance letter was obtained from Addis Ababa Health Bureau Ethical Reviewing committee. Those pregnant women who gave a written consent were selected and enrolled as a study subjects for testing of HBsAg and HIV. The testing was done in the laboratory. Pregnant women with HIV positive results got post counseling and referred to Anti-Retroviral treatment (ART) for further management and those pregnant women that were positive for HBsAg were counseled and got health education for better management. There were no potential benefits in participating in this study as compensation. However, they were informed that their participation will help improve management of pregnant women and newborn and that there will not be potential risks except little pain during drawing venous blood and not forced to participate without free will. Confidentiality was the first concern and no one except the interviewers know the participant that take part in the study and the answers are not be given by the participant marked with a especial study number only, and not the name. All information obtained was held securely and stored on paper, and computer files.

6.11. Dissemination of Results

The result of this study was presented and submitted to the Department of Medical Laboratory Sciences (DMLS), School of Allied Health Sciences (SAHS), College of Health Sciences (CHS), Addis Ababa University (AAU) as well as to the study sites to serve as a reference material in the library laboratory. The principal investigator will present the study abstract in local professional associations and other international associations for a continuous medical educational events or conference .The summary of the thesis will be submitted to peer reviewed journal for publication.

Current national testing algorithm of HIV

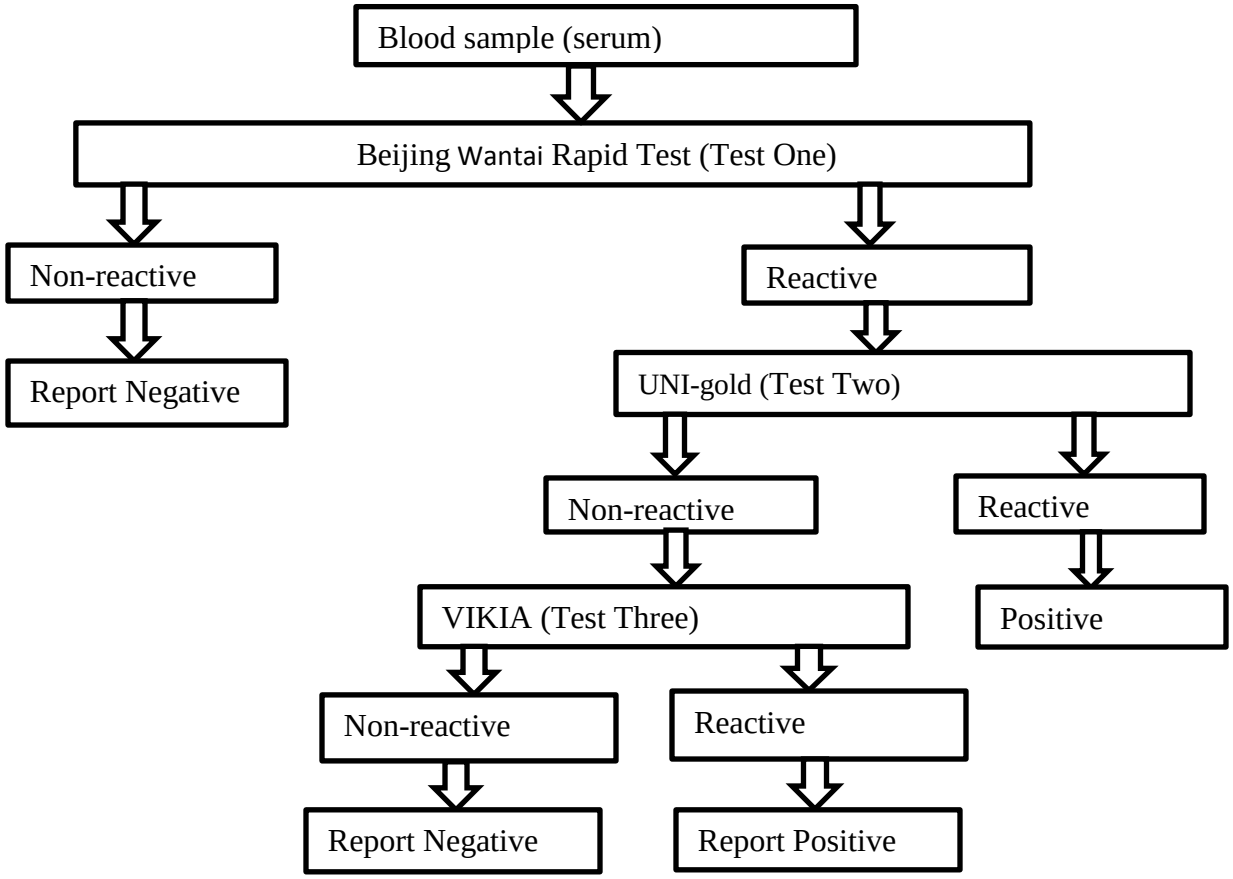

Figure- 2 Current National Testing algorithm for HIV

6.12. Work flow chart for the test procedure.

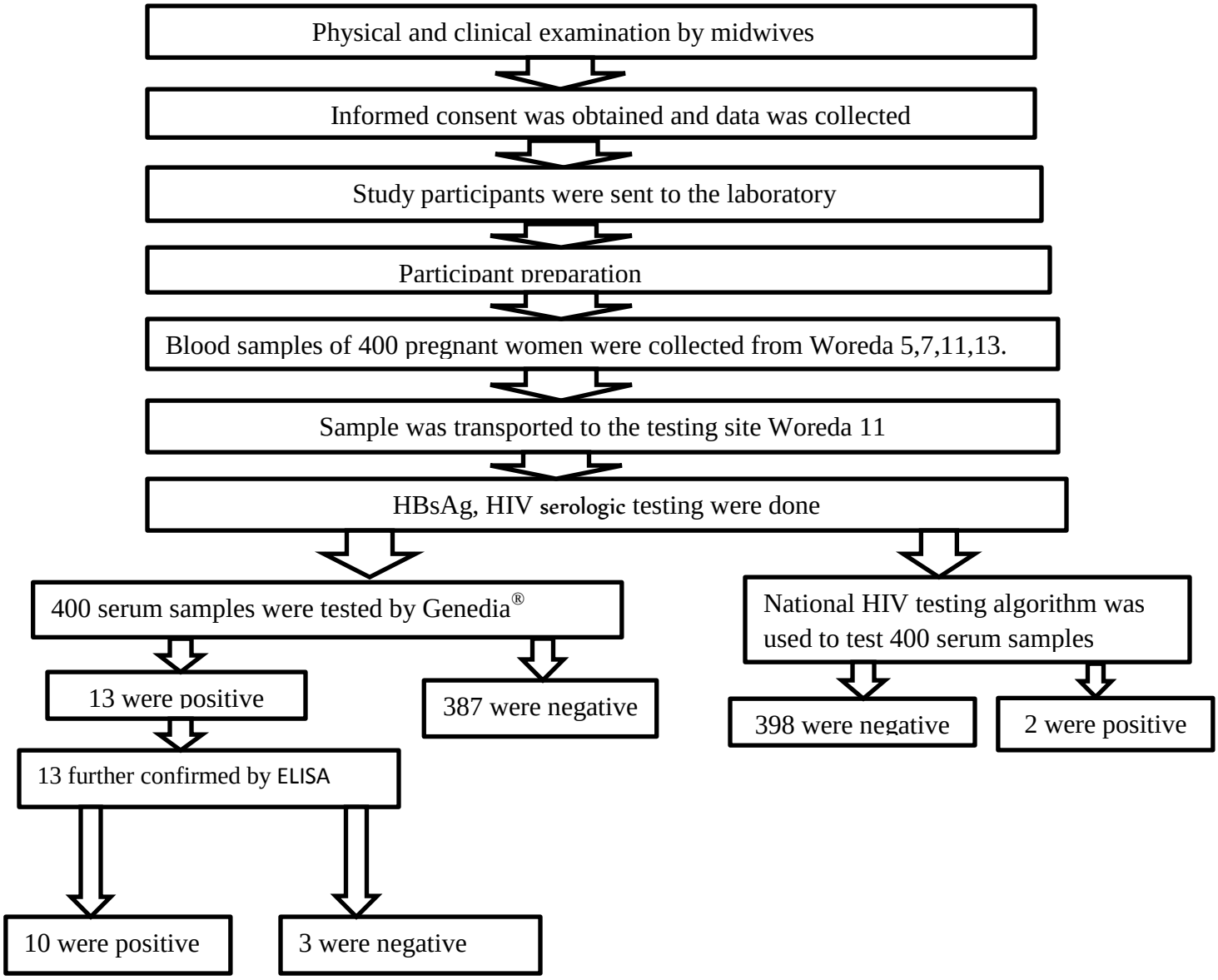

Figure-3: Diagrammatic representation of the work flow of the research

7. Results

7.1 Socio-demographic characteristics

A total of 400 pregnant women attending four health centers in Yeka sub-city were enrolled in this study. Their age ranges between 18 and 39 years with a mean and standard deviation of 26.8 (4.6). The majority were in the age group 26-35 years (54.8%), primary school level (46.0%), and house wives (55.5%) while 4.8% of them were unmarried. Table 1 summarized socio-demographic characteristics of the study participants.

Table-1. Socio-demographic characteristics of pregnant women attending selected health centers in Yeka sub-city, Addis Ababa, Ethiopia, February- March, 2018 (n=400)

Variables	Frequency	Percent
Age (in Years)		
18-25	161	40.3
26-35	219	54.8
>35	20	5.0
Education level		
Illiterate	53	13.3
Primary school (1-8)	184	46.0
Secondary (9-12)	115	28.8
Diploma and above	48	12.0
Marital Status		
Unmarried	19	4.8
Married	378	94.5
Widowed	2	0.5
Divorced	1	0.3
Occupation		
House wife	222	55.5
Self employed	70	17.5
Private employed	75	18.8
Government employed	33	8.3

7.2 Seroprevalence of HBV and HIV

Figure 4 displays seroprevalence of HBV and HIV infection among pregnant women attending selected health centers. Of a total of 400 pregnant women in this study, 13 participants were positive using rapid HBsAg while ten of them remain positive using ELISA for HBsAg. Two pregnant mothers were HIV positive as determined by the national algorithm for HIV testing. Of the two, one had HBV co-infection.

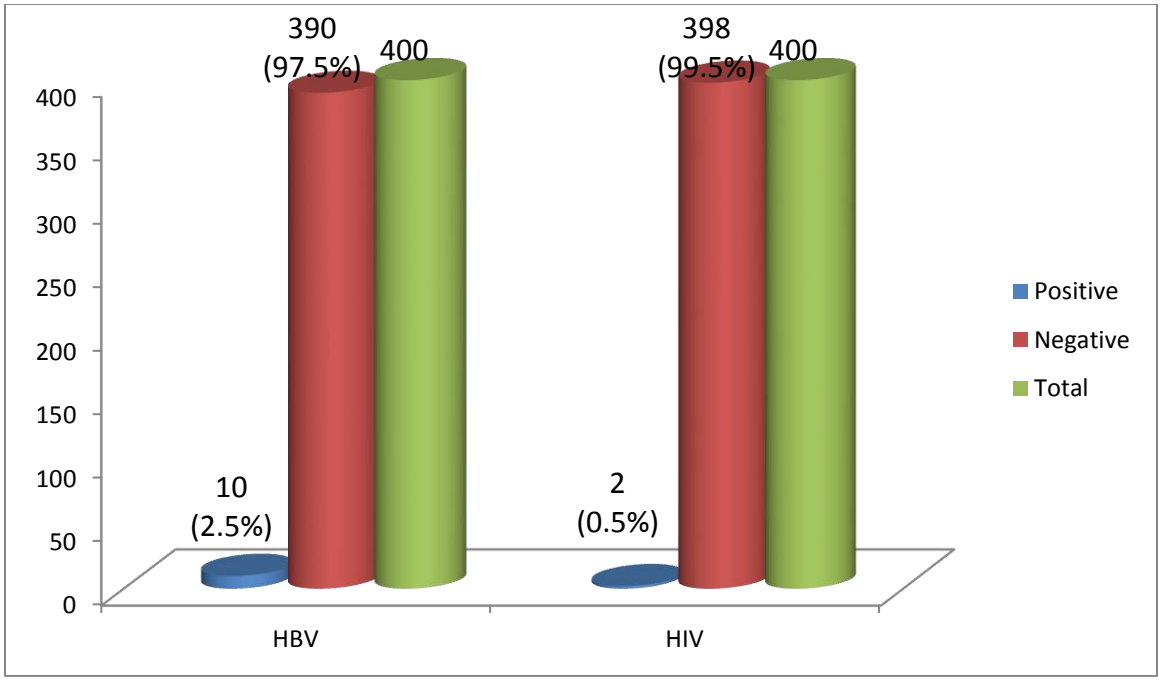

Figure 4. Seroprevalence of HBV and HIV among pregnant women attending selected health centers in Yeka sub-city, Addis Ababa, Ethiopia, February- March, 2018 (n=400)

7.3 Factors associated with HBV infection

7.3.1 Seroprevalence of HBV and analysis of risk factors.

The difference in the prevalence of HBsAg among different age categories was not statistically significant which is (p= 0.401). Age groups among 26-35 were the highest detection followed by 18-25 as illustrated in table 2. High prevalence was detected in those in primary school, who were married, and house wives.

With respect to risk factors associated with HBsAg, a bivariate analysis showed significant associations in pregnant women with multiple sexual partner, those with history of blood transfusion , who had undergone surgery and had contact with HBV positive person with a p-value (p=0.046), (p=0.042), (p=0.002) and (p=0.009), respectively as indicated in table 3. And the associated factors like sharp object injury, ear piercing, tattoo, hospital admission, tooth extraction, history of abortion, did not have a significant association to acquire HBV infection.

On the other hand only history of surgery showed a significant associations with HBV infection, with a p-value of (p=0.034) in a multivariate analysis done as indicated in table 4.

Table-2. Bivariate analysis of HBV and socio-demographic factors among pregnant women attending selected health centers in Yeka sub-city, Addis Ababa, Ethiopia, February-March, 2018 (n=400)

Variables	HBV infection		COR	P value	AOR	P value
	Positive	Negative				
Age (in Years)				0.401		
18-25	2	159	0.381 (0.78 – 1.85)	0.233		
26-35	7	212	0.239 (0.21 – 2.75)	0.252		
>35	1	19	1.0 (reference)			
Education level				0.831		
Illiterate	0	53				
Primary school (1-8)	6	178	1.29 (0.25 - 6.60)	0.760		
Secondary (9-12)	2	113	2.45 (.33 -17.96)	0.376		
Diploma and above	2	46	1.0(reference)			
Marital Status				1.0		
Unmarried	0	19	NA	1.0		
Married	10	368	1.0	1.0		
Widowed	0	2	NA	1.0		
Divorced	0	1	NA			
Occupation				0.772		
House wife	6	216	NA	0.998		
Self employed	3	67	NA	0.998		
Private employed	1	74	NA	0.998		
Government employed	0	33	NA			

AOR: adjusted odd ratio **COR**: crude odd ratio **NA**: not applicable

Table-3. Bivariate analysis of HBV and possible risk factors among pregnant women attending selected health centers in Yeka sub-city, Addis Ababa, Ethiopia, February-March, 2018 (n=400)

Variables	HBV infection		COR	P value
	Positive	Negative		
Sharp Object injury				
Yes	3	69	1.99(0.50-7.91)	0.326
No	7	321	1.0(reference)	
Multiple sex				
Yes	4	59	3.74(1.02-13.65-)	0.046
No	6	331	1.0(reference)	
Tatoo				
Yes	2	91	0.82(0.17-0.39.83)	0.806
No	8	299	1.0(reference)	
Contact with HBV positive person				
Yes	3	24	6.54(1.59-26.88-)	0.009
No	7	366	1.0(reference)	
Blood transfusion				
Yes	1	4	10.72(1.09-105.75)	0.042
No	9	386	1.0(ref)	
Hospital Admission				
Yes	2	49	1.74(0.36-8.43-)	0.492
No	8	341	1.0(reference)	
Tooth Extraction				
Yes	4	100	1.93(0.53-6.99)	0.315
No	6	290	1.0(reference)	
History of surgery				
Yes	3	16	10.02(2.37-42.41)	0.002
No	7	374	1.0(reference)	
Gravida				
One	7	182	2.42(0.49-11.85)	0.369
Two	2	126	3.15(0.38-26.05)	
3 and above	1	82	1.0(reference)	
History of Abortion				
Yes	4	83	2.47(0.68-8.94)	0.170
No	6	307	1(reference)	

COR: crude odd ratio

Table-4.Multivariable Logistic regression analysis of HBV and possible risk factors among pregnant women attending selected health centers in Yeka sub-city, Addis Ababa, Ethiopia, February-March, 2018 (n=400)

Variables	HBV infection		AOR	P value
	Positive	Negative		
Multiple sex				
Yes	4	59	2.56 (0.61-10.77)	0.201
No	6	331		
Blood transfusion				
Yes	1	4	5.75 (0.39-2.52483.48)	0.20
No	9	386		
History of surgery				
Yes	3	16	5.56 (1.14-27.27)	0.034
No	7	374		
Contact with HBV positive person				
Yes	3	24	4.23 (0.89-20.61)	0.69
No	7	366		

AOR: adjusted odd ratio

7.4 Factors associated with HIV infection

7.4.1 Seroprevalence of HIV and analysis of risk factors

Of a total of 400 pregnant women in this study, 2 were found to be HIV positive with a prevalence rate of 0.5 % as showed in figure 4.

Both belong to the age group 26-35 as shown in Table 5.

Both HIV positive participants are married, had sharp object injury, undergone ear piercing and had multiple sexual partners as shown in Table 5.

Table-5. Frequency of HIV by socio-demographic and possible risk factors among pregnant women attending selected health centers in Yeka sub-city, Addis Ababa, Ethiopia, February-March, 2018 (n=400)

Variables	HIV infection	
	Positive	Negative
Age (in Years)		
18-25	0	161
26-35	2	217
>35	0	20
Education level		
Illiterate	1	52
Primary school (1-8)	1	183
Secondary (9-12)	0	115
Diploma and above	0	48
Marital Status		
Unmarried	0	19
Married	2	376
Widowed	0	2
Divorced	0	1
Occupation		
House wife	1	221
Self employed	0	70
Private employed	1	74
Government employed	0	33
Sharp Object injury		
Yes	2	70
No	0	328
Multiple sex		
Yes	2	61
No	0	337
Ear piercing		
Yes	2	357
No	0	41
History of contact with HIV positive person		
Yes	1	26
No	1	372
Tooth Extraction		
Yes	1	103
No	1	295
History of Abortion		
Yes	1	86
No	1	312

7.5. Discussions

Hepatitis B virus and HIV are posing a paramount health impact especially in pregnant women and their neonates. Co-infection rate of hepatitis B virus and HIV is common which leads to increased morbidity and mortality as compared to HIV or HBV mono-infections (8). Monitoring the prevalence of HIV and HBV infection during antenatal care will help to design and implement timely interventions aimed at preventing perinatal transmission. This study aimed at determining the prevalence and associated factors of HBV and HIV among pregnant women attending selected health facilities in Yeka sub-city, Addis Ababa, Ethiopia.

The overall prevalence of HBsAg was 2.5 % (10/400), which falls in the intermediate level set by World Health Organization (WHO) (2%-7%)(1). The finding of current study was lower than several studies conducted by Chernet *et al*,2015in Dawuro zone, SNNPR, Southwest Ethiopia which was 3.5% (26), another study conducted in southern Ethiopia by Metaferia Y *et al*.,2015, 7.8% (27), Zenebe *et al*.,2013 from Bahir Dar Northwest Ethiopia 3.8% (8), Tegegne *et al*.,2012 in Addis Ababa Ethiopia 3.0% (28), and another study in the same area Desalegn *et al*.,2014,6%(18). The finding of the study is also lower than researches conducted from other African countries, Chinenye Gloria A .*et al.*,2013from southwest Nigeria 8.3% (25), Lem Edith A. *et al*,2015 from Bamenda Northwest Cameroon 6% (33) , J.M. Kabinda *et al.*,2013 in Democratic republic of Congo 5.9% (35),in Mwumvaneza M, *et al*, 2017 in Rwanda 3.7% (36). It is also lower compared to the rest of countries outside Africa, Papaevangelou *et al.*,2006 in Greece 2.89%(16), Ibrahim B.*et al*,2012 Jazan region Kingdom of Saudi Arabia 4.1%(24).On the other hand the current finding was higher than studies conducted by Moghdasifar .*et al*,2015 in Iran 1.2%(23), Vandana *et al.*,2015in India 2.37% (4), and another study in the same area by Jindal N *et al.*,2012 in India 2.4% (32).

The overall seroprevalence of HIV in the current finding which is 0.5% is lower than studies documented by Desalegn *et al.*,2014 in Addis Ababa Ethiopia 4.2% (18), and Zenebe *et al*.,2013 Bahir Dar Northwest Ethiopia 6.6% (8). One of the probable reason why the finding in this study is lower than other studies could be those pregnant mothers who are already on PMTCT follow up were not captured as they attend in a separate clinic.

Furthermore Lem Edith A.*et al.*,2015 in Bamenda Northwest region Cameroon 6.6%(33) , Thompson Joseph A.*et al.*,2014in Southwest Nigeria 5.16 % (10), and even it was very high in a

research conducted by Joel M.*et al*,2014in Dar es salaam 17.2% (34), J.M. Kabinda *et al.*,2013 in Democratic Republic of Congo 4.1% (35), Mwumvaneza M,*et al*.,2017 in Rwanda 4.1%(36), and outside Africa, angelica E, *et al*.,2010in Brazil 0.7% (29). On the other hand the finding was higher than researches documented by Vandana *et al*., 2015 India 0.03 %(4), and it was comparable with studies conducted by Jindal N, *et al*., 2012 in India 0.4% (32).

HBV and HIV co infection had a prevalence of 0.25% which is lower than a research conducted by Thompson Joseph A, *et al.*, 2014 in Ekiti Southwest Nigeria 0.70%(10),Joel M, *et al.*, 2014 in Dar es Salaam 2.8%(34). The highest prevalence was documented by Zenebe *et al*., 2013 from Bahir Dar Northwest Ethiopia 19.0% (8). This co-infection rate implicates that both HIV and HBV share same route of transmission

With respect to age and prevalence of HBsAg, the current finding showed the higher Sero positivity among age groups 26-35 and 18-25 years is the second which was in agreement with researches conducted by Chinenye Gloria A *et al.*, 2013 from Southwest Nigeria (25), Angelica E, *et al*.,2010 in Brazil (29), Thompson Joseph A, *et al.*, 2014 in Ekiti Southwest Nigeria (10), in Rwanda (Mwumvaneza M *et al*.,2017).This high Seropositivity among those age groups shows the presence of possible risk factor to be infected by HBV. A multivariate analysis done on associated risk factors showed that history of surgery had a significant association with HBV infection with (AOR=5.56, 95% CI = 1.14-27.27); .p-value (p=0.034).

The observed HBV infection distribution variation in different geographical areas might be due to variation in socio-demographic characteristics of cultural and behavioral differences, the level of knowledge and attitude for the risk factors of HBV infection. The study design and study areas may contribute for the variation in seroprevalence of HBV infection in different geographical locations.

8. Strength and Limitations of the study

- HBsAg rapid screening positive results were confirmed by Enzyme Linked Immunosorbent Assay (ELISA).
- Sero markers like HBeAg and IgM for HBcAb that enable to determine acute infections were not used in this study.

9. Conclusion and recommendation

9.1. Conclusion

The current finding indicated a 2.5% seroprevalence rate of HBV infection among study participants of pregnant women which was an intermediate level, according to the world health organization's classification and an HIV prevalence of 0.5%. HBV, HIV co-infection was 0.25%. As indicated in this study, history of surgery had a significant association with HBV infection as well as high prevalence of HBV was observed among reproductive age groups. Hence, antenatal screening tests for HBsAg and HIV must be accessible for all pregnant women. There should be a mechanism to facilitate vaccination for HBV infection for all pregnant women.

9.2 Recommendation.

The prevalence of current study indicates that screening of all pregnant women for HBsAg and HIV and the way they prevent the risk factors for the exposure of HBV and HIV should be strengthened. Population based studies with additional serological markers and molecular techniques are required so as to design a working strategy for evidence based intervention and implement control measure. Further research works should be conducted at country level to consolidate the fight against HBV, and HIV infection among pregnant women. Hence, screening of pregnant women for HBV, HIV is a corner stone for disease detection, diagnosis, prevention and intervention.

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11. Annexes

Annex 11.1. Information sheet

Title of the Study

Seroprevalence of HBV, HIV and associated factors among Pregnant Women in selected health centers of Yeka Sub City, in Addis Ababa, Ethiopia.

Purpose of the study

The purpose of the study is to determine the seroprevalence of HBV, HIV viral infection and to assess the associated factors among pregnant mothers of 18 and above years.

What will it mean if you decide to take part in the study?

If you agree to participate in this study, you will participate in this interview in a private place. The interview will last for about 15 minutes. During the interview, you will be asked to respond to questions related to HBV, HIV infections and their associated factors. The recorded data will not contain your names or other identifying information. They will just be labeled with a study number. In addition; 5 ml blood (one tea spoon) will be collected for HIV and HBV testing.

The results will assist policy makers, planners and health service providers for making considerations regarding the associated factors, transmission and seroprevalence of HBV and HIV infections among pregnant women. It will also help to contribute in the subsequent efforts to improve prevention, diagnosis, treatment and support of HBV and HIV in relation to Pregnant Women in the community.

Risks and discomforts

There is no possible risk associated with participating in this study. But there is a little pain during drawing venous blood which will be collected by professional phlebotomists. You are free to decline answering any question that you do not wish to answer and you may leave our interview at any time you want to. The interview will take 15 minutes.

Potential benefits

You will not receive any payment for participating in this study as compensation. However, your participation will help to identify the magnitude and associated factors of HIV and HBV infections among pregnant women which will help improve the management of pregnant women and their newborns. You will be provided the results for free through your attending clinician

Confidentiality

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

Voluntary Participation

Your participation is voluntary. You may withdraw from the interview at any time without giving a reason and without any penalty. You are not forced to participate without your free will.

Contact information

If you have any questions, you may consult the PI or supervisors by the following addresses:

Please, contact: Yibeltal Getaneh,

Department of Medical laboratory sciences

Addis Ababa University

Cell phone: 0929136829

Email: yibget78@gmail.com

ቅጽ 11.2: ስለጥናቱ ማስተዋወቂያና በጥናቱ ለመሳተፍ ፈቃደኝነት መጠየቂያ የአማርኛ ቅጽ

የጥናቱ ርእስ

እርጉዝ እናቶች ላይ የጉበት በሽታን(ሄፓታይተስ ቢ)፣ኤች አይቪ የሚያመጡ ረቂቅ ተህዋሥያን ስርጭት (መጠን)ና ተያያዥ ምክንያቶች ላይ የሚደረግ ጥናት

የጥናቱ አላማ

በረቂቅ ተህዋስያን የሚመጣ የጉበት በሽታን(ሄፓታይተስ ቢ)፣ኤች አይቪ ፣ስርጭትና አጋላጭ ምክንያቶችን 18 እና በላይ እድሜ ባሉ እርጉዝ እናቶች ላይ ማጥናት ነው፡፡ጥናቱ የሚካሄደው በአዲስ አበባ ከተማ የካ ክፍለ ከተማ በተመረጡ ጤና ጣቢያዎች ላይ ይሆናል፡፡

በጥናቱ ላይ ለመሳተፍ ፍቃደኛ ነዎት;

በዚህ ጥናት ላይ ለመሳተፍ ፈቃደኛ ከሆኑ ቃለ መጠየቁ ሚሰጥራዊ ብሆነ ቦታ ይደረግልዎታል፡፡ቃለ መጠይቁ 15 ደቂቃ ይወስዳል፡፡እርሰዎንም በእርጉዝ እናቶች ላይ የጉበት በሽታን(ሄፓታይተስ ቢ)፣ኤች አይቪረቂቅ ተህዋሥያን ስርጭት (መጠን)ና አጋላጭ ምክንያቶች ተያያዥነት ያላቸውን ጥያቄዎች እንጠይቀዎታለን፡፡ከዚህም በተጨማሪ ለሄፓታይተስ ቢ እና ኤች አይቪ ምርመራ 5 ሚሊ ሊትር ወይም አንድ ማንኪያ ደም ይሰጣሉ፡፡ ከዚህ ጥናት የሚገኘዉ ዉጤት ለፖሊሲ አውጭዎችና አስፈጻሚዎች እንዲሁም ለማህበረሰቡ፤ ለአጋላጭ ሁኔታዎችና ስለመከላከያ መንገዶች ለማወቅ ይረዳል፡፡ በማህበረሰቡ ዉስጥ ለሚገኙ ነፍሰ ጡር እናቶችም አስፈላጊውን ድጋፍ ፤ምርመራና ህክምና ማድረግ የስችላል፡፡በሌላ በኩልም ስለበሽታው ግንዛቤና ጥንቃቄ ለማገኘት ይረዳል፡፡

በጥናቱ በመሳተፍዎ የሚመጣ ችግር

በጥናቱ በመሳተፈዎ ምክንያት የሚመጣበዎት ምንም አይነት ችግር አይኖርም፡፡ ነገር ግን 5 ሚሊ ሊትር (አንድ ማንኪያ) የደም ናሙና ለመዉሰድ መርፌ ሲገባ ከሚፈጥረዉ የቅጽበት የህመም ስሜት በስተቀር የጎላ ችግር አያመጣም፤ ምቾት ካልተሰማዎት ባለሙያ እንዲያይዎት ይደረጋል፡፡

በጥናቱ በመሳተፍዎ የሚያገኙት ጥቅም

ጥናቱ ለእርሰዎ ቀጥተኛ የሆነ ጥቅም ባይኖረውም ለፖሊሲ አውጭዎችና አስፈጻሚዎች እንዲሁም ለማህበረሰቡስ ለአጋላጭ ሁኔታዎችና ስለመከላከያ መንገዶች ለማወቅ ይረዳል፡፡ በሌላ በኩልም ስለበሽታው ግንዛቤና ጥንቃቄ ለማገኘት ይረዳል፡፡ የደምዎ ናሙና በላብራቶሪ ሲመረመር ምንም አይነት ችግር ካሳ የባለሙያ ምክር ይሰጥዎታል፡፡

እርሰዎንም በዚህ ጥናት እንዲሳተፉ በትህትና እንጠይቀዎታለን፡፡ በዚህ ጥናት በመሳተፈዎ የምናገኘው መረጃ ለጥናታችን ውጤታማነት እነዲሁም በጥናቱ ውጤት ላይ ከፍተኛ አስተዋፅዎ ይኖረዋል፡፡ ስለዚህም በዚህ ቃለ-መጠይቅ በመሳተፈዎ ምስጋናዬ የላቀ ነው፡፡

የጥናቱ ሚስጥራዊነት

በጥናቱ ውስጥ ስምዎም ሆነ የመለያ ቁጥርዎ በማንኛውም ሁኔታ አይገለጽም፤ ስለሆነም የሚሠጡት መረጃ ሙሉ በሙሉ ሚስጢራዊነቱ እንደጠበቀ በወረቀት እንዲሁም በኮምፒውተር ይቀመጣል፡፡በዚህ ጥናት መሳተፍዎን የሚያዉቀው በጥናቱ ወቅት ቃለ መጠይቅ ያደረገልዎት ሰው ብቻ ነው፡፡የሚሰጡትም መልስ በሚስጥራዊ ቁጠር ስለሚመዘገብ የሚያስከትለው ችግር አይኖርም፡፡ በዚህ ጥናት የሚሰጡት መረጃ ሙሉ በሙሉ ሚስጥራዊ እነደሆነ እንገልጽልዎታለን፡፡

ነጻ ፍቃደኝነት

ስለዚህ በጥናቱ ለመሳተፍ የእርሠዎ ሙሉ ፈቃድ አስፈላጊ ነው፡፡ በተጨማሪም ለመመለስ የማይፈልጉዋቸው ጥያቄዎች ካሉጥያቄዎችን ለመመለስ አይገደዱም፡፡ አንዲሁም በጥናቱ ላለመሳተፍ ከፈለጉ በማንኛውም ጊዜ ማቋረጥ ይችላሉ፡፡ በጥናቱ ባለመሳተፈዎ በርሰዎ ላይ የሚያስከትለው ወይም የሚያመጠው ምንም አይነት ጉዳት የለውም፡፡

መረጃ ለመስጠት

ቃለመጠየቁን በተመለከተ ወይንም አጠቃላይ ስለጥናቱ ማንኛውንም አይነት ጥያቄና አስተያየተ ቢኖረዎት በሚከተሉት አድራሻዎች መጠቀም ይችላሉ፡፡

ይበልጣል ጌታነህ፡ አዲስ አባበ ዩኒቨርስቲ ላቦራቶሪ ሳይንስ ዲፓርትመንት

ስልክ፡092916829

ኢሜይል፡yibget78@gmail.com

Annex 11.2. Consent form

Consent form (English Version).18 and above years of pregnant women.

Consent form for pregnant women of 18 and above years, I have read the information above, or it has been read to me. I have been given the opportunity to ask questions and my questions have been answered to my satisfaction. I voluntarily consent that I would participate in this study understanding that I have the right to withdraw from the study at any time and my decision will not affect the service I am getting from the health facility.

Print name of participant, date and signature or thumb impression of participant

_____ ____ /____ /____(dd/mm/yy) _____

Print name of researcher, date and signature of researcher

_____ _____/____/_____ (dd/mm/yy) _____

ስምምነት ማረጋገጫ ቅፅ ከ 18 ዓመትና በላይ ነፍሰጡር እናቶች

ከላይ በመግቢያው ላይ የተጠቀሰውን መረጃ አንብቢያለሁ ወይም በቃል የተሰጠኝን ማብራሪያ ተረድቻለሁ፡፡ በዚህ መሰረት ከእኔ የሚጠበቅብኝን ድርሻ በሚገባ አውቄያለሁ እናም በዚህ ጥናት ላይ በመሳተፌ ሊከሰቱ የሚችሉትን ሁኔታዎች ተገንዝቢያለሁ፡፡ ከዚህ ጥናት በማንኛውም ሠዓት ያለምንም ቅድመ ሁኔታና ምክንያት እራሴን ከተሳታፊነት የማግለል ሙሉ መብት እንዳለኝ ተረድቻለሁ፡፡ ይህን ውሳኔዬን ተከትሎ በእኔም ሆነ በቤተሰቦቼ ላይ በምንፈልገው የጤና አገልግሎት ላይ ምንም አይነት አሉታዊ ተጽዕኖ እንደማይደርስብኝ ተረድቻለሁ፡፡ በመሆኑም ስለጥናቱ ማብራሪያ የተሰጠ መሆኑን በተለመደው ፊርማዬ አረጋግጣለሁ፡፡

የተሳታፊው ስም----------ፊርማ ----------ቀን----------

የጥናት አድራጊው ስም---------- ቀን----------ፊርማ

Annex 11.3. Survey questionnaire English Version

Questionnaire to "Determine Seroprevalence of HBV,HIV and identifying associated factors among Pregnant Women in selected Health Center of Yeka Sub City, Addis Ababa, Ethiopia 2017.

- Facility Name _____ Year _____
- Address (Sub City) _____ Telephone_____
- Data Collector Name _____ Date _____Signature_____

Participants' General Information

- Participant Code_____
- Sub City_____
- Woreda_____

I. Socio-demographic Information

1. Age_____

2. Education Level A. Illiterate B. Primary School (1-8) C. Secondary School (9-12) C. Diploma And Above

3. Marital Status A. Unmarried B. Married C. Widowed D. Divorced

4. Occupation A. House wife B. Self employed

C. Private Employed D. Government employed E. Others

II. Associated factors for acquiring HBV, HIV in the Study Subjects

5. Sharp object injury A. Yes B. No

6. Multiple sexual partners A. Yes B. No

7. Tattooing A. Yes B. No

8. Ear Piercing A. Yes B. No

9. History of Contact with HIV, HBV Infected Person A. Yes B. No

10. What about your sexual partner exposure for HBV,HIV? A. Yes B. No C. Not Known

III. **Did you receive any of the following medications?**

11. Surgery A. Yes B. No

12. Tooth extraction A. Yes B. No

13. Hospital admission A. Yes B. No

14. Blood transfusion A. Yes B. No

IV. **History of pregnancy and related questions**

15. Pregnancy Status

A. First gravid B. Second gravid C. Third and above gravid

16. What about your sexual partner exposure for HBV, HIV? A. Yes B. No C. Not Known

17. Have you yourself experienced any pregnancy related problems? A. Yes B. No

18. Abortion/Miscarriage A. Yes B. No

19. If "yes" for question number 19, how many times.

A. One Time B. Two Times C. Three Times D. Four Times And Above

20. Did you take HBV vaccination (Hepatitis B Virus). A. Yes B. No

21. HIV Status A. Negative B. Positive C. not known

22. HBV Status A. Negative B. Positive C. Not known

V. HBsAg and HIV serological tests laboratory finding during the study period.

1. HIV1/2 A. Positive B. Negative

2. Hepatitis B virus (HBsAg) A. positive B. negative

VI. ELISA confirmatory test

1. Positive 2. Negative

Annex 11.4. Survey Questioner **(Amharic Version)**

የመረጃ መሰብሰቢያ መጠይቅ ፎርም(ቅጽ)

በአዲስ አበባ ዩኒቨርሲቲ ፤ የጤና ሳይንስ ኮሌጅ ፤ የአላይድ ጤና ሳይንስ ትምህርት ቤት፤ የህክምና ላቦራቶሪ ዲፓርትመንት

ይህ መጠይቅ ለተሳታፊዎች(ወላድ እናቶች)፤አጠቃላይ መረጃና የኤች አይቪ ቫይረስና የሄፓታይቲስ ቢ በሽታ ስርጭት የሆኑትን ምክኒያቶች ለማወቅ የሚደረግ መጠይቅ ነዉ::መጠይቁን የሚያስሞላው በጥናቱ ጊዜ የሚመረጠው አዋላጅ ሲሆን፤መጠይቁ የሚሞላው ጥናቱ በሚካሄድበት ቦታ ነው:: ለጥናቱ መሳካት ያግዘን ዘንድ ጥያቄዎችን በጥንቃቄ እነዲሞሉልን በትህትና እንጠይቃለን::

የተቋሙ ስም_____ ዓ.ም

_____

አድራሻ (ክፍለ ከተማ)_____ ስልክ ----------

ዳታ ሰብሳቢ ስም_____

ቀን_____ ፊረማ_____

.አጠቃላይ የተሳታፊ መረጃን በተመለከተ

መለያ ቁጥር/ኮድ----------

ክፍለ-ከተማ----------ወረዳ----------

፪.ዲሞግራፊክ መረጃ

1.እደሜ_____

2. የትምህርት ደረጃ ሀ. ያልተማረች ለ. 1ኛ ደረጃ (1-8) ሐ. 2ኛ ደረጃ (9-12) መ. ዲፕሎማ እና ከዛ በላይ

3. የጋብቻ ሁኔታ ሀ. ያላገባች ለ. ያገባች ሐ. ባል የሞተባት መ. የፋታች

4. የሙያ ዘርፍ ሀ. የቤት እመቤት ለ. የግል ተቀጣሪ ሐ. የግል ሰራተኛ መ. የመንግስት ስራተኛ ሠ. ሌሎች

፫. በኤች አይቪ ቫይረስ፤ ሔፓታይቲስ ቢ ቫይረስ ለመያዝ ወሳኝ የሆኑ ምክኒያቶች

5. ስለታም ነገር ቆርጥዎት ያውቃል ሀ. አዎ ለ. አላውቅም

6. ከአንድ በላይ የትዳር ጉደኛ ኖርዎት ያዉቃል ሀ. አዎ ለ. አላውቅም

7. ጆሮዎን ተበስተው ያውቃሉ ሀ. አዎ ለ. አላውቅም

8. ንቅሳት ተነቅሰው ያውቃሉ ሀ. አዎ ለ. አላውቅም

9. በ ሄፕታይትስ ፤ኤች ኣይ ቪ በሽታ ከተያዘ ሰው ጋር ንክኪ አድርገው ያውቃሉ ሀ. አዎ ለ. አላውቅም

10. የትዳር አጋርዎስ ለ ኤች አይቪ ወይም ለጉበት በሽታዎቸ ተጋልጠዉ ያዉቃሉ ሀ. አዎ ለ. አያዉቅም

፬. ከሚከተሉት ህክምናዎች የትኞችን ተጠቅመው ያዉቃሉ

11. ከሌላ አካል ደም ተቀብለው ያውቃሉ ሀ. አዎ ለ. አላውቅም

12. ሆስፒታል ውስጥ ታመው ተኝተው ያውቃሉ ሀ. አዎ ለ. አላውቅም

13. የጥርስ ነቀላ አድርገው ያውቃሉ ሀ. አዎ ለ. አላውቅም

14. የቀዶጥገና ህክምና አድርገው ያውቃሉ ሀ. አዎ ለ. አላውቅም

፭. ከ እርግዝና ጋር የተያያዙ ጥያቄዎች

15.የእርግዝና ሁኔታ ሀ. የመጀመሪያ ለ. ሁለተኛ ሐ . ሶስተኛ መ. አራትና ከዚያ በላይ

16. ከ እርግዝና ጋር የተያያዙ ችግሮች አጋጥሞዎት ያዉቃል ሀ. አዎ ለ. አያዉቅም

17. የትዳር አጋርዎስ ለ ኤች አይቪ ወይም ለጉበት በሽታዎቸ ተጋልጠዉ ያዉቃሉ ሀ. አዎ ለ. አያዉቅም

18. ዉርጃ አጋጥሞዎት ያውቃል ሀ. አዎ ለ. አያውቅም

19. አዎ ከሆነ መልሶ ለስንት ጊዜ ሀ. አንድ ጊዜ ለ. ሁለት ጊዜ ሐ. ሶስት ጊዜ መ. አራት ጊዜ

20. የጉበት በሽታ/ ከትባት ተከትበዉ ያዉቃሉ ሀ. አዎ ለ. አላዉቅም

21. የኤች አይቪ ቫይረስ ሁኔታ በደም ዉስጥ ሀ. አለ ለ. የለም ሐ. አላዉቅም

22. የጉበት በሽታ/ሄፕታይትስ ቢ/ በ ደም ዉስጥ፡፡ ሀ. አለ ለ. የለም ሐ. አላዉቅም

፮. በጥናቱ ወቅት የተገኘ የ ሄፓታይትስ ቢ እና ኤች አይ ቪ ቫይረስ ውጤት

1. ኤች አይ ቪ ቫይረስ ½ ሀ. አለ ለ. የለም

2. ሄፓታይትስ ቢ ቫይረስ ሀ. አለ ለ. የለም

፯ ኤሊዛ ከንፊርመተሪ ምርመራ

ሀ. አለ ለ .የለም

12. Standard operating procedures (SOPs) for sample collection.

- Briefly explain the procedure of blood drawing and reassure the client.
- Wear clean, sterile, powder free, protective rubber glove make the client in a stable position.
- Make ready syringe with needle and cotton.
- Tie the tourniquet around the arm above the bend on the elbow and position the tourniquet 7.5cm to10cm above the puncture site.
- Tell the client to clench her fist.
- Examine the phlebotomy site using tip of the index finger to feel the vein and decide exactly where to apply the puncture.
- Swab the punctured site with cotton in small outward circles without alcohol. Do not touch the swabbed area.
- Insert the needle and withdraw approximately 4ml of peripheral blood in an EDTA tube
- Let the client to open the clenched fist.
- Untie and release the tourniquet.
- Withdraw the needle and cover the punctured site with cotton and make the client hold or hold by applying pressure for 3 minutes or until adequate hemostasis is visible.
- Label tubes with the client's identification number immediately after the specimen is obtained.
- Dispose used materials properly in the safe container and tells the client to discard the cotton assuring the stopping of the bleeding.
- Ship the samples from collection site to area of analysis having strict adherence, triple packaging, labeling and maintain the optimum temperature.
- Samples should be kept in the refrigerator at 2-8^{o}c or frozen at -20^{o}c based on the time required for storage, within 48 hours or longer respectively.

ELISA (Enzyme Linked Immunosorbent Assay) test for HBsAg

Principles

For detection of HBsAg ,the method uses "sandwich "ELISA method in which, polystyrene micro well strips are pre coated with monoclonal antibodies specific to HBsAg virus. Patient's serum or plasma sample is added to the micro-well together with a second antibody conjugated with the enzyme horseradish peroxidase (the HRP-conjugate initiates blue color production of the colorless chromogen. The sulfuric acid were stop the reaction and result in yellow color formation. The amount of color intensity can be measured instrumentally as optical density by spectrophotometer at wavelength of 450 nm and it is proportional to the amount of antigen captured in the wells and to the sample.

Procedure summary	Amount added
Add the sample	50ul(microliter)
Add HRP-conjugate	50ul(microliter)
Incubate	60minutes
Wash	5 times
Coloring	50 ul A+ 50 ul B (substrate solutions)
Incubate	15 min
Stop the reaction	50 ul stopping solution(sulpheric acid)
Read the absorbance	450nm

Interpretation of results.

Each micro plate 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.

Negative results (s/c.o.<1):samples giving an absorbance less than the cut-off value are considered negative (no HBsAg has been detected with HBsAg ELISA kit).

Positive results (s/c.o≥1): samples giving an absorbance greater than or equal to the cut-off value are considered initially reactive.

Borderline: samples with absorbance to cut-off ratio between 0.9 and 1.00 are considered borderline samples and retesting is recommended. S= the individual absorbance (OD) of each specimen.

13. Declaration

I, the undersigned, declare that this MSc thesis 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 thesis have been duly acknowledged.

.

M.Sc. candidate: Yibeltal Getaneh (B.Sc.)

Signature: _____

Date of submission: _____

This proposal has been submitted with our approval as advisors.

Advisors:

Aster Tsegaye (MSc, PhD)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.

Kassu Desta (MSc, PhD candidate.)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.