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SERO-PREVALENCE AND ASSOCIATED RISK FACTORS OF HBV AND HIV CO- INFECTIONS AMONG PREGNANT WOMEN ATTENDING ANTENATAL CARE AT SHAHURA HEALTH CENTER, ALEFA DISTRICT, NORTHWEST ETHIOPIA

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BY

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By

Mezgebekal Alemu Mekonnen

A thesis submitted to the Department of Biology in partial fulfillment of the requirements for the Degree of Master of Science in Biology (Biomedical Sciences)

Advisor: Endalkachew Nibret (PhD)

June, 2021

Bahir Dar, Ethiopia

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Approval Sheet

As a thesis research advisor, I hereby certify that, I have read and evaluated the thesis prepared under my guidance, by Mezgebekal Alemu Mekonnen entitled "Sero-prevalence and associated risk factors of HBV and HIV Co- infections among pregnant women attending antenatal care at Shahura Health Center in Alefa District, Central Gondar, northwest Ethiopia". I recommended this thesis to be submitted as fulfilling the requirements for the degree of Master of Science in Biology (Biomedical Sciences).

Endalkachew Nibret (PhD)	----------	----------
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As an advisor member of the examining board for the final MSc thesis open defense, we certify that we have read and evaluated the thesis prepared by Mezgebekal Alemu Mekonnen and examined the candidate. We recommended that the thesis to be accepted as fulfilling the requirements for the degree of Master of Science in Biology (Biomedical Sciences).

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External Examiner	Signature	Date

Declaration

I declare that, this thesis is my original work under the supervision of Dr. Endalkachew Nibret, and not been presented in any university in fulfillment of MSc program, and all the sources used for the manuscript are acknowledged.

Name of student candidate: Mezgebekal Alemu Mekonnen

Signature----------

Date of submission-------/------/----------

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List of Abbreviations and Acronyms

AIDS- Acquired Immunodeficiency Syndrome

ANC -Antenatal Care

AOR -Adjusted Odds Ratio

COR- Crude Odds Ratio

HBsAg- Hepatitis B Surface Antigen

HBV- Hepatitis- B Virus

HIV- Human Immunodeficiency Virus

PMTCT- Prevention of Mother to Child Transmission

SPSS- Statistical Package for the Social Science

WHO-World Health Organization

Abstract

HBV is a major health problem worldwide and cause acute or chronic hepatitis, which can lead to the development of extensive liver scarring (cirrhosis), liver failure, liver cancer, and death. HIV is also the leading cause of death in women of reproductive age globally. The current study was amide to assess the seroprevalence and associated risk factors of HBV and HIV co-infections among pregnant women attending antenatal care at Shahura Health Center in Alefa District, northwest Ethiopia. A cross sectional study was conducted among 384 pregnant women attending antenatal care at Shahura Health Center from January to March 2021. Simple random sampling technique was used to pick and include the study subjects in this study. A pre-tested structured questionnaire was used to collect socio-demographic characteristics and potential associated factors. HBsAg and HIV were determined using rapid screening tests. Descriptive statistics and logistic regression analysis were done to describe and analyze the data using SPSS version 21 and p-value less than 0.05 was considered statistically significant. Seroprevalence of 7% (27/384) and 1.8% (7/384) were found for HBsAg and HIV, respectively, with co-infection rate of 1.04% (4/384). Out of the risk factors considered in the present study, an injury by sharp object (Adjusted Odds Ratio (AOR)= 3.05; 95% CI: 1.16- 8.01), having multiple sexual partners (AOR =2.66; 95% CI:1.07- 6.45), contact with HIV, HBsAg infected person (AOR=3.62; 95% CI: 1.15-11.38), blood transfusion (AOR= 7.39; 95% CI: 1.69-32.26) and abortion (AOR=3.53; 95% CI:1.20- 10.37) were independent explanatory risk factors for HBV infection($p<0.05$). However, in HIV infection, having multiple sexual partners (AOR=6.81; 95% CI: 1.22-38.12) was the only independent explanatory risk factor ($p<0.05$). The findings of this study (e.g., intermediate infection rate of HBsAg) implicates still a need for strong commitment and continuous work in prevention and control of HBsAg and HIV transmission among pregnant women in the study area.

Keywords: Ethiopia, HIV, HBsAg, Pregnancy, Risk factor, Shahura town, Seroprevalence

CHAPTER ONE

1. INTRODUCTION

1.1. Background of the study

Viral hepatitis is an inflammation of the liver. It is a major health problem worldwide and cause acute or chronic hepatitis, which can lead to the development of extensive liver scarring (cirrhosis), liver failure, liver cancer, and death. It is the 10th leading cause of death and the leading cause of liver cancer worldwide (Kamenya *et al*., 2017). The WHO estimates the prevalence of hepatitis infection at 2 billion globally with 350 million patients developing chronic infection (WHO, 2017). Viral hepatitis type B is a common and immensely serious disease caused by the hepatitis B virus (HBV), a partially double stranded DNA virus of the hepadnaviridae family. Chronic hepatitis B virus infection results in liver cirrhosis and hepatocellular carcinoma (Lavanchy, 2004).

The world's one-third population had HBV infection, and 500,000-700,000 people die every year (Shepard *et al*., 2006). The world can be divided into three areas where the prevalence of chronic HBsAg infection is: high (>8%), intermediate (2-8%), and low (<2%) (WHO, 2017). In Africa, about 70–90% of infants, infected before 1 year of age, develop chronic HBV infection, liver cirrhosis, hepatocellular carcinoma, and early death in children (Breakwell *et al.,* 2017). In 2015, the global prevalence of HBsAg infection in the general population was 3.5%. Its prevalence was the highest, 6.1%, in the African region (WHO, 2017).

The prevalence of HBsAg is 8% in West Africa and 5-7% in other parts of Africa. Nearly 70–95% of the adult population in Africa had past exposure to HBsAg infection with sero-prevalence of 6–20% (WHO, 2017). 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. Ten percent of infants born to women with acute HBsAg 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 (Mahadevapp *et al.,* 2016). In Ethiopia, the HBsAg prevalence

rate among pregnant women varies between 3% and 7.8% (Sefinew Molla *et al.,* 2015). Women of childbearing age can potentially transmit HBsAg to their babies. They transmit an infection to newborn usually during birth or soon after birth following close contact. Newborns that exposed to HBsAg will have almost 85–90% risk of developing chronic liver diseases (WHO, 2017) .In Ethiopia, the rate of HBsAg transmission from infected mother to the newborn is not well studied. However, one study revealed that 75% of newborns born from HBsAg infected women were positive with hepatitis B surface antigen (Dessie Tegegne *et al.,* 2014)

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 there were 430 000 new pediatric infections (Gisslen *et al*., 2017). 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.

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 (WHO, 2017). Both HIV and HBV share common ways of transmission in humans, which accounts for the high frequency of HIV- HBV co-infections. HIV and HBV have common risk factors like injectable drug use and blood transfusion (Yohannes Zenebe *et al.,* 2014), especially, the two most significant infectious agents are transmitted by sexual intercourse and positive mothers to fetus or newborns. Co-infection rate of hepatitis B virus and HIV is common which leads to increase morbidity and mortality as compared to HIV or HBV mono-infections (Muriuki *et al.,* 2013). 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 (Muriuki *et al.,* 2013). Moreover, in countries where the viruses are highly endemic, the rate can be as high as 20-25% (Muriuki *et al.,* 2013). The dual magnitude of HBV and HIV infection in Ethiopia was revealed by many seroepidemiological researches conducted in different segments of the country with regional variation. In a study from a rural hospital in Southern Ethiopia, the seroprevalence was 6.1 % for HBsAg and 1.8 % for HIV. Co-infection

with HIV-1 and HBsAg was detected in one patient (prevalence: 0.6 %) (Ramos, 2011). This study aimed to assess the seroprevalence and associated risk factors of HBsAg and HIV co-infections among pregnant women attending antenatal care at Shahura Health Center in Alefa District, central Gondar northwest Ethiopia.

1.2. Statement of the problem

Hepatitis B and Human immunodeficiency viruses exert a high toll worldwide. Both Hepatitis B virus and Human immunodeficiency virus are important public health problem. HBV causes hepatic cancer and death. Understanding the knowledge and practice about HIV/HBV co infection helps in formulating a strategy for prevention and treatment of HIV and HBV. Continuous monitoring of the level of HBV, HIV among pregnant women is important as treating those HBV, HIV infected mothers before delivery prevent transmission around birth (WHO, 2017).

Both HBV and HIV can lead to chronic disease, cancer, and death and neither can be eradicated with the use of current therapies (Kourtis. *et al*., 2012). Hepatitis B transmission is similar to that of HIV; therefore, co-infection with HIV and hepatitis B is not unusual (Firnhaber *et al*., 2008). 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 (Zelalem Desalegn *et al*., 2016). A cross-sectional study conducted in Bahir Dar northwest Ethiopia (Yohannes Zenebe *et al.,* 2014) on sero-prevalence 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%.

Studies in Ethiopia recommended incorporation of routine antenatal care (ANC) screening program for Hepatitis B (Kindie Mitiku *et al.,* 2018). However, regular antenatal screening of pregnant women is not common and compulsory in Ethiopia (Kindie Mitiku *et al.,* 2018). The absence of regular HBsAg screening program could be partly explained by lack of awareness on the overall load of hepatitis B among pregnant women in Ethiopia by health professionals and policymakers. Therefore, this study is conducted to give a quantitative estimate of the load

of HBsAg infection among pregnant women as a step to use for a better understanding of its epidemiology in Ethiopia and inform policymakers to take practical action at the policy level.

Although several studies have been conducted on the seroprevalence and associated risk factors of HBsAg and HIV mono-infections separately among pregnant women in different parts of Ethiopia, there are still lack of awareness on the overall magnitude of hepatitis B and HIV co-infections among pregnant women in the study area, Shahura town and co-infections among pregnant women has not been documented and published. Moreover, information about the seroprevalence and associated risk factors of HBsAg and HIV mono-infections. Therefore, this study was aimed to assess the seroprevalence and associated risk factors of HBsAg and HIV co-infections among pregnant women attending antenatal care at Shahura Health Center.

Research questions

1. What was the seroprevalence of HBsAg and HIV mono-infections among pregnant women in the study area in the study period?
2. What was the HBsAg /HIV co-infection rate among pregnant women in the study area?
3. What were the major potential risk factors associated with HBsAg and HIV co-infections among pregnant women in the study area?

1.3. Objectives

1.3.1. General objective of the study

The general objective of this study was to assess the seroprevalence and associated risk factors of HBV and HIV co-infections among pregnant women attending antenatal care at Shahura Health Center in Alefa District, northwest Ethiopia.

1.3.2 Specific objectives

The specific objectives of the present study were to: -

- Determine the seroprevalence of mono-infections of HBV and HIV among pregnant women.
- Determine the seroprevalence of HBV and HIV co-infection among pregnant women.

- Identify the major explanatory risk factors of HBV and HIV co-infection among pregnant women in the study area.

1.4. Significance of the study

This study will provid**e** information about the current prevalence and possible risk factors of HBV and HIV co- infections among pregnant women in the study area. It might also be helpful in effective planning and designing prevention, control and intervention strategies of HBV and HIV co-infections among pregnant women. Moreover, the current study may serve as baseline information for further studies on areas related to prevalence and associated risk factors of HBV and HIV co- infections among pregnant women.

1.5 Limitations of the Study

The study was limited to only pregnant women visiting Shahura Health Center during the study period, January to March, 2021. It did not include pregnant women who attended antenatal care in other health centers in the district.

1.6 Conceptual frame work

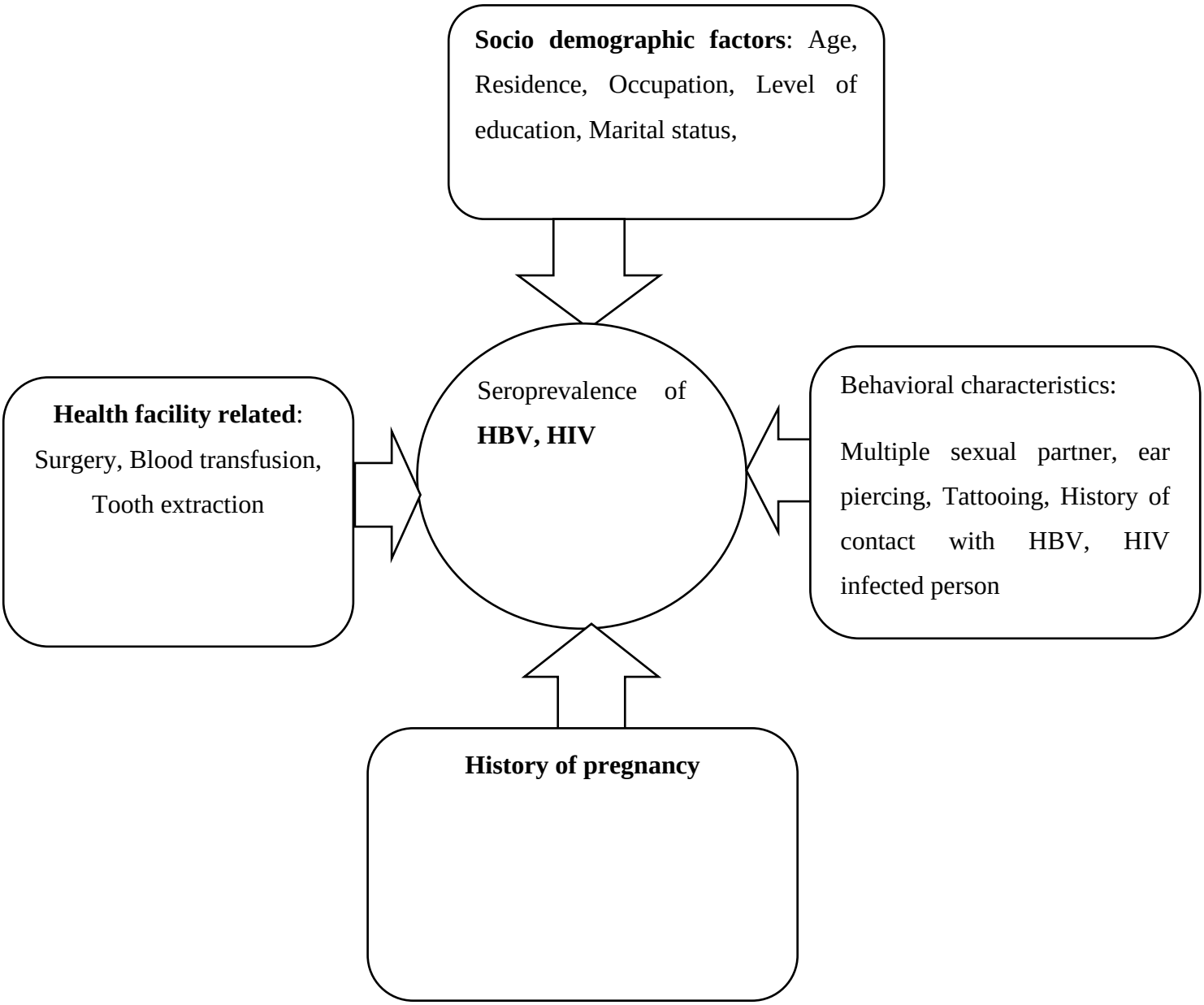

Figure 1. Conceptual framework of the study, Shahura Health Center, Ethiopia, 2021

CHAPTER TWO

2. LITERATURE REVIEW

Hepatitis B virus and Human immunodeficiency virus (HIV) infections are posing huge health impact throughout the world and the problem is higher in developing countries, very particularly, in Africa. The two most significant viruses share similar ways of transmission in humans, which accounts for the high frequency of HIV-HBV co-infections (Zelalem Desalegn *et al.*, 2016). 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 (Joseph *et al*.,2015). The prevalence of HIV and HBV infection in pregnant women can represent the majorities of the population in the communities because pregnant women are the interface for their sexual partners and infants. 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 (Alter, 2017).

2.1. Magnitude of HBV infection

Hepatitis B Virus (HBV) is a DNA virus and was first identified in the1965s (Shepard *et al*., 2006). The Hepatitis B Virus is a blood-borne virus and roughly 50 – 100 times more infectious than HIV (Tunje Tang *et al*., 2019). Hepatitis B virus (HBV) infection (acute and chronic) is one of the most common causes of human liver disease, and most people usually stay unaware of their infection status and present when the disease is advanced (Addisu Alehegn *et al*., 2020). It is the 10th leading cause of death and the leading cause of liver cancer worldwide. Assuming that women of reproductive age constitutes 25.3% of the world's population adults chronically infected may include 65 million women of childbearing age who can potentially transmit HBV to their babies. In addition, a proportion of these adults would benefit from long-term, if not lifelong, treatment, particularly those above 30 years of age, those who have cirrhosis, and those with HIV infection. This proportion of patients who would benefit from treatment is not well known. In community-based studies, reports range from less than 5% to about 10%. In health-care facility-based studies, the proportion is higher (WHO, 2017).

The prevalence of HBV infection varies greatly in different regions of the world and it is highly endemic in areas such as sub-Saharan Africa, Asia, the Pacific Basin, parts of the Middle East and the Amazon Basin (Dessie Tegegne *et al*., 2014). A study which was conducted South America the overall prevalence of HBV infection was low risk populations ranges from 0.4% to 13% Infectious Diseases Section, (1996). Different reports showed different prevalence of HBV infection among pregnant women in different parts of the world. According to a hospital- based cross-sectional study conducted in Felege Hiwot Referral Hospital, northwest Ethiopia, the seroprevalence of hepatitis B infections was found to be 4.4 %. (Zelalem Desalegn *et al.*, 2016).

A cross-sectional study which was conducted in Addis Ababa to investigate seroprevalence and transmission of Hepatitis B virus among delivering women revealed that 8/265 (3.0 %) of mothers were positive for Hepatitis B Virus surface antigen (Zelalem Desalegn *et al.*, 2016). The prevalence of HBV infection among pregnant women was reported 4.1% in Saudi Arabia and 4.6% in Pakistan (Bani *et al.*, 2012). On the other hand, lower prevalence of HBV infection was reported in India (0.9%) (Manisha *et al.*, 2011). other study done among Pregnant Women with Maternal and Perinatal Outcome in Government Medical College Jammu; Government Medical College Srinagar, India showed overall prevalence of 4% HBsAg among pregnant and non-pregnant women. The carrier rate of HBsAg among pregnant women was higher i.e. 5% as compared to non-pregnant i.e. 2%. 3 patients had history of blood transfusion and 66.6% of them were HBsAg positive. 3 patients had history of jaundice in the past. 2 out of these i.e. 66.66% were HBsAg positive. These findings suggest that blood and blood products are the commonest methods of spread and history of previous jaundice as one of the major risk factors to be looked for during routine screening of patients for hepatitis B.

All the HBsAg positive pregnant patients delivered live babies at term vaginally without any major complication. The cord blood of 60% babies was positive for HBsAg delivered from HBsAg positive mothers and these values were statistically highly significant (Rita *et al.*, 2012). Iran (0.7%). (Mohebbi *et al.*, 2011) and Egypt (1.75%). (Mortada *et al.*, 2013). However, higher prevalence of HBsAg were reported in Cameroon 7.7%, (Fomulu *et al.*, 2013), Yemen 10.8%, (Murad *et al.*, 2013), Mali 8.0%, (MacLean *et al.*, 2012), Nigeria 9.3%

(Pennap *et al*., 2011). Other studies conducted in University of Ilorin teaching hospital in Nigeria on the similar study population showed that prevalence of hepatitis B virus infection was found to be 16% but 5.6% in Sudan (Elsheikh *et al.,* 2007). Knowledge of the prevalence of chronic HBV infection among pregnant women is critical to understand the epidemiology of the disease. Studies and systematic reviews have found that the prevalence of HBsAg among pregnant women in a collection of African nations varies from 2.4–16% (Mutagoma *et al*., 2017) for example, a study conducted among Sudanese pregnant women found a prevalence of 5.6% (Elsheikh *et al*., 2007). Studies conducted in different sites of Ethiopia reported varied prevalence rates. In Jimma town the overall prevalence of HBsAg was 3.7% and high proportion of HBsAg positivity was recorded among the illiterate (61%) (Awole Mohammed *et al.,* 2005). Another study which was conducted in Gondar town and Dessie town, the overall prevalence of HBV was 4.9% and 7.3%, respectively (Moges Tiruneh 2008).

A study carried out in Northern Uganda (Bayo *et al*., 2014) showed the prevalence to be 11.8%. Although there had been a number of studies that have examined the epidemiology of HBV in South Western Uganda, no studies on factors associated and prevalence of Hepatitis B among pregnant women had been carried out in South Western Uganda.

The primary method of transmission reflects the prevalence of chronic hepatitis B virus infection in a given area. Risk factors identified included previous histories of surgical procedures, delivery outside the hospital and blood transfusion (Oladimeji *et al., 2013*). In low prevalence areas such as the continental United States and Western Europe, where less than 2% of the population is chronically infected, injection, drug abuse and unprotected sex are the primary factors, although other factors may be important. In moderate prevalence areas, which includes; Eastern Europe, Russia, and Japan were 2-7% of the population is chronically infected; the disease is predominately spread among children. In high prevalence areas such as China and South East Asia, transmission during child birth is most common, although in other areas of high endemicity such as Africa, transmission during childhood is a significant factor. The prevalence of chronic hepatitis B virus infection in areas of high endemicity is at least 8%-20% (Obeagu, 2018). Cirrhosis of the liver which is liver cancer may result from hepatitis B. The hepatitis B virus primarily interferes with the functions of the liver by replicating liver hepatocytes (Obeagu, 2018).

2.2. Load of HIV infection

Epidemiological studies throughout the world have shown four modes of HIV transmission. Unsafe sexual contact, transfusion of contaminated blood, use of contaminated syringes and from infected mother to the newborn child are the major routes of transmission of HIV. HIV infected women can transmit HIV to her fetus or infant before, during, or after birth. A pregnant woman with HIV infection has an approximately 20-40% chance of passing the virus to her fetus or newborn baby. There is evidence that infection can occur as early as the first 12-15 weeks of gestation. 50% of perinatal infections are in utero or during the birth process. It is estimated that a large number of perinatal infections occur through breastfeeding (Rajesh, 2008). Approximately 90% of all new pediatric infections occur during the perinatal period, of which 50% occur in relation to labour and delivery (Bailey *et al*., 2018).

Antiretroviral treatment (ART) as part of prevention of mother-to-child transmission (PMTCT) is a key strategy to combat the African HIV epidemic and decreases vertical transmission rates from as high as 45% to < 5% in breastfeeding populations (UNAIDS, 2015). PMTCT has yielded remarkable results, with an estimated 93% of pregnant women in eastern and southern Africa living with HIV receiving antiretroviral prophylaxis, resulting in mother-to-child transmission rates of less than 10%. Nevertheless, western and central Africa appear to lag behind, with fewer than half (48%) of pregnant women living with HIV in 2017 receiving PMTCT services (UNAIDS, 2015).

A previous study among pregnant women conducted from 2002 to 2006 found that 5.7% were HIV-1 infected, and 2.4% were HIV-2 infected, including 0.7% HIV-1/2 dually infected women (Gianelli *et al*., 2010). 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 (Soares *et al.*, 2011). In Ethiopia, the overall of prevalence of HIV among

pregnant women attending anti-natal care (ANC) was 5.3%. Moreover, the prevalence was higher in pregnant women from urban (9.5%) than rural areas (2.2%) (WHO, 2017).

A cross sectional study conducted on the utilization of HIV testing services among pregnant mothers in low income primary care settings in northern Ethiopia. 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 (Yihun Mulugeta *et al.*, 2017).

2.3. Load of HBV and HIV co-infection

HIV and HBV are blood-borne pathogens, and because of their shared modes of transmission, people at risk for HIV infection are also at risk for HBV infection (WHO, 2016; Djuidje *et al*., 2017). Hepatitis B virus (HBV) and Human immunodeficiency virus (HIV) infections are posing huge health impact throughout the world and the problem is higher in developing countries, very particularly, in Africa. The two most significant viruses share similar ways of transmission in humans, which accounts for the high frequency of HIV-HBV co-infections (Zelalem Desalegn *et al*., 2016). Co-infection with hepatitis B virus and HIV leads to increased morbidity and mortality as compared to independent HIV and HBV infections. In areas where HBV infection is either endemic or intermediate to high, the prevalence rate of HIV/HBV co-infection is recorded as high as 10–20 %. The prevalence rate can be as high as 20–25 % in countries where the viruses are highly endemic (Zelalem Desalegn *et al*., 2016). Co-infection of HBV and HIV among pregnant women attending ANC in Zambia was 31.3%, 9.0% in Ivory Coast, and 4.9% in Uganda. In North Region of Cameroon, the prevalence of HIV and HBV co-infection was 1.5%. HBV-related liver diseases are more progressive in HIV co-infected patients than in patients with HBV infection alone (Mutagoma *et al.*, 2017).

In a cross-sectional survey conducted on 13,121 pregnant women in Rwanda, (Mutagoma *et al*., 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%.

A cross-sectional study conducted in Bahir Dar Northwest Ethiopia (Yohannes Zenebe *et al*., 2014) 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 (Yohannes Zenebe *et al*, 2014).

A study conducted in Bamenda northwest region, Cameroon to determine sero-prevalence 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%) (Edith *et al*., 2015).

Another study from Bahir Dar which has included a total of 318 pregnant women was carried out to determine seroprevalence and risk factors of hepatitis B virus and human immunodeficiency virus infection among pregnant women. Overall, 21/318 (6.6 %) and 12/318 (3.8 %) of the pregnant women were positive for HIV and HBsAg, respectively. Of these, HIV/HBV co-infection rate was 4 (19.0 %) (Zelalem Desalegn *et al.,* 2016). The two most significant infectious agents are transmitted by sexual intercourse and positive mothers to fetus or newborns. Studies conducted in Ethiopia have revealed that HBV and HIV are endemic with regional variation (Zelalem Desalegn *et al.,* 2016).

In Ethiopia as part of other sub-Saharan Africa countries, the prevalence of HIV and liver disease 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 (Zelalem Desalegn *et al.*, 2016).

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 at Shahura Health Center.

2.4 Modes of Transmission and associated risk factors of HBV and HIV co infections

Prenatal transmission from mother to child and household contact with a person infected with HBV are the primary modes of transmission in areas with intermediate- or high-prevalence of HBV such as Asia, the South Pacific, sub-Saharan Africa, and certain populations in the Arctic, South America, and the Middle East (Aparna *et al*.,2015). Other less common modes of transmission include chronic hemodialysis, certain occupational exposures, blood transfusion, and organ transplant (rare). Tattooing with shared, contaminated needles or needle-like devices is another potential mode of HBV transmission that specifically affects pregnant women (Abdul *et al*.,2012).

HBV is viable for at least seven days on environmental surfaces and can be transmitted by sharing contaminated household items such as razors and tooth brushes (Elizabeth and Ramsey ,2011). A cross-sectional study conducted from January, 2012 to April, 2012 in Woldia, Northern Ethiopia reported that sharing of sharp materials in pregnant women. Similarly, those having shared any sharp material were 2.8 times more likely to be infected with HBV infection than those who never share sharp materials among pregnant women (AOR:2.807, 95%CI: 1.038 – 7.592). Across sectional study was conducted from March 2013 to April, 2013 at Bahir Dar city, Northwest Ethiopia, reported that Previous history of blood transfusion (AOR = 3.7, 95% CI, 9.02-14.84), body tattooing (AOR = 5.7, 95% CI, 1.24-26.50), history of surgery (AOR = 11.1, 95% CI, 2.64-46.88) and unsafe injection (AOR = 5.6, 95% CI, 1.44-22.19) were significantly associated with HBV infection (Yohannes Zenebe *et al.,* 2014).Mother to child transmission of HIV is the spread of HIV from a woman living with HIV to her child during pregnancy ,childbirth(labor and delivery)or breastfeeding .This mother to child transmission of HIV is perinatal transmission of HIV.

Across-sectional study was conducted from February to May, 2015 among pregnant women at Bahar Dar Felege HIwot Referral Hospital in Ethiopia, reported that history of nose piercing (COA 5.9; 95 % CI 1.2–29.9) and sexually transmitted infection (COR 4.3; 95 % CI 1.1–16.4) were significantly associated with HBV infections.

2.5 Diagnosis of HBV and HIV coinfections

Acute HBV infection may be subclinical, symptomatic, but self-limited, or fulminant. Subclinical (asymptomatic) disease usually occurs when HBV is acquired prenatally or in early childhood or in the immune suppressed. Mild to moderate symptoms occur in approximately 30–50% of persons infected as adults, and include fever, jaundice, anorexia, nausea, abdominal pain, and malaise. Arthritis, serum sickness, and a nonspecific rash may also occur with acute HBV infection and, when present, are helpful diagnostically. Acute HBV infection is confirmed by the serologic detection of IgM anti-HBcAg and HBsAg. The detection of HBsAg alone is not diagnostic for acute HBV infection, since pregnant women with asymptomatic chronic HBV infection can be newly infected with other pathogens that cause acute hepatitis. IgM anti-HBcAg may persist at detectable levels for up to two years in a small subset of acutely infected pregnant women.

2.6 Treatment of HBV and HIV coinfections

The aims of treatment of chronic hepatitis B are to achieve sustained suppression of HBV replication and remission of liver disease. The ultimate goal is to prevent cirrhosis, hepatic failure, and HCC" (AASLD, 2009).and HCC” (AASLD, 2009).

Some medications are “primary” treatments, meaning first line therapies and others are “secondary” treatments. Secondary treatments are used if primary treatment is contraindicated or if primary treatment has failed, particularly in the case of resistance.

Primary Treatments:

- Interferon-α (INF-α): Standard treatment up to 12-24 weeks, many side effects (flu-like, fatigue, neutropenia), injected medication, cannot be used in patients with decompensated liver disease,if planning on becoming pregnant, less chance of resistance, may cause neutropenia.

- Pegylated Interferon-α (peg INF-α): Standard treatment is 48 weeks. Many side effects (flu-like, fatigue, neutropenia). More convenient administration and sustained viral suspension than INF-α. Injected. Cannot be used in decompensated state.
- Lamivudine: Standard treatment 48-52 weeks. Cost effective for oral medication, high incidence of resistance, may be used during pregnancy, used for patients coinfected with HIV, can be used in patients with decompensated liver disease.
- Entecavir: Standard treatment 48 weeks. High efficacy primary treatment, low rate of resistance, can be used in patients with decompensated liver failure.

Secondary Treatments (or may be primary treatments if above primary treatments not indicated):

- Adefovir: Standard treatment 48 weeks. Good for patients with lamivudine-resistant HBV, but lower efficacy rate at eradicating the virus (only 25% response in some studies/patients).
- Tenofovir: Standard treatment is 48 weeks. Higher potency than adefovir and is effective at suppressing lamivudine-resistant HBV and wild-types.
- Telbivudine: Standard treatment is 52 weeks. Better efficacy than lamivudine and adefovir, but has same resistance and is expensive. Limited role as a primary therapy

2.7 Control and prevention of HBV and HIV coinfections

Prevention strategies include primary prevention of new infections (i.e. vaccines and postexposure prophylaxis), secondary prevention of HBV and HIV transmission by appropriate sexual and sanitary practices, and tertiary prevention of the pathological consequences of chronic HBV and HIV by anti-viral treatment (Elizabeth and Ramsey ,2011).

CHAPTER THREE

3. MATERIALS AND METHODS

3.1 Description of the study area

The study was conducted at Shahura Health Center located in Shahura town of Alefa District. Shahura is the town of Alefa District in Central Gondar zone, Amhara Regional State, northwest Ethiopia. It is bounded on the north by Takusa, on the east by Lake Tana, on the west by Quara and on the southwest by Mirabgojjam Zone (Figure 2). It is located at latitude $12^0$14'N and longitude $36^0$19'E in north western part of the country and it is about 685 km away from Addis Ababa.

The average temperature of the District is 22 $^{\circ}$C with the average rainfall which ranges from 1400-2200 mm (Alefa District Administration Office). Farmers widely produce cereals like teff, sorghum, and maize and they raise animals. About 80% of the farmers raise both crops and livestock, while 15.4% only grow crops and 4.75% only raise livestock. Based on 2007 national census conducted by Central Statistical Agency of Ethiopia (CSA) the population of Alefa District is 233,917, among these 119,518 of them are males and 114,399 of them are females (Alefa District Plan commission based on projection).

Shahura has one primary hospital (Shahura Primary Hospital) and 5 health centers in five kebele towns. The Shahura Health Center serves for 7 rural kebeles and 2 town kebeles. The total population, who received medical services since July 2020/2021 are 60797. Among these, 30,520 of them are males and 30,277 of them are females in the District (Health Center population data, 2020/2021).

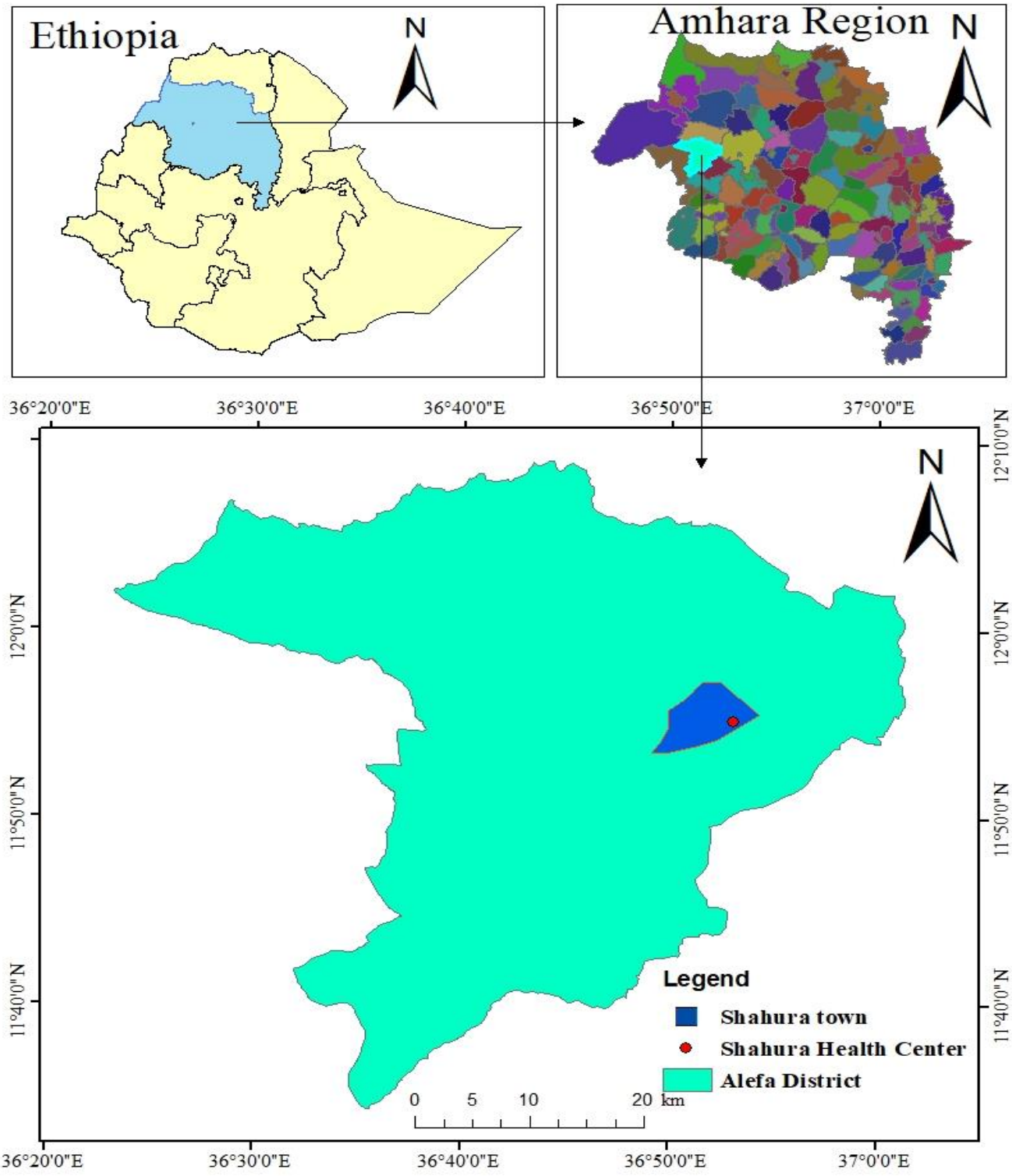

Figure 2. -Map of the study area (source: Alefa District Land Administration Office,2021)

3.2 Study design and period

A cross-sectional study was conducted from January to March 2021 to determine seroprevalence and associated risk factors of HBV and HIV co- infections among pregnant women attending antenatal care at Shahura Health Center, Alefa district, northwest Ethiopia.

3.3 Source and study population

3.3.1 Source population

All pregnant women attending antenatal care at Shahura Health Center were considered as a source of population.

3.3.2 Study Population

Pregnant women attending antenatal care at Shahura Health Center and fulfilling the inclusion criteria during the study period were the study population.

3.4 Inclusion and exclusion criteria

3.4.1 Inclusion criteria

All pregnant women, who were attending antenatal care at Shahura Health Center and who were willing to provide blood sample, socio demographic and potential risk factor information at a time of data collection were included in the study.

3.4.2 Exclusion criteria

Pregnant women, who were vaccinated against HBV infection, who were referred from other health institutions (who already knew HBV and HIV status), and those who severely ill were excluded from the study.

3.5 Sample size determination

In the estimation of sample size, statistical formula for sample size calculation for seroprevalence and associated risk factors of HBV and HIV co-infections among pregnant women attending antenatal care was used. Since the seroprevalence and associated risk factors of HBV and HIV co-infections among pregnant women attending antenatal care in the study

area was not known the sample size of the proposed study was calculated as a function of the 50% prevalence (Naing *et al*., 2006).

$n=Z^2P\ (1-P)/d^2$ Where: n=the sample size (respondents to be interviewed and give blood samples)

d=the precision of the study (5%)

Z=the standard normal deviation corresponding to 95% CI which is 1.96

P= seroprevalence of hepatitis B and HIV among pregnant women in Shahura Health Center was assumed to be 50%, since there has not been report on seroprevalence.

Thus: At 95%, Z=1.96, d=5%=0.05, P=50%=0.5, and Q=1- 0.5=0.5

Hence

$N = \frac{1.96 \times 1.96 \times 0.5 \times 0.5}{0.05 \times 0.05}$N=384.

Therefore, a total of 384 pregnant women were participated in the current study.

3.6 Sampling technique

Simple random sampling method was employed to include 384 study participants. Before sample collection, a brief explanation of the aims of study was given to volunteers and information was collected using a pre-structured questionnaire which contained questions related to socio-demographic characteristics, associated factors and history of pregnancy.

3.7 Data collection methods

3.7.1. Questionnaire survey

Structured questionnaire was used to collect data from pregnant woman, which was pre-tested in simple randomly selected pregnant women before the main study in other health center other than Shahura Health Center. An explanation about the aim of the study was given by the investigator to all voluntary participants, then after obtaining written consent from each of the study participants, information about risk factors of HBV and HIV infections was gathered from each pregnant woman. The questionnaires were distributed to study subjects and were filled by themselves. Those study subjects who could not read and write the questionnaires

were helped by the investigator to fill out the questionnaires. The questionnaires were developed in English and their response was translated to local language, Amharic.

3.7.2Blood Sample Collection and Examination

3.7.2.1 Laboratory analysis

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. 5 ml of venous blood was drawn by a sterile needle from each participant by the laboratory technician. Yellow top serum separator tube was used to collect blood. The specimens were labeled properly using participants' code. Standard operating procedure 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 15 minutes and the following testes were used in laboratory analysis.

3.8. HIV test

HIV testing was carried out following the national testing algorithm (Figure 3).

3.8.1 STAT PAK kit: It is manufactured by CHEMBIO DIAGNOSTIC SYSTEMS, INC.MEDFORD, USA. The Chembio HIV 1/2 STAT-PAK™ Assay is a single-use immunochromatographic test for the detection of antibodies to Human Immunodeficiency Virus Type 1 (HIV-1) and Type 2 (HIV-2) in finger stick whole blood, venous whole blood, serum or plasma specimens. The Chembio HIV 1/2 STAT-PAK™ assay is intended for use as a point of- care test to aid in the diagnosis of infection with HIV-1 and HIV-2. This test is suitable for use in multi-test algorithms designed for the statistical validation of rapid HIV test results. When multiple rapid HIV tests are available, this test should be used in appropriate

multi-test algorithms. In this test based on Ethiopian Public Health Institution, there are standard operating procedures and universal safety precautions.

Biological principles of the test

The Chembio HIV 1/2 STAT-PAK™ Assay employs a unique combination of a specific antibody binding protein which is conjugated to colloidal gold dye particles and HIV-1/2 antigens which are bound to the solid phase membrane. The venous or capillary (fingerstick) whole blood, serum or plasma is applied to the SAMPLE (S) well of test device followed by the addition of Running Buffer. The Buffer facilitates the lateral flow of the specimen and test reagents and promotes the binding of the antibodies to the antigen.

The specimen/buffer mixture migrates along the test strip by capillary action, reconstituting the conjugate. If present, the antibodies bind to the colloidal gold conjugated antibody binding protein. In a reactive sample, the dye conjugated-immune complex migrates on the nitrocellulose membrane and is captured by the antigens immobilized in the TEST (T) area producing a pink/purple line. In the absence of HIV-1 and HIV-2 antibodies, there is no pink/purple line in the TEST (T) area. The sample continues to migrate along the membrane and produces a pink/purple line in the CONTROL (C) area containing immunoglobulin G antigens. This procedural control serves to demonstrate that specimen and reagents have been properly applied and have migrated through the device. The sensitivity and specificity of the Chembio HIV 1/2 STAT-PAK™ Assay is 98.2% - 100% and 99.6% - 100%, respectively.

3.8.2 ABON kit

Test principle:

The HIV 1/2/O Tri-line Human Immunodeficiency Virus Rapid Test Device test strip is pre-coated with HIV-1 and subtype O antigens on T1 test line and HIV-2 antigen on T2 test line. Firstly, specimen and then buffer is added to the specimen well, thus starting the migration of the specimen/buffer. The specimen/buffer passes the conjugate pad which contains a mixture of HIV-1 envelope and capsid antigens and HIV-2 envelope antigen. These detection antigens are conjugated to latex particles. If present, the HIV-1 of HIV-2 antibodies react and bind to the detection antigen-conjugate. The antibody/antigen-conjugate mixture then migrates further

and binds to antigens present on the test lines. If the specimen contains antibodies to HIV-1, the specimen will bind to the T1 test line and produce a line, if specimen contains antibodies to HIV-2, the specimen will bind to the T2 test line. As liquid continues to migrate down the test strip, the control line will appear. If the control line is present, in addition to either or both test lines, then the test is reactive for HIV1/2 antibodies. If the specimen does not contain HIV-1 or HIV-2 antibodies, no colored lines will appear for either of the test lines region indicating a non-reactive result. It should be noted that the appearance of colored lines at T1 and T2 is highly unlikely to be indicative of co-infection with HIV-1 and HIV-2 but rather is a result of cross-reactivity between antigens.

A colored line will appear in the control line region if the migration of liquid has been successful and must be present for the test to be valid. Its presence does not confirm sufficient specimen addition. If used as a first line (screening) assay, any reactive specimens should be referred for additional testing using another method to confirm reactivity. Depending on the prevalence of disease, this may require one or two additional reactive results on at least two other assays. It is manufactured by ABON Biopharm Hangzhou Co., Ltd, P.R. China. The sensitivity and specificity of the ABON kit is 99.7% - 100% and 99.6% - 99.9%, respectively and also its accuracy is 99.7%-100%.

3.8.3 SD BIOLINE kit

Biological principles of the test

The SD BIOLINE HIV-1/2 3.0 kit is a rapid, qualitative test for detection of antibodies to all isotypes (IgG, IgM, IgA) specific to HIV-1including Subtype-O and HIV-2 simultaneously in human serum, plasma or whole blood. The SD BIOLINE HIV-1/2 3.0 test contains a membrane strip, which is pre-coated with recombinant HIV- 1 capture antigen (gp41, p24) on test band 1 region and with recombinant HIV- 2 capture antigen(gp36) on test band 2 region, respectively. The recombinant HIV 1/2 antigen (gp41, p24, and gp36)-colloid gold conjugate and the specimen sample move along the membrane chromatographically to the test region (T) and form a visible line as the antigen –antibody –antigen gold particle complex forms with high degree of sensitivity and specificity. This test device has a letter of 1,2 and C as test line 1 (HIV-1), test Line 2(HIV-2) and control Line on the surface of the device. Both the test

Lines and control Line in result window are not visible before applying any sample. The control Line is used for procedural control. Control line should always appear if the test procedure is performed properly and the test reagents of control Line are working. It is manufactured by STANDARD DIAGNOSTICS, INC, Republic of Korea. The sensitivity and specificity of the kit is 98%-100% and 99.8%-100%, respectively.

3.8.4 Current national testing algorithm of HIV

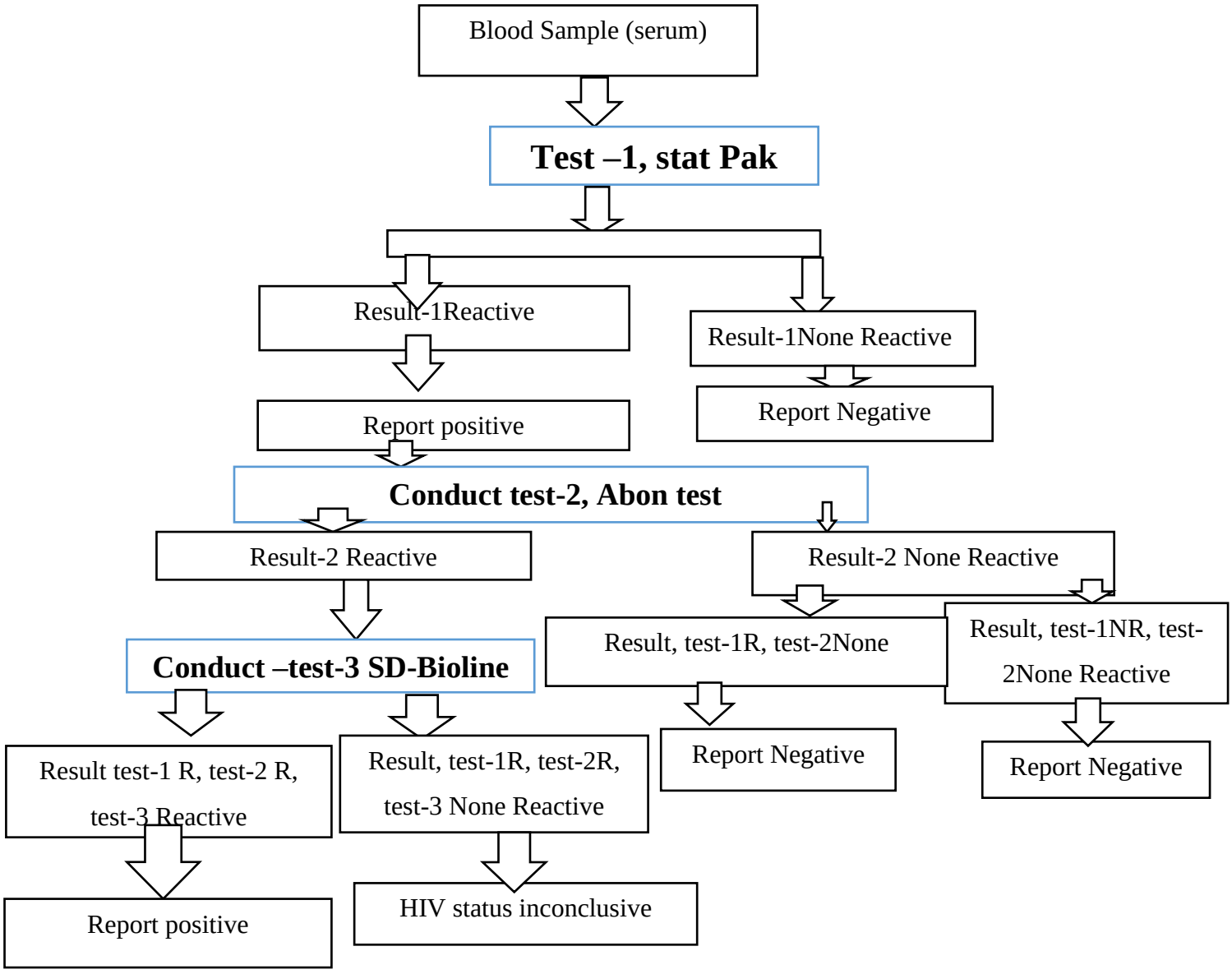

Figure 3. Current National Testing algorithm for HIV (Source: MoH ,2011 EC)

3.9 HBsAg Test

Principles: - HBsAg Rapid Test Cassette (serum/plasma/whole blood) is a lateral flow chromatographic immunoassay for the qualitative detection of hepatitis B surface antigen (HBsAg) in human whole blood, serum or plasma. It is intended to be used as a screen test and as an aid in the diagnosis of infection with hepatitis B virus (HBV). The presence of HBsAg in serum or plasma is an indication of an active hepatitis B infection, either acute or chronic. All serum samples were tested for HBsAg using rapid diagnostic kits which work in the principle of immune chromatography following standard operating procedure of the laboratory. The quality of test results was maintained using internal quality control of the test kits and by using known negative and positive samples. The sensitivity and specificity of the kit is 99.4% and 99.5%, respectively and as well as its accuracy is 99.5%. It is manufactured by ACON® LABORATORIES, INC. San Diago, USA.

3.10 Variables

The independent variables of the study were maternal age, history of contact with infected person, tooth extraction, residence, education level, sharp object injury, abortion, hospital admission, ear piercing, occupational status, marital status, surgery, body tattooing, history of blood transfusion, history of multiple sexual partners, history of pregnancy whereas prevalence of HBV and HIV infections among pregnant women attending antenatal care at Shahura Health Center were the dependent variables.

3.11 Quality assurance and control

To make sure that the questionnaire was appropriate and understandable for pregnant women in the study area, pre-testing of 5% the questionnaire was done among pregnant women in another health center prior to the study. The collected data was checked daily for consistency and accuracy. Standardized procedures were strictly followed during blood sample collection, storage and analytical process. Positive and negative controls were run alongside of the laboratory test.

3.12 Data analysis and interpretation

The quantitative data was cleaned and entered into SPSS version 21 for analysis. After the analysis the descriptive part was presented in the form of tables, percentages and graphs. Univariate logistic regression analysis was done and the variables with less than 0.25 p-value in the univariate logistic regression analysis was taken as candidates for the multivariable logistic regression analysis (Bursac *et al.*, 2008). In the multivariate logistic regression analysis, a p-value of less than 0.05 and Adjusted Odds Ratio (AOR) with a 95% CI was taken to declare independent explanatory factors for HBV/HIV mono- infections or co-infections among study subjects in the study area.

3.13 Ethical consideration

The study protocol together with consent form was submitted to Ethical Clearance Committee of Science College, Bahir Dar University for proposal approval. After getting ethical clearance (Appendix-VI), letter of support was written to Shahura Health Center. The subjects were informed about the study and written informed consent was obtained from all of the participants before collecting the blood samples. Participation in the study was on voluntary basis and study subjects were free to withdraw from the study before and after collection of blood samples without losing any of the benefits they were supposed to obtain from the health center. Study subjects who were positive for either of the viruses or both were advised to see their medical doctors for regular follow up and appropriate treatment.

3.14 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.

Hepatitis refers to an inflammation of the liver commonly caused by viruses and possibly also by other causes.

HIV is a virus that attacks the immune system.

3.15 Work flow chart for the test procedure

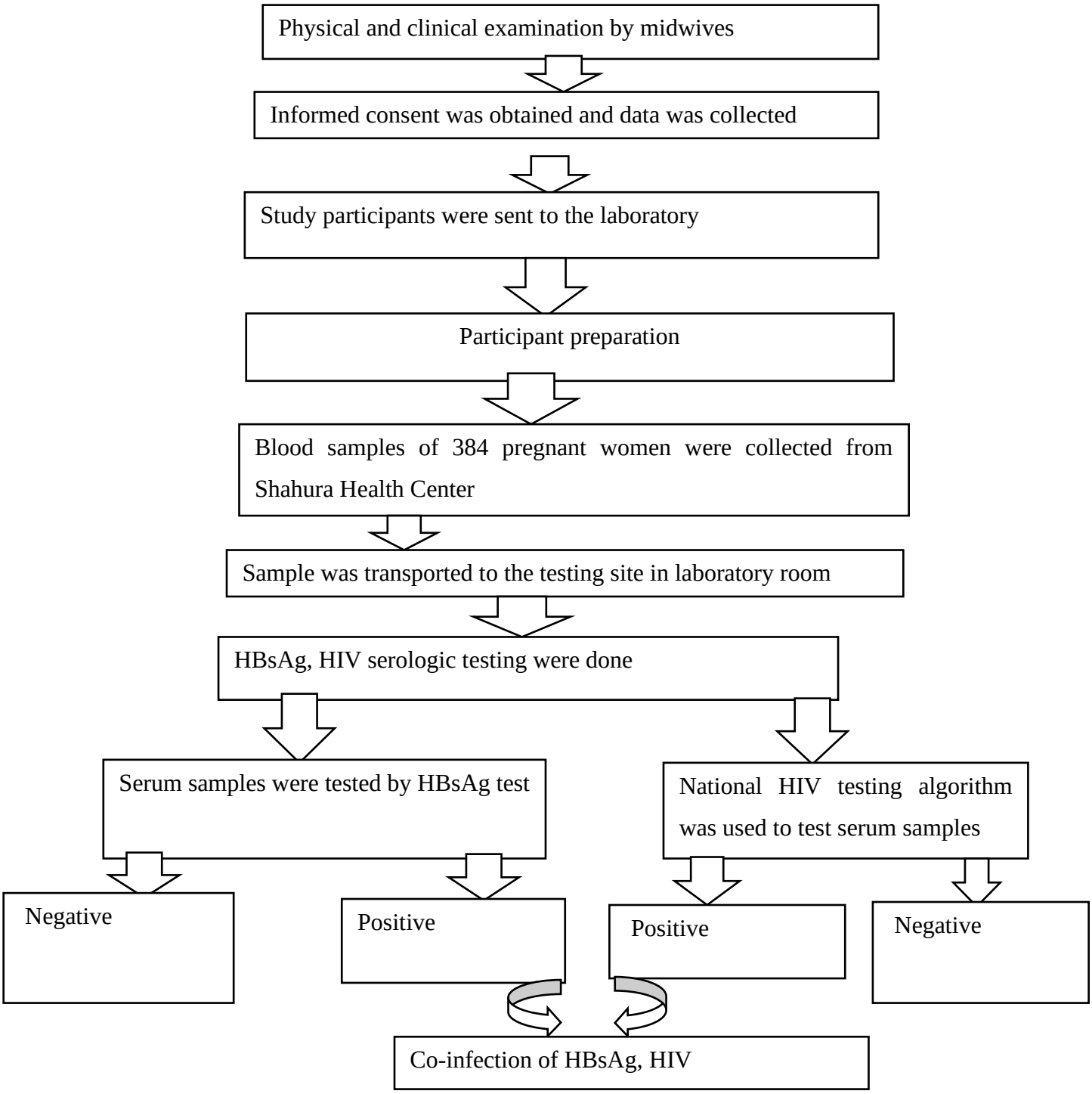

Figure 4. Diagrammatic representation of the work flow of the research

CHAPTER FOUR

4. RESULTS

4.1 Socio-demographic characteristics

A total of 384 pregnant women were included in the current study with a total of 100% response rate. Their age ranged from 16 to 43 years with a mean and standard division of 26.1± 5.5. The majorities were married (86.7%), housewives (65.9%), in age group of 20-29 years (64.6%), urban resident (52.9%), and illiterate (31.3%).

Table 1**:** Socio-demographic characteristics of pregnant women attending at Shahura Health Center from January-March 2021(n=384)

Variables	**HIV Status**		HBsAg Status		**Total n (%)**
	Positive n (%)	**Negative n (%)**	**Positive n (%)**	**Negative n (%)**	
Age (years)					
<20	0(0.0)	30(100)	2(6.7)	28(93.3)	30(7.8)
20-29	6(2.4)	242(97.6)	20(8.1)	228(91.9)	248(64.6)
30-39	1(1.0)	96(99)	5(5.2)	92(94.8)	97(25.3)
>=40	0(0.0)	9(100)	0(0.0)	9(100.0)	9(2.3)
Residence					
Rural	2(1.1)	179(98.9)	12(6.6)	169(93.4)	181(47.1)
Urban	5(2.5)	198(97.5)	15(7.4)	188(92.6)	203(52.9)
Educational level					
Illiterate	1(0.8)	119(99.2)	10(8.3)	110(91.7)	120(31.3)
Primary school	2(2.3)	86(97.7)	5(5.7)	83(94.3)	88(22.9)
Secondary school	1(0.9)	108(99.1)	9(8.3)	100(91.7)	109(28.4)
Diploma and above	3(4.5)	64(95.5)	3(4.5)	64(95.5)	67(17.4)
Marital Status					
Single	2(8.3)	22(91.7)	2(8.3)	22(91.7)	24(6.3)
Married	4(1.2)	329(98.8)	23(6.9)	310(93.1)	333(86.7)
Widowed	1(6.7)	14(93.3)	1(6.7)	14(93.3)	15(3.9)
Divorced	0(0.0)	12(100)	1(8.3)	11(91.7)	12(3.1)
Occupation					
House Wife	3(1.2)	250(98.8)	18(7.1)	235(92.9)	253(65.9)
Private	4(5.1)	75(94.9)	7(8.9)	72(91.1)	79(20.6)
Gov. Employed	0(0.0)	52(100)	2(3.8)	50(96.2)	52(13.5)

Table 2. Sero-prevalence of HIV and HBV coinfection among pregnant women attending antenatal care at Shahura Health Center from January-March 2021(n=384)

Variables	HIV Status		HBsAg Status		Total n (%)
	Positive n (%)	Negative n (%)	Positive n (%)	Negative n (%)	
Sharp object injury					
Yes	1(1.4)	72(98.6)	14(19.2)	59(80.8)	73(19.0)
No	6(1.9)	305(98.1)	13 (4.2)	298(95.8)	311(81.0)
Multiple sexual partners					
Yes	4(5.1)	75(94.9)	12(15.2)	67(84.8)	79(20.6)
No	3(1.0)	302(99.0)	15(4.9)	290(95.1)	305(79.4)
Body tattooing					
Yes	1(1.6)	118(99.2)	7(5.9)	112(94.1)	119(31.0)
No	6(1.9)	259(97.7)	20(7.5)	245(92.5)	265(69.0)
Ear-piercing					
Yes	5(2.0)	243(98.0)	16(6.5)	232(93.5)	248(64.6)
No	2(1.5)	134(98.5)	11(8.1)	125(91.9)	136(35.4)
History of contact with HIV, HBsAg infected person					
Yes	0(0.0)	26(100.0)	6(23.1)	20(76.9)	26(6.8)
No	7(2.0)	351(98.0)	21(5.9)	337(94.1)	358(93.2)
Surgery					
Yes	0(0.0)	36(100.0)	1(2.8)	35(97.2)	36(9.4)
No	7(2.0)	341(98.0)	26(7.5)	322(92.5)	348(90.6)
Tooth extraction					
Yes	1(1.6)	61(98.4)	4(6.5)	58(93.5)	62(16.1)
No	6(1.9)	316(98.1)	23(7.1)	299(92.9)	322(83.9)
Hospital admission					
Yes	0(0.0)	57(100.0)	5(8.8)	52(91.2)	57(14.8)
No	7(2.1)	320(97.9)	22(6.7)	305(93.3)	327(85.2)
Blood transfusion					
Yes	1(7.7)	12(92.3)	6(46.2)	7(53.8)	13(3.4)
No	6(1.6)	365(98.4)	21(5.7)	350(94.3)	371(96.6)
Gravidity					
1st gravida	3(2.9)	99(97.1)	8(7.8)	94(92.2)	102(26.6)
2nd gravidae	2(1.6)	123(98.4)	10(8.0)	115(92.0)	125(32.6)
3rd and above	2(1.3)	155(98.7)	9(5.7)	148(94.3)	157(40.9)
Sexual partner 's HIV, HBsAg exposure					

Variables	HIV Status		HBsAg Status		Total n (%)
	Positive n (%)	Negative n (%)	Positive n (%)	Negative n (%)	
Yes	0(0.0)	11(100.0)	1(9.1)	10(90.9)	11(2.9)
No	7(1.9)	366(98.1)	26(7.0)	347(93.0)	373(97.1)
Pregnancy related problems					
Yes	1(1.4)	70(98.6)	8(11.3)	63(88.7)	71(18.5)
No	6(1.9)	30798.1 ()	19(6.1)	294(93.9)	313(81.5)
Abortion					
Yes	2(5.9)	32(94.1)	9(26.5)	25(73.5)	34(8.9)
No	5(1.4)	345(98.6)	18(5.1)	332()94.9)	350(91.1)

4.2 Sero-prevalence of HBsAg and HIV

Figure 5 displays seroprevalence of HBsAg**,** HIV, and co-infection of both among pregnant women attending at Shahura Health Center. Out of 384 pregnant women in this study, 27(7%) participants were HBsAg positive whereas 7(1.8%) of pregnant participants were HIV positive. Among these, four of the pregnant participants were co-infected by HBsAg and HIV.

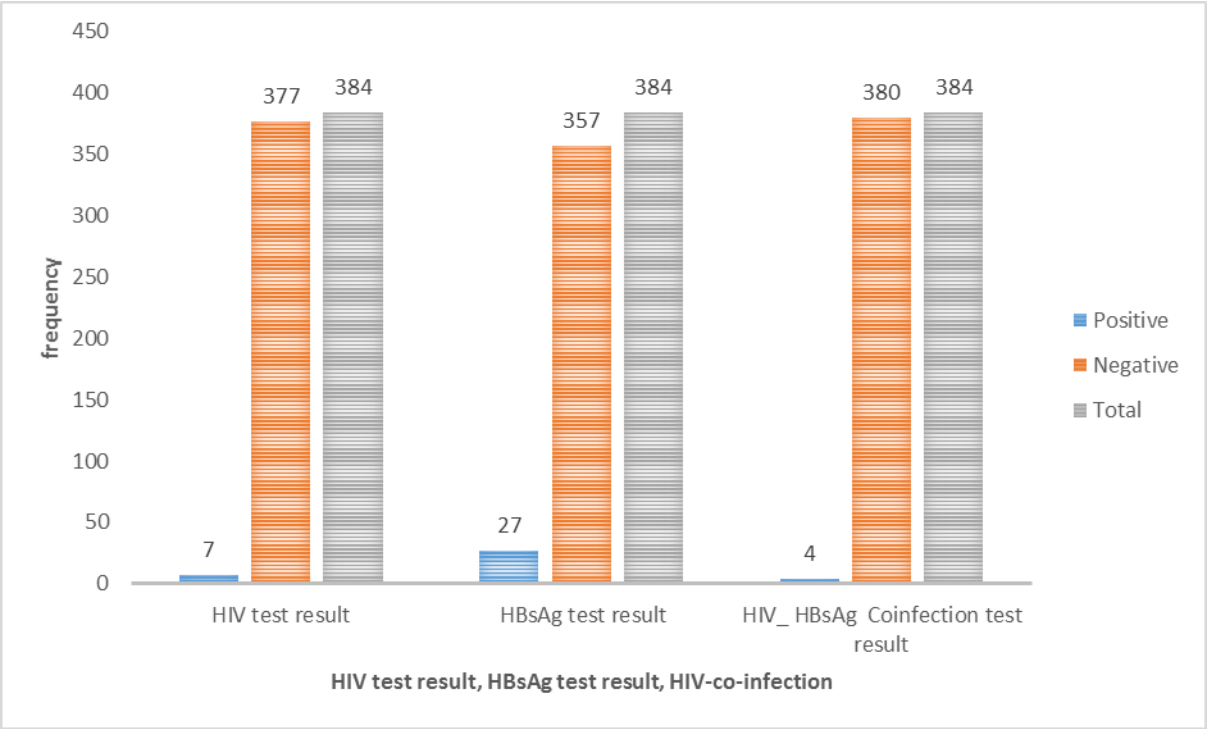

Figure 5. Seroprevalence of HBsAg, HIV and Co-infection of HBsAg, HIV among pregnant women attending antenatal care at Shahura Health Center

4.3 Univariate logistic regression analysis of risk factors potentially associated with sero-prevalence of HBsAg

Table 3 shows the univariate analysis of HBsAg result with its factor variables. From this analysis, there was statistically significant association between injury due to sharp object and HBsAg infection ($p<0.05$). The odds of HBsAg infection among pregnant women who were injured with sharp object were five times compared with those pregnant women who were not injured with sharp object (COR: 5.44; 95% CI: 2.43-12.17).

The variable, multiple sexual partners was significantly associated with HBsAg ($p <0.05$) (COR: 3.46; 95% CI: 1. 55-7.74). This implied that the odds of HBsAg infection among pregnant women who had multiple sexual partners were three times compared with those who had no multiple sexual partners.

Contact with HBsAg and HIV infected person was significantly associated with HBsAg ($p <0.05$) (COR: 4.81; 95%CI: 1. 75-13.26). This result showed that the odds of infection among

pregnant women who had contact with HBsAg and HIV infected persons were about five times compared with those who had no history of contact with HBsAg and HIV persons.

There was statistically significant association between blood transfusion and HBsAg ($p<0.05$). The likelihood of HBsAg positivity was 14 times higher among pregnant women who received blood from donors by transfusion than in those who did not receive blood by transfusion (COR: 14.29; 95%CI: 4. 41-46.30).

Abortion showed statistically significant association with HBsAg ($p <0.05$) (COR: 6.64; 95%CI 2.71-16.29). This result showed that the likelihood of HBsAg positivity among pregnant women who aborted during pregnancy was about seven times higher compared with those who did not abort.

Table 3. Univariate analysis of risk factors potentially associated with HBsAg among pregnant women attending antenatal care at Shahura Health Center from January -March 2021(n=384)

Variables	**HBsAg Test Result**			
	Positive	**Negative**	COR(CI)	*P*-value
Sharp object injury				
Yes	14	59	5. 44(2.43, 12.17)	0.000
No	13	298	1	
Multiple sexual partner				
Yes	12	67	3.46(1.55, 7.74)	0.002
No	15	290	1	
Body tattooing				
Yes	14	112	0. 77(0.32, 1.86)	0.556
No	13	245	1	
Ear-piercing				
Yes	16	232	0.78(0.35, 1.74)	0.549
No	11	125	1	
History of contact with HIV, HBsAg infected person				
Yes	7	20	4.81(1.75, 13.26)	0.000
No	20	337	1	
Surgery				
Yes	1	35	0.35 (0.05, 2.67)	0.315
No	26	322	1	
Tooth extraction				
Yes	4	58	0. 90(0.30, 2.69)	0.846
No	23	299	1	
Hospital admission				
Yes	5	52	1.33(0.48, 3.68)	0.579
No	22	305	1	
Blood transfusion				

Yes	6	7	14.29(4.41, 46.30)	0.000
No	21	350	1	
Gravidity				
1st gravida	8	94	1.40 (0.52, 3.76)	0.504
2nd gravidae	10	115	1.43(0.56, 3.64)	0.452
3rd and above gravidae	9	148	1	
Sexual partner's HIV HBsAg, exposure				
Yes	1	10	1.34(0.16, 10.38)	0.787
No	26	347	1	
Pregnancy related problems				
Yes	8	63	1.97(0.82, 4.69)	0.128
No	19	294	1	
Abortion				
Yes	9	25	6.64(2.71, 16.29)	0.000
No	18	332	1	

Note: Statistically significant at p-value < 0.05, COR=crude odds ratio, CI= confidence interval

4.4 Multivariate logistic regression analysis of risk factors potentiality associated with sero-prevalence of HBsAg

From univariate logistic regression analysis result, we found six potential risk factors (variables) such as sharp object injury, multiple sexual partners, history of contact with HIV, HBsAg infected person, blood transfusion, pregnancy related problem, and abortion with P-value ≤ 0.25 which were supposed to be selected and entered into multivariable logistic regression to control the confounding effects. After adjusting for confounding effects, in the final multivariate logistic regression model, only five variables were found to be independent explanatory variables of HBsAg infection among pregnant women in the study area ($p<0.05$) (Table 4).

In the multivariate logistic regression analysis, pregnant women who were injured with sharp objects were three times more likely to be HBsAg positive compared with those without injury by sharp object (AOR: 3.05; 95% CI: 1.16 -8.01).

The odds of HBsAg positivity were about three times higher in pregnant women who had multiple sexual partners than in those who had no multiple sexual partners (AOR: 2.67;95%CI: 1.07-6.45).

The chance of being infected with HBsAg was about four times higher in pregnant women who had history of contact with HIV, HBsAg infected persons than in those who had no history of contact (AOR: 3.62; 95% CI: 1.15-11.38).

The odds of HBsAg positivity were seven times higher in pregnant women who had history of blood transfusion than in those without history of blood transfusion (AOR: 7.4; 95% CI: 1.69-32.26).

The odds of HBsAg positivity were about four times higher in pregnant women who had history of abortion than in those who had no history of abortion (AOR: 3.53; 95% CI: 1.20-10.37). This implies that participants who aborted during pregnancy were highly likely that they had been exposed to HBsAg.

Table 4. Multivariate logistic regression analysis of HBsAg and possible risk factors among pregnant women attending antenatal care at Shahura Health Center from January-March 2021(n=384)

Variables	HBsAg positive	HBsAg negative	COR(CI)	*P*-value	AOR (CI)	*P*-value
Sharp object injury						
Yes	14	59	5. 44(2.43, 12.17)	0.000	3.05(1.16, 8.01)	0.024
No	13	298	1		1	
Multiple sexual partners						
Yes	12	67	3.46(1.55, 7.74)	0.002	2.66 (1.07, 6.45)	0.036
No	15	290	1		1	
History of contact with HIV, HBsAg infected person						
Yes	7	20	4.81(1.75, 13.26)	0.000	3. 62(1.15, 11.38)	0.002
No	20	337	1		1	
Blood transfusion						
Yes	6	7	14. 29(4.41, 46.30)	0.000	7.39(1.69, 32.26)	0.008
No	21	350	1		1	
Pregnancy related problems						
Yes	8	63	1. 97(0.82, 4.69)	0.128	0. 93 (0.32, 2.72)	0.893
No	19	294	1		1	
Abortion						
Yes	9	25	6.64(2.71, 16.29)	0.000	3.53(1.20, 10.37)	0.022
No	18	332	1		1	

Note: Statistically significant at p-value < 0.05, AOR= adjusted odds ratio, COR=crude odds ratio, CI= confidence interval

4.5 Factors associated with HIV infection and co-infection of HIV and HBsAg

4.5.1 Seroprevalence of HIV and analysis of risk factors

Of the total 384 pregnant women in this study, seven were found to be HIV positive with a prevalence rate of **1.8%** (Figure- 5). Six of them belonged to the age group of 20-29 years whereas the remaining one pregnant woman belonged to age group of 30-39 years. Of the total of HIV positive participants, four were married, two were single and one was widow (Table 5).

Table 5. Risk factors potentially associated with HIV infection among pregnant women attending antenatal care at Shahura Health Center from January -March 2021(n=384)

Variables	Total n (%)	HIV positive n (%)	COR (95%CI)	*P*-value	AOR (CI)	*P*-value
Age (years)						
<20	30(7.8)	0(0)	-	-	-	-
20-29	248(64.6)	6(85.7)	-	-	-	-
30-39	97(25.3)	1(14.3)	-	-	-	-
>=40	9(2.3)	0(0)	-	-	-	-
Residence						
Rural	181(47.1)	2(28.6)	0.44(.09-2.31)	0.333	0. 73(.08-6.83)	0.78
Urban	203(52.9)	5(71.4)	1			
Educational level						
Illiterate	120(31.3)	1(14.3)	0. 18(0.02-1.76)	0.140	0. 26(0.01-5.44)	0.385
Primary school	88(22.9)	2(28.6)	0. 50(0.08-3.06)	0.450	1.06(0.12-9.34)	0.961
Secondary school	109(28.4)	1(14.3)	0. 20(.02-1.94)	0.164	0. 25(.02-2.73)	0.253
Diploma and above	67(17.4)	3(42.9)				
Marital Status						
Single	24(6.3)	2(28.6)	-	-	-	-
Married	333(86.7)	4(57.1)	-	-	-	-
Widowed	15(3.9)	1(14.3)	-	-	-	-
Divorced	12(3.1)	0(0)	-	-	-	-
Occupation						

House Wife	253(65.9)	3	-	-	-	-
Private	79(20.6)	4	-	-	-	-
Gov. Employed	52(13.5)	0	-	-	-	-
Sharp object injury						
Yes	73(19)	1(14.3)	0. 71(0.08-5.96)	0.749	0.44(0.04-4.81)	0.504
No	311(81)	6(85.7)	1		1	
Multiple sexual partners						
Yes	79(20.6)	4(57.1)	5. 37(1.18-24.50)	0.03	6. 81(1.22-38.12)	0.029
No	305(79.4)	3(42.9)	1			
Body tattooing						
Yes	119(31)	1(14.3)	0.37(0.04-3.07)	0.354	0. 37(0.04-3.56)	0.392
No	265(69)	6(85.7)	1			
Ear-piercing						
Yes	248(64.6)	5(71.4)	1. 38(0.25-7.20)	0.703	2. 38(0.38-15.06)	0.356
No	136(35.4)	2(28.6)	1		1	
History of contact with HIV, HBsAg infected person						
Yes	26(6.8)	0(0)	-	-	-	-
No	358(93.2)	7(100)	-	-	-	-
Surgery						
Yes	36(9.4)	0(0)	-	-	-	-
No	348(90.6)	7(100)	-	-	-	-
Tooth extraction						
Yes	62(16.1)	1(14.3)	0.86(0.10-7.30)	0.893	1.06(0.11-10.76)	0.958
No	322(83.9)	6(85.7)	1		1	
Hospital admission						
Yes	57(14.8)	0(0)	-	-	-	-
No	327(85.2)	7(100)	-	-	-	-
Blood transfusion						
Yes	13(3.4)	1(14.3)	5. 07(0.57-45.47)	0.147	4.57(0.36-58.70)	0.243
No	371(96.6)	6(85.7)	1		1	
Gravidity						
1st gravidae	102(26.6)	3(42.9)	2. 35(0.39-14.30)	0.354	3.03(0.38-24.19)	0.295
2nd gravidae	125(32.6)	2(28.6)	1.26(0.18-9.07)	0.818	1. 14(0.13-9.71)	0.904
3rd and above gravidae	157(40.9)	2(28.6)	1		1	
Sexual partner ‘s HIV, HBsAg exposure						
Yes	11(2.9)	0(0)	-	-	-	-
No	373(97.10)	7(100)	-	-	-	-
Pregnancy related problems						
Yes	71(18.5)	1(14.3)	0.73(0.09-6.17)	0.773	0. 54(0.05-6.39)	0.623
No	313(81.5)	6(85.7)	1		1	

Abortion						
Yes	34(8.9)	2(28.6)	4.31(0.80-23.12)	0.088	3. 49(0.48-25.32)	0.216
No	350(91.1)	5(71.4)	1		1	

4.5.2 Sero prevalence of HIV-HBsAg co-infection and analysis of risk factors.

Of the total of 384 pregnant women in this study, four were found to be both HIV and HBsAg positive at the same exposure with a prevalence rate of 1.04 %. Three of them belonged to the age group of 20-29 years whereas the remaining one pregnant woman belonged to age group of 30-39 years. Of the total of HIV_ HBsAg co-infection positive participants, three were urban dwellers, while the one was rural resident undergone ear piercing (Appendix- V).

CHAPTER FIVE

5. Discussion

Hepatitis B virus and HIV are posing a vital health impact especially in pregnant women and their neonates. Co-infection rate of hepatitis B virus and HIV is common which leads to increase morbidity and mortality as compared to HIV or HBV mono-infections particularly in developing countries (Yohannes Zenebe *et al.,* 2014). 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 sero-prevalence and associated risk factors of HBV and HIV co-infection among pregnant women attending at Shahura Health Center.

The overall seroprevalence of HBsAg in the current study was 7% (27/384). This was in agreement with the prevalence of 7.3% in Gondar town, northwest Ethiopia (Moges Tiruneh 2008). 6.9% in Deder Hospital, eastern Ethiopia (Abdi Umare *et al*., 2015)

The finding of current study was lower than several studies conducted in different countries. In Ethiopia, the study conducted in Hawassa University Referral Hospital, southern Ethiopia was 7.8% (Metaferia Yeshi *et al*.,2016). In different countries, for instance, higher prevalence of HBsAg in pregnant women was reported in Yemen 10.8% (Murad *et al.,* 2013), Nigeria 9.3% (Pennap *et al*., 2011), Mali 8.0% (MacLean *et al.,* 2011). Other studies conducted in the University of Ilorin teaching hospital in Nigeria on the similar study population showed that prevalence of hepatitis B virus infection was found to be 16% (Elsheikh *et al.,* 2007). A study carried out in Northern Uganda (Bayo *et al.,* 2014) showed the prevalence to be 11.8%).

On the other hand, the present finding showed a higher prevalence rate compared to those reported from different parts of Ethiopia. For instance, it was higher than 3.8% from Bahir Dar town, northwest Ethiopia (Yohannes Zenebe *et al*., 2014), 3.0% from Addis Ababa Ethiopia and 3.5% from Dawuro zone, SNNPR, southwest Ethiopia (Chernet Asrat *et al*., 2017) and 6% another study in Dawuro zone, SNNPR, southwest Ethiopia (Zelalem Desalegn *et al*., 2016). Studies which were conducted in different sites of Ethiopia, In Jimma town the overall prevalence of HBsAg was 3.7% (Awole Mohammed *et al*., 2005). Another study which was

conducted in Dessie town the overall prevalence of HBsAg was 4.9% (Moges Tiruneh 2008). In different parts of Ethiopia, the prevalence showed 5.3% in Debre Tabor general hospital (Fisseha Walle *et al*., 2008), 5.5% in Tigray (Tadele Araya *et al*.,2017), 3% in St. Paul's millennium medical college and Selam Health Center (Dessie Tegegne, 2014). This might be due to the difference in sampling method, risky socio cultural and risky behavioral practice and methods used to screen HBsAg infection among studies.

The prevalence of HBsAg was also higher when compared to the rest of Africa and other world countries in Iran (0.7%) (Mohebbi. *et al.*, 2011) and Egypt (1.75%) (Mortada *et al.*, 2013) and in Greece 2.89% (Papaevangelou *et al.*, 2006). On the same way, the current finding was higher than studies conducted in Iran 1.2% (Moghdasifar *et al*, 2016). The prevalence of HBsAg infection among pregnant women was reported 4.1% in Saudi Arabia and 4.6% in Pakistan (Bani *et al.*, 2012). On the other hand, lower prevalence of HBsAg infection was reported in India 0.9%(Manisha *et al*., 2011), 0.14% to 0.97% in USA (Euler, 2003).0.9% in Brazil (Souza, 2012) 1% in Kenya (Ngerecia, 2016), 1.5% in Libya (El-Magrahe, 2010) 1.6% in Saudi Arabia (Alrowaily and Ferwanah, 2008) and 2.1% in North Turkey (Brett, 2012) was reported. This variation may be due to regular screening and vaccination for HBsAg in more developed countries. Whereas the high prevalence of HBV infection among pregnant women in the study area were due to unprotected sexual practice, sharing or using of sharp objects, lack of awareness about transmission methods and risky socio cultural and risky behavioral practice,

The overall seroprevalence of HIV in the current result was 1.8%. This indicates lower than studies documented in Addis Ababa Ethiopia 4.2% (Zelalem Desalegn *et al.*, 2016), and Bahir Dar northwest Ethiopia 6.6% (Yohannes Zenebe *et al*., 2014). One of the possible reasons why the finding in this study is lower than other studies could be the inability to include other pregnant mothers attending other separate clinics in Alefa district. The present HIV among studied subjects was also lower than 6.6% reported from Bamenda, northwest region Cameroon (Edith *et al*., 2015).

On the other hand, the current finding about HIV was higher than reports from India 0.03% (Bansal *et al.*, 2015), rural hospital in Mali 0.4 % (MacLean *et al*.,2011), in Brazil 0.09%-

0.7% (Costa *et al*.,2009). This higher prevalence of HIV in the present study was attributed to age of study participants where majority were between 20-39 years-old and importantly, it is a time when most of the women start to participate in risk sexual behaviors.

The seroprevalence of HBsAg and HIV co- infection among studied pregnant women in the study area was 1.04% which is lower than what were reported from Dare Salaam 3.9 % (Rashid *et al.*, 2014) and Nigeria (9.5%) (Bassey *et al*.,2009). The highest seroprevalence of co-infection of both viruses was documented in Bahir Dar town, northwest Ethiopia (19.0%), (Yohannes Zenebe *et al*., 2014). However, the current study was higher than the study done in Southern Ethiopia (0.6%) (Ramos *et al*.,2011). This co-infection rate implicates that both HIV and HBsAg share the same route of transmission. Differences in findings among various studies can be explained by variations in geography, socio-economic conditions, sexual practice, and the number of study population and the level of awareness about the transmission of HIV and HBsAg.

In this study, there was no statistically significant association between the socio-demographic characteristics and hepatitis B surface antigen and HIV positivity. In the multivariate logistic regression analysis, pregnant women who were injured with sharp objects were three times more likely to be HBsAg positive compared with those without injury by sharp object (AOR: 3.05; 95% CI: 1.16 -8.01). Among explanatory variables, previous history of piercing with sharp materials (AOR = 3.0, 95% CI 1.17- 7.80)

Having multiple sexual partners was a recognized mode of transmission of hepatitis B infections and other sexually transmitted infections, hence the finding in this study that women with more than one sexual partner had an association with HBV and HIV infections. The odds of HBsAg positivity were about three times higher in pregnant women who had multiple sexual partners than in those who had no multiple sexual partners (AOR: 2.67;95%CI: 1.07-6.45). This finding was consistent with the fact that the possibility of having multiple sexual partners was considered as one of the most predisposing factors of HBsAg infection. This study identified different associated risk factors for HBV infection (Table 4). Having a history of multiple sexual partners was found to be an important risk factor to acquire HBVand HIV infection. This might be because pregnant women who had multiple sexual partners were more likely to get HBV and HIV transmission through unprotected sex with different partners than

those who had a single partner. This implies that there may be unsafe sexual practices within the community which is a serious practice to expose people not only to hepatitis but also to HIV and other sexually transmitted diseases. This finding is similar to other studies (Sefinew Molla *et al*.,2015).

The chance of being infected with HBsAg was about four times higher in pregnant women who had history of contact with HIV, HBsAg infected persons than in those who had no history of contact (AOR: 3.62; 95% CI: 1.15-11.38).

In this study having a history of blood transfusion was an independent risk factor for hepatitis B virus infection. The odds of HBsAg positivity were seven times higher in pregnant women who had history of blood transfusion than in those without history of blood transfusion (AOR: 7.387; 95% CI: 1.69- 32.26). A similar study, which was conducted in Egypt identified this variable as significant associated factor (Kamal *et al*.,2010). Such pregnant women who had a previous history of blood transfusion were about four times more likely to be positive for HBsAg (AOR = 3.70, CI, 9.02-14.84). In fact, in Ethiopia, blood and blood products have been screened for HBV and HIV**.**

The odds of HBsAg positivity were about four times higher in pregnant women who had history of abortion than in those who had no history of abortion (AOR: 3.53; 95% CI: 1.20-10.37). This implies that participants who aborted during pregnancy were highly likely that they had been exposed to HBsAg. History of abortion (AOR = 6.6, 95% CI 2.50- 17.71) were significantly associated with HBV infection. Abortion is directly related to sexually active women, and one of the most known modes of transmission for HBV is exposure to sexual intercourse. Deliberate termination of pregnancy is the result of unwanted pregnancy which in turn could be because of unwanted sexual contact. Therefore, abortion significance could be because of sexual transmission of hepatitis B virus.

CHAPTER SIX

6. Conclusion and Recommendation

6.1 Conclusion

The current finding indicated a 7% seroprevalence rate of HBsAg infection among studied pregnant women in Shahura Health Center and the level of prevalence is an intermediate one, according to the world health organization's classification. On the other hand, the HIV seroprevalence (1.8%) among these study subjects is a little bit higher than the nation prevalence rate. The seroprevalence of HBsAg and HIV co- infection was 1.04%. As indicated in this study, out of risk factors considered, injury by sharp object, contact with HBV and HIV infected persons, having multiple sexual partners, history of blood transfusion and abortion were significantly associated with HBV infection and were explanatory variables among the study subjects in study area. A high prevalence of HBsAg was observed among reproductive age groups.

6.2 Recommendation

The seroprevalence of current study indicates that screening of all pregnant women for HBsAg and HIV and the way to prevent the risk factors for the exposure of HBsAg 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 implementation of control measures. Hence antenatal screening tests for HBsAg and HIV must be accessible for all pregnant women; there should be a mechanism to facilitate vaccination for HBsAg infection for all pregnant women. Thus, health education about these risk factors in particular and the mode of transmissions and prevention of HIV and HBsA in general should be given. Further research works should be conducted at district and higher levels to consolidate the fight against HBsAg and HIV infection among pregnant women in the region, hence, screening of pregnant women for HBsAg, HIV is a corner stone for disease detection, diagnosis, prevention and intervention.

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Appendices

Appendix-I - Written consent form

Name of study participant ----------Code----------

Dear participant of this study

I am Mezgebekal Alemu and am an MSc student at Biology Department of Science College, Bahir Dar University and have proposed to study the sero- prevalence of HBsAg and HIV co-infections among pregnant women attending antenatal care at Shahura Health Center. So, you are invited to be part of this study by providing blood sample and associated risk factor related information. There is no health problem in participating in this study and the result obtained from your blood sample is strictly secured. Participation in this study is completely voluntary and you have a right to withdraw from participating in this study at any time. Refusal to participate will not result in loss of medical care or any other benefits you receive. However, participating in this study is extremely important for predicting the prevalence of HBsAg and HIV co- infections that could seriously affect the health of pregnant women. If you have understood the explanation, we are asking you kindly to participate in the study. Please put your signature as illustrated bellow

Participant's Name----------signature----------Date----------

Wittiness's Name----------signature----------Date----------

Investigator's Name----------signature----------Date----------

የስምምነት ቅጽ በአማርኛ

ኤች አይቪ እና ሄፓታይተስ ቢ ላይ ጥናትና ምርምር ልሰራ ነኝ፡፡እርስዎ ፈቃደኛ ከሆኑ በጥናቱ ላይ እንዲሳተፉ ተጋብዘዋል፡፡በጥናቱ ላይተሳታፊ ከሆኑ እርስዎ ላይ ለምርምራ የሚያገለግል የደም ናሙና በመስጠት እንዲተባበሩኝ በትህትና እጠይቅወታለሁ፡፡ የእርስዎ በዚህ ጥናት መሳተፍ የእርስዎ ሙሉ ፈቃድ ሲሆን በጥናቱ ላይ ላለመካተትም ሆነ ከተካተቱም በኋላያ ለምንም ቅድመ ሁኔታ ፈቃደኝነትዎን የማንሳት መብትዎ ሙሉ በሙሉ የተጠበቀ ነዉ፡፡እርስዎ በጥናቱ ያለመካተት መወሰንዎ በእርስዎ ላይ ያገኙት የነበረን የህክምና ተጠቃሚነትንም ሆነ ሌላ ችግር ፈጽሞ ሊያስከትልብዎት አይችልም፡፡ ለእርስዎ የተባለዉን ነገር በትክክል ተረድተዉ ጥያቄ ካልዎት መጠየቅ እና ማብራሪያ የማግኘት መብት አለዎት፡፡ስለጥናቱ አላማ ምርመራ እና ሂደት ተገልጾልኛል፡፡ ምክንያት ሳያስፈልገኝ ከጥናቱ ላለማቋረጥ ተረድቻለሁ፡፡ ይህ ስምምነት ቅጽ በአፍ መፍቻ ቋንቋየ አንብቤ/ተነቦልኝ በትክክል ተረድቼ በራሴ ፈቃደኝነት በጥናቱ ለመሳተፍተ ስማምቻለሁ፡፡ለዚህም በፊርማየ አረጋግጣለሁ፡፡

የጥናቱተሳታፊስም----------ፊርማ----------ቀን----------

የምስክርስም----------ፊርማ----------ቀን----------

የአጥኝዉስም----------ፊርማ----------ቀን----------

Appendix-II Questionnaire

Questionnaire prepared to assess the seroprevalence and associated risk factors of HBsAg and HIV co-infections among pregnant women attending at Shahura health center. Therefore, your

honest response is vital. All information given in this questionnaire will be handled confidentially. Please circle the letter of your choice. Code----------

I. Socio-demographic Information

1. Age_____ A. Below 20 A. 20-29 B. 30-39 C. 40 and above

2. Residence A. Rural B. Urban

3. Education Level A. Illiterate B. Primary School C. Secondary School D. Diploma& Above

4. Marital Status A. Single B. Married C. Widowed D. Divorced

5. Occupation A. House wife B. Private C. Government employed

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

6. Sharp object injury A. Yes B. No

7. Multiple sexual partners A. Yes B. No

8. Body tattooing A. Yes B. No

9. Ear Piercing A. Yes B. No

10. History of Contact with HIV, HBsAg infected person A. Yes B. No

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**.**

III. 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 HBsAg, HIV? A. Yes B. No

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

18. Abortion/Miscarriage A. Yes B. No

Appendix-III Questionnaire form for clinicians

This will be filled by medical doctors or nurses after the study subjects have undergone medical examination

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

Code of the study subject: ……. Circle either of the two choices depending on laboratory result

1. HIV1/2 A. Positive B. Negative

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

Appendix -IV Standard Operating Procedures

1. Collect test items and other necessary lab supplies.

2. Remove device from package and label device with client identification number.

3. Collect approximately 5ul of specimens using a new disposal loop or pipette.

4. Dispense the sample in the center of sample well.

5. Add 3 drops of buffer, holding vial vertically over the sample well.

6. Wait for 10 minutes before reading the results.

7. Read and record the results and other pertinent information on the work sheet.

Stat – Pak Test Results

1. Reactive:2 lines of any intensity appear in both the control and test areas.

2. **Non-Reactive:**1 line appears in the control area and no line in the test area.

3. **Invalid:** no line appears in the control area. Do not report invalid results. Repeat test with a new test device even if a line appears in the test area.

Appendix –V

Table5. Factor associated with HIV_ HBsAg co-infection positivity among pregnant women attending antenatal care at Shahura Health Center from January-March 2021(n=384)

Variables	Total n (%)	HIV_ HBsAg co-infection positive n (%)	COR (95%CI)	*P*-value	AOR (CI)	*P*-value
Age Category						
<20	30(7.8)	0(0)	-	-	-	-
20-29	248(64.6)	3(75)	-	-	-	-
30-39	97(25.3)	1(25)	-	-	-	-
>=40	9(2.3)	0(0)	-	-	-	-
Residence						
Rural	181(47.1)	1(25)	0.37(0.04-3.59)	0.392	0. 61(0.003-9.12)	0.769
Urban	203(52.9)	3(75)			1	
Educational level						
Illiterate	120(31.3)	1(25)	0.27 (0.02-3.07)	0.293	0. 18(0.003- 9.12)	0.390
Primary school	88(22.9)	0(0)	-	-	-	-
Secondary school	109(28.4)	1(25)	0.30(0.03- 3.38)	0.331	0.26(0.02-3.95)	0.330
Diploma and above	67(17.4)	2(50)	1		1	
Marital Status						
Single	24(6.3)	1(25)	-	-	-	-
Married	333(86.7)	2(50)	-	-	-	-
Widowed	15(3.9)	1(25)	-	-	-	-
Divorced	12(3.1)	0(0)	-	-	-	-
Occupation						
House Wife	253(65.9)	2(50)	-	-	-	-
Private	79(20.6)	2(50)	-	-	-	-
Gov. Employed	52(13.5)	0(0)	-	-	-	-
Sharp object injury						
Yes	73(19)	1(25)	1. 43(0.15-13.91)	0.76	0. 71 (0.03-17.40)	0.835
No	311(81)	3(75)	1		1	
Multiple sexual partner						
Yes	79(20.6)	2(50)	3. 94 (0.55-28.38)	0.174	2.34 (0.27-20.41)	0.442
No	305(79.4)	2(50)	1		1	
Body tattooing						
Yes	119(31)	0(0)	-	-	-	-
No	265(69)	4(100)	-	-	-	-
Ear-piercing						
Yes	248(64.6)	4(100)	-	-	-	-
No	136(35.4)	0(0)	-	-	-	-
History of						

contact with HIV, HBsAg infected person						
Yes	26(6.8)	0(0)	-	-		
No	358(93.2)	4(100)				
Surgery						
Yes	36(9.4)	0(0)	-	-	-	-
No	348(90.6)	4(100)	-	-	-	-
Tooth extraction						
Yes	62(16.1)	1(25)	1.74 (0.18-17.04)	0.633	3.17(0.22-45.24)	0.395
No	322(83.9)	3(75)	1		1	
Hospital Admission						
Yes	57(14.8)	0(0)	-	-	-	-
No	327(85.2)	4(100)	-	-	-	-
Blood Transfusion						
Yes	13(3.4)	1(25)	10.22(0.99-105.60)	0.051	8.06(0.31-211.51)	0.211
No	371(96.6)	3(75)	1		1	
Pregnancy Status						
1st gravidae	102(26.6)	1(25)	0. 77(0.10-8.57)	0.830	0.72(0.06-9.53)	0.806
2nd gravidae	125(32.6)	1(25)	0. 63(0.06-6.98)	0.703	0.38(0.03-5.68)	0.484
3rd and above gravidae	157(40.9)	2(50)	1		1	
Sexual Partner HIV, HBsAg exposure						
Yes	11(2.9)	0(0)	-	-	-	-
No	373(97.10)	4(100)	-	-	-	-
Pregnancy Related Problems						
Yes	71(18.5)	1(25)	1.48 (0.15-14.40)	0.738	2.18(0.12-38.62)	0.597
No	313(81.5)	3(75)	1		1	
Abortion						
Yes	34(8.9)	2(50)	10. 88 (1.48-79.81)	0.019	8.65(0.84-89.44)	0.070
No	350(91.1)	5(50)	1		1	

Appendix –VI

ሳይንስ ኮሌጅ
የድህረ ምረቃ፣ምርምርና ማህበረሰብ
አገልግሎት ም/ዲን
ባሕር ዳር ዩኒቨርሲቲ
ባሕር ዳር - ኢትዮጵያ

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ቁጥር: PRCSVD/74/2013
ቀን: 21/05/2013

Ethical Clearance Approval Form

Applicant's Name: Mezgebekal Alemu

Research Title	Sero-prevalence and associated risk factors of HBV and HIV co-infections among pregnant women attending antenatal care at Shahura Health Center in Alefa District, Central Gondar, northwest Ethiopia
Researcher (s) Name (s)	Mezgebekal Alemu

Thank you for submitting your application for ethical clearance, which was considered at the College of Science Research Ethics Committee meeting on 15 January 2021. The committee has reviewed your ethical application, issues pertaining to participants, consent form, debriefing, and relevant questionnaires.

The researcher should keep the confidentiality of the identity of research participants and data that will be obtained from them. Any serious adverse events or significant changes which occur in connection with this study and /or which may alter its ethical consideration must be reported immediately to the committee for a possible ethical amendment.

We are therefore pleased to inform you that the College's Ethical Clearance Committee has approved your study from an ethical point of view.

With kind regards

Tsegaye Kassa (PhD)
The Graduate, Research and Community Services V/Dean
College of Science

Dr. Tsegaye Kassa Gogie
P.G.R.C.S. Vice Dean

CC//
- Dean office
- The Graduate, Research and Community Services V/Dean
- Department of Biology

College of Science