ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

SEROPREVALENCE AND RISK FACTORS OF HBV, HIV AND SYPHILIS INFECTIONS AMONG PREGNANT WOMEN ATTENDING AT GANDHI MEMORIAL HOSPITAL, ADDIS ABABA, ETHIOPIA.

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LIST OF ABBREVIATIONS

- AAU Addis Ababa University
- AIDS Acquired immunodeficiency syndrome
- ANC Antenatal clinic
- ART Anti-Retroviral Treatment
- ARV Anti-Retroviral
- CD4 Cluster of Differentiation 4
- CD8 Cluster of Differentiation 8
- CDC Centre for disease control
- DC Data Collector
- DNA Deoxy Ribonucleic Acid
- DRERC Departmental Research and Ethical Review Committee
- ELISA Enzyme linked immunosorbent assay
- EMA Ethiopian Medical Association
- EMLA Ethiopian Medical Laboratory Association
- EPHA Ethiopian Public Health Association
- GMH Gandhi Memorial Hospital
- MTCT Mother to Child Transmission
- HAART Highly active antiretroviral therapy
- HBV Hepatitis B virus
- HBsAg Hepatitis B surface antigen
- HCC Hepatocellular carcinoma
- HIV Human immunodeficiency virus
- HRP Horse Radish Peroxidase
- HSV-2 Herpes simplex virus type 2
- LFT Liver function test
- AOR Adjusted Odds Ratio
- PMTCT Prevention of Mother-to-Child Transmission
- PICT Provider initiative counseling and testing

- RNA Ribonucleic acid
- RPR Rapid plasma reagin
- SOPs Standard Operating Procedures
- SPSS Statistical Package for the Social Sciences
- STD Sexually transmitted diseases
- STI Sexually transmitted infection
- TPPA *Treponema pallidum* particle agglutination assay
- UNAIDS The Joint United Nations Programme on HIV/AIDS
- VCT Voluntary Counseling and Testing
- VDRL Venereal Disease Research Laboratory
- WHO World Health Organization

OPERATIONAL DEFINITIONS

- **Acute infection**: is an active infection developed rapidly after exposure (less than six month of exposure).
- **Chronic HBV infection**: Persistent (long-term) infection with Hepatitis B Virus developed after six month of exposure.
- **Co-infection**: is the presence of dual infection concurrently.
- **Hepatitis B surface antigen (HBsAg):** A marker present in persons who are currently infected with HBV (i.e. persons with both recent infection and chronic infection)
- **Prevalence:** is the number of cases of a disease in a specific place at a specific time
- **Seronegative:** is the status of an individual who gives a negative reaction to a serological test
- **Seropositive:** is the status of individual who gives a positive reaction to a serological test
- **Sexually transmitted infections (STIs**): – Infectious disease which is passed from one person to another through intimate sexual contact with infected person.

ABSTRACT

Background

Sexually transmitted infections (HIV, HBV and Syphilis) are major public health problems worldwide with a highly prevalent in developing countries like Asia and Sub-Saharan Africa. All of these common sexually transmitted infections share similar mode of transmission which is horizontal and perinatal (vertical) transmission. Their complication isn't only restricted to the pregnant women but also to their newborn infants; rather they affects concurrently.

Objective

This study has undertaken to determine sero-prevalence and risk factors of HBV, HIV and syphilis infections among pregnant women attending at Gandhi Memorial Hospital, Addis Ababa, Ethiopia from January to April 2014.

Method

A descriptive-correlational study design conducted among 403 pregnant women attending at Gandhi Memorial Hospital, Addis Ababa, Ethiopia between January and April 2014. Participants enrolled consecutively after consenting and an interview have conducted to obtain information regarding risk factors. Blood have collected and screened for hepatitis B surface antigen using rapid screening test strip and cassette device. After all; the final positive sample for HBsAg confirmed by enzyme linked immunosorbent assay (ELISA). Moreover; Antibodies to HIV-1/2 tested based on the national testing algorism but *Trepollema pallidum* antibodies were by Syphilis Rapid Test Strip (Quick Test™ Syphilis Serum/ Plasma/Whole Blood Strip). Finally the data entered using Epi Info version 3.5.1 and exported to SPSS version 16 so as to recode, clean, validate and analyze.

Results

A total of 403 pregnant women with the mean age of 24.8 (SD±5.99) years old enrolled in the study. Overall, 21/403 (5.2%) and 20 /403 (5%) of the pregnant women were positive for HIV and HBsAg, respectively. The co-infection of HIV-HBV was 0.5% (2/403). However; no cases of Syphilis detected positive. In relation to the risk factors; history of sex with multiple sexual partners, pre-exposure to STI and low level of monthly income were significantly associated with both HBV and HIV, while each infection found to have additional different risk factors;

these includes: receiving of blood through donation, ear piercing and history of abortion for HBV infection and sharing different sharp materials and contact history with infected person for HIV infection alone.

Conclusion

This study showed that HIV infection was similar with Addis Ababa HIV prevalence whereas; HBV infection was intermediate according to WHO classification. Syphilis was non-existing and HBV-HIV co-infection was very low. Therefore; not only for HIV but also for HBV infection needs to be screened for all pregnant women during pregnancy.

Key terms: HIV, HBsAg, Syphilis, Seroprevalence, risk factors, pregnant women.

1. Introduction

1.1. Back ground information

Sexually transmitted infections (STIs) are a group of infectious or communicable diseases in which their primary mode of transmission is through sexual contact and they are among the major causes of illnesses throughout the world particularly in the developing countries. They are extraordinarily common with an estimated 340 million new cases of "curable" infections (including syphilis) occurring each year worldwide in men and women aged 15–49 years. In addition to the "curable" STIs, there are also millions of viral STIs that occur annually (including human immunodeficiency virus and hepatitis B viruses) that cannot be eradicated through currently available medications [1].

Currently such STIs (HIV, HBV and syphilis) have continued to be a public health problem in both developed and developing countries that causes acute illness, infertility, long-term disability and death, with severe medical and psychological consequences for millions of women and infants. There is tangible scientific evidence that a person with an untreated sexually transmitted infections (STIs), particularly involving ulcers or discharge, is at an increased risk of passing on or acquiring HIV during sex due to the presence of broken skin or membranes allowing the virus to enter or leave the body [2].

1.1.1. Human immunodeficiency virus (HIV)

Human immunodeficiency virus (HIV) is an RNA virus that belongs to a family of viruses known as retroviruses and causes acquired immunodeficiency syndrome (AIDS). The name comes from the fact that these viruses can convert their RNA into a DNA copy using an enzyme known as reverse transcriptase. The virus is a highly variable which mutates very readily meaning it have many different strains, even within the body of a single infected person [3]. HIV infects vital cells in the human immune system such as helper T cells (specifically CD4+ T cells) and its infection leads to low levels of CD4+ T cells through different mechanisms [4]. There are two types of HIV: HIV-1 and HIV-2 and their main transmission routes are through unsafe sexual contact, contaminated blood and blood products and vertically from mother to child. The geographical distribution of type-1 is predominant throughout the world where as type-2 is relatively uncommon and is concentrated in West Africa [5].

1.1.2. Hepatitis B Virus (HBV)

Viral hepatitis type B is a serious disease caused by hepatitis B virus (HBV) and it is under Hepadnaviridae family. Infection with hepatitis B virus (HBV) is a serious public health problem worldwide and leads to a wide spectrum of clinical presentations, ranging from asymptomatic carrier state to acute self-limiting infection or fulminant hepatic failure, chronic hepatitis with progression to cirrhosis and hepatocellular carcinoma (HCC) . Presence of Hepatitis B surface antigen (HBsAg) in serum is the first sero-marker to indicate active HBV infection, either acute or chronic [6].

Acute disease usually occurs when the immune response is well preserved and it lasts for six months. On the other hand the chance of developing chronic infection depends on the status of individuals and their related immunities where; patients with an immunodeficiency are more likely to develop a chronic disease and becoming a source for new infections. In addition the likelihood where HBV infection become chronic can depends upon the age at which a person is infected, infants and young children being the most likely to develop chronic infection[6, 7].

HBV is carried in blood and other body fluids, including saliva, tears, semen and vaginal secretions that's why exposing to either of this transmitting fluids can be a source of acquiring HBV infection. Depending on the epidemiological pattern within a geographic area, the main ways of transmission are sexual intercourse, parenteral contact or infection of the baby at birth from an infected mother. [7].

1.1.3. Syhpilis

Syphilis is one of the sexually transmitted infections and is a reportable disease caused by *Treponema pallidum* which is a fragile spiral bacterium with 6-15 micrometer long by 0.25 micrometer in diameter. Its small size makes it invisible on light microscopy; therefore, it must be identified by its distinctive undulating movements on dark field microscopy. The bacteria can survive only briefly outside of the body; thus, transmission almost always requires direct contact with the infectious lesion through acquired or congenital [8].

Syphilis passes through a series of frequently overlapping stages which are primary, secondary, latency, and tertiary. Primary syphilis is characterized by a single, painless chancre that begins about 21 days after exposure where as secondary syphilis presents with an array of dermatological lesions and eruptions that can occur 4 to 10 weeks after exposure. Moreover; Latent (hidden) stage of syphilis begins when primary and secondary symptoms disappear without treatment and its infection remains in the body for 10 to 20 years. On the other hand tertiary syphilis usually occurs one to ten (1-10) years after the onset of initial infection, though in some cases it may take up to 50 years [9, 10].

1.1.3.1. Congenital Syphilis

In contrast to most other congenitally acquired STIs, syphilis is transmitted *in utero* by transplacental passage of the *Treponema*. The risk of transmission is related to the maternal *Treponema* load; hence early maternal infection carries a much greater risk of transmission than late infection. Indeed, almost all babies born to mothers with untreated primary or secondary infection will be infected, but the likelihood of transmission and the severity of infection will fall with subsequent pregnancies as the mother's *Treponema* load will fall with time. Syphilis in pregnancy has a number of potential outcomes: spontaneous abortion, which may occur during the second and early third trimester; early congenital syphilis, which is a syndrome similar to adult secondary disease and occurs during the first 2 years of life; late congenital syphilis, a syndrome which usually presents around puberty and is similar to late adult disease; and latent congenital syphilis, in which neither signs nor symptoms are present and the diagnosis is made on incidental positive syphilis serology. Even if congenital syphilis passed from mother to child through the placenta during fetal development or birth, it can be prevented by early detection of maternal infection and treatment at least 30 days before delivery [11].

1.2. Statement of the problem

Sexually transmitted infections (STIs) are major public health problem throughout the world, especially in developing countries. They are highly prevalent among pregnant women in Africa and cause significant maternal and prenatal morbidity. In addition they have been associated with a number of adverse pregnancy outcomes including abortion, stillbirth, preterm delivery, low birth weight & congenital infections [12].

Therefore; diagnosing and treating of these devastating etiologic agents at an early stage may result in preventing the spread of such infections not only for pregnant women but also for their new born infants. On the other hand pregnant women are relatively considered as unselected population, for whom prevalence data may be extended to the general sexually active heterosexual population [13].

I. Human immunodeficiency virus (HIV)

Globally, 35.3 million people were living with HIV in all adult aged 15-49. Out of this globally estimated infection of HIV, there were around 1.6 million [1.5 million–1.9 million] deaths related to the acquired immune deficiency syndrome (AIDS) and 2.3 million new infections (6,300 new HIV infections per day). Women represent about half (52%) of all people living with HIV worldwide and the infection is leading cause of death among women of reproductive age HIV prevalence among the adult population in the Sub-Saharan Africa and Caribbean sub region was estimated to be 4.7% and 1% respectively, while the global adult prevalence was estimated at 0.8% [14, 15].

According to HIV/AIDS Estimates and Projections in Ethiopia, the total people who are living with HIV in 2012 were 759,268 with the overall prevalence of 1.3% in the adult general population (men and women) where as the adult male prevalence were 0.9% as well as women were 1.8% [16].

On the other hand the latest ANC sentinel surveillance data shows that HIV prevalence varies widely between urban and rural settings. This is confirmed by Demographic and Health Surveys where; urban adult HIV prevalence was 4.2% while rural adult HIV prevalence was 0.6%. ANC

results also document with a wide variations among urban and rural settings not only in different parts of the country but also among different administrative regions. According to the Ethiopian Demographic and Health Surveys, HIV prevalence ranges from 0.9% in SNNP and 1.0% in Oromia region to 5.2% in Addis Ababa and 6.5% in Gambella region [17].

Even if the introduction of highly active antiretroviral therapy (HAART) to pregnant women increases life expectancy as well as reduces the rate of mother-child-transmission of HIV, its efficacy is compromised when the patients are co-infected with other viruses such as hepatitis B virus (HBV) and/or with *Treponema pallidum* the etiologic agent of syphilis. HIV and these other pathogens share major routes of transmission especially the heterosexual route and mother-to child transmission [18].

Mother-to-child transmission of HIV can take place in utero, during labour, at delivery and postnatal through breastfeeding. The risk of transmitting from mother to child depends on maternal viral load (plasma HIV ribonucleic acid (RNA) level), mode of delivery, duration of rupture of membranes, cervico-vaginal viral load, low CD4 cell count, maternal symptomatic HIV disease/AIDS, co-infections and viral subtype as well host genetic factors [19].

II. Hepatitis B virus (HBV)

It is estimated that, worldwide more than 2 billion people have been infected by HBV and 350 million people have develop chronic infections [20]. In addition; out of the chronic carriers of HBV, 50 million people are estimated to reside in sub-Saharan Africa [21]. The virus is highly contagious and relatively easy to transmit from infected to no-infected individuals by blood-to blood contact, during birth, unprotected sex and by sharing needles and has a relatively higher prevalence in the tropics. It is 50 - 100 times more infectious than HIV and 10 times more infectious than hepatitis C virus (HCV) with many carriers not realizing that they are infected with the virus and thus referred to as a "silent killer"[22].

Mother to child transmission (MTCT) of HBV has great importance for two main reasons. On the one hand, it is a numerically important mode of transmission; and on the other hand, the earlier an individual is infected the more likely to cause chronic infection [23]. In highly

endemic areas, up to 75% of chronic carriers acquire HBV by vertical transmission [24]. Infections of infants have a chance of 90% developing chronic infection. In contrast, if an individual is an adult at the time of infection, there have been 6% to 10% chances of developing chronic infection which have much lower probability [25].

III. Syphilis

Syphilis is still serious public health issue in the modern world both in developed and developing country. Approximately 12 million new cases of syphilis detected each year and more than 2 million occur in pregnant women [14, 18]. It transmits by sexual contact, blood transfusion and by vertical transmission. The risk of contracting syphilis through a sexual contact with a person that has primary or secondary syphilis is 30–50% [1, 26].

Worldwide, a million pregnancies are adversely affected each year by syphilis because of maternal infection. About 270 000 babies are born with congenital syphilis, 460 000 pregnancies end with abortion or perinatal death and 270 000 babies are born prematurely or with low birth weight. Furtherlly, vertical transmission can occur at any time during pregnancy and at any stage of syphilis but it is more common in primary (50%) and secondary syphilis (50%), compared with early latent (40%), late latent (10%) and tertiary syphilis (10%). 70-100% of infants born to untreated infected mothers are infected and the risk of transmission is correlated with the extent of spirochete presence in the circulation [11, 27].

IV. Co-infection

A. HIV/HBV Co-infection

Co-infection with HBV and HIV is becoming common and a growing public health concern because both viruses share similar transmission routes. In HIV infected individuals, HBV infection prevalence is approximately ten times higher than in the general population. Individuals with HIV who contract acute hepatitis B are more likely to develop chronic hepatitis B than individuals who contract acute hepatitis B without HIV [4].

B. HIV/SYPHILIS Co-infection

Syphilis and HIV co-infection is now increasingly common all over the world. One of the major concerns regarding the coexistence of HIV and syphilis is that syphilis, as other genital ulcer diseases, might facilitate HIV acquisition and transmission due to interfering with the natural mucosal and epithelial barriers and by causing local inflammation. Furthermore, syphilis has been found to decrease (at least transiently) CD4 T-cell counts and increase plasma viral load in patients chronically infected with HIV where both of which have been linked to increase in HIV transmission [10].

In general; HIV, HBV and Syphilis infections has been causing a serious pregnancy related problems with high morbidity and mortality effect. As a result; Understanding the burden of these infections in pregnant women is an integral part to develop strategy for prevention and to emphasis on providing integrated service. Therefore; this study was providing a data on the current burden of these infections. On the other hand integrated service for diagnosis of such infections particularly HBV infection isn't well established in all health service of the City Administration. Thereby providing integrated services to pregnant women is a crucial issue in the current health system and in achievement of Millennium Development Goals.

1.3. Significance of the study

HIV, HBV and Syphilis infections are the major causes for the development of different complications during pregnancy worldwide. Especially HIV infection in humans is one of the most destructive pandemics in recorded history. The impact of HIV and AIDS, on society in Africa is immense, placing great strain on resources and undermining already fragile economies. On the other hand; though the mechanism isn't well known infection with syphilis and HBV has been also shown to increase the risk of HIV acquisition by proliferating CD4 cells which enhances HIV replication. Moreover, as the body's immunity is lowered, STI infections are enhanced and severity increased. In addition utero maternal-infantile transmission is an important and common transmission route of these common STIs (HIV, HBV & syphilis) that result in major disease in infants and congenital defects; thus, Understanding the proportion of new infections attributable to these STIs and the role they play in fanning the HIV-1 pandemic were provide insight into current epidemic and to develop strategies aimed at interrupting the transition that would largely reduce the number of new infections and would mitigate suffering imposed by the disease on the individuals, families and the society. The result of this study may be used by programme managers, health planners or policy makers and other concerned bodies to initiate the relevant intervention measures and screening packages in pregnant women.

Generally, this study used to:

- ✓ Find out the current burden of HIV, HBV and syphilis in pregnant women
- ✓ Identify the possible risk factors for acquisition of these infections in pregnant women
- ✓ Identify a gap that help to design a strategy for prevention of HIV, HBV & Syphilis infection transmission in pregnant women
- ✓ Draw the attention of stake holders to focus on such life threatening but preventable infections.
- ✓ It may also serve as a base line data for the coming researchers who are interested in related topics.

2. Literature Review

Seroprevalence of HIV, HBV and syphilis infections is well recognized worldwide but has been reported to be more common in developing countries especially in Africa and Asia. Perhaps it's wide spread all over the world; different epidemiological studies suggest that the prevalence of such common sexually transmitted infections has been shown to vary from one country to other among different groups. In addition to this the focus has now shifted to the management of concurrent illnesses such as chronic HBV infections and syphilis, which have the potential to increase long-term morbidity and mortality [13].

In a cross-sectional study conducted in Brazil in 2001 by Angelica E *etal,* to determine the seroprevalence of HIV, HBV and Syphilis on 1608 pregnant Women at their first Visit to Public Antenatal clinics showed with an overall prevalence of HIV infection of 0.8%, hepatitis B virus carriers 1.1% and syphilis 3%. In this study the potential risk factors were also assessed where condoms never use was 52.8% and those that have blood transfusion history were 1.5% [28].

Other study done on 200 pregnant women whose age range from 14 to 29 years in Rio de Janeiro, Brazil in 2004 to determine the prevalence of HIV infection and other STIs, who seek HIV testing and investigate risk factors for these infection showed that the prevalence of HIV was 8% and syphilis 6.5% and indicates the rate of infection was higher in young women [29].

In Spain, a study conducted on 2,929 pregnant women showed that antibodies against *T. pallidum* were not detected in any case, where as HBsAg was found in 11 patients (0.4%) in which six of them (54.5%) were not aware of their condition. In addition to this an anti-HIVantibodies were detected in two intravenous drug abusers who were aware of their condition [30].

In India a study conducted byJindal Neerja *et al* in 2012 to determine the prevalence of four major sexually transmitted infections (HIV, HBV, HSV-2 and Syphilis) in 500 asymptomatic pregnant women shows with an overall prevalence of 4.8 % and the highest prevalence was HBV (2.4 %) followed by HSV-2 (2 %) and HIV (0.4 %) but no woman tested positive for syphilis and multiple infections [31].

A similar study done in Andhra Pradesh, India to estimate the prevalence of HIV-1/2, Hepatitis B surface antigen, antibody to *Treponema pallidum* and to emphasize the need of strengthening the existing awareness programmes for HIV, the Prevalence rate for antenatal women showed 11 (7.4%) that were positive for infections of HIV, HBV and syphilis. HIV was positive in 1 (0.7%), HBV 2 (1.3%) and RPR test for syphilis was reactive in 8 (5.4%) [32].

In a cross sectional study conducted in Bangladesh; mainly to document on the prevalence of reproductive tract infections (RTI) and sexually transmitted infections (STI) as well as to identify associated risk factors among pregnant women showed with a prevalence of 2.9% for antibody of *Treponema pallidum* and 7.6% (62/2328) were positive for HBsAg but HIV test were not done in this study. Furthermore; the risk factors identified were a husband not living at home or suspected of being unfaithful [33].

In South Africa Studies suggest that; the national prevalence of syphilis steadily declined from 11.2% in 1997 to 2.8% in 2007 and HIV prevalence increased from 17.0% to 28.0% in pregnant women attending ANC. Similar study done by Thu-Ha D *et al* in 2013 on integration of Preventing Mother-To-Child Transmission of HIV and Syphilis Testing and Treating in 2379 pregnant women seeking Antenatal Care Services shows with a prevalence of HIV 14% and syphilis infection 5% [34].

In a cross-sectional survey conducted among 3,839 women attending ante-natal clinics in Northern Tanzania to assess the trends in HIV & syphilis prevalence and correlates of HIV infection by Kumogola *et al*, 2010, shows with an overall prevalence of 7.6% for HIV and 6.4% for syphilis [35].

Other study conducted on seroprevalence of HBV and HIV infection among 384 rural pregnant women in Cameroon showed that seroprevalence of HBsAg was 5.4% and HIV-1 was 3.5%.In this study RPR reactivity for syphilis was also investigated that shows with an overall prevalence of 15.8% [36]. After four years, in a similar study conducted in Cameroon in 4100 pregnant women the seroprevalence of HIV was 4.2% (95%CI: 3.6%-4.8%) and antibodies to *Treponema*

pallidum was 17.4% (95% CI: 16.3%-18.6%). In this two study conducted in different period (four your of difference) there is an increase in seroprevalence of both HIV and antibodies to *Treponema pallidum* [37].

A study conducted on seroprevalence of hepatitis B surface antigen and human immunodeficiency virus in 1120 pregnant women where their age range from 16-50 years and attending ante-natal clinics in Anambra state, Nigeria in 2004 by Christy Nkiru Ezegbudo showed that 8.6% (96/1120) were seropositive for HIV, 9.3% (104/1120) for HBsAg and 0.7% for both HIV and HBV co-infection [38].

Similar cross-sectional study conducted in Niger Delta, Nigeria by Buseri F *etal*, in 2010 to determine the seroprevalence of serological markers of Human immunodeficiency virus-antibody (HIV-Ab), Hepatitis B surface antigen(HBsAg) and antibodies to *T. pallidum* in a total of 1,000 pregnant women aged 15-44 years shows with an overall seroprevalence of HIV, HBsAg and syphilis was found to be 4.1%, 5.3% and 5.0% respectively [13].

In a research conducted in Annaba (Algeria) by Aidaoui M *et al,* 2008, to assess the prevalence and risk factor of infection for HIV among pregnant women shows with an overall prevalence of 5.3/1000 (CI 95%; 3.12-8.37) of HIV. In this study a total of 3044 pregnant women were included and the biomarkers of hepatitis B virus infection and syphilis were also assessed to determine the possible mode of contamination, by blood or sexual transmission. As a result the prevalence of HBsAg were 24.7/1000 (CI 95%; 19.6-30.7) and syphilis prevalence was 2.6/1000 (CI 95%; 1.2-5) [39].

In Ethiopia, a cross-sectional study conducted to determine the seroprevalence of syphilis and HIV-1 among pregnant women at the University of Gonder Teaching Hospital shows with syphilis prevalence of 1% and antibodies against HIV-1 were 9.6%. In this study only one subject (2.2%) was found to be positive for both HIV-1 and syphilis [40].

Similar study done on assessing the magnitude of Seroprevalence of multiple sexually transmitted infections among 480 pregnant women attending in Gonder Health Center, shows with a seroprevalence of 11.9%, 7.3%, 1.3% and 2.3% for HIV, HBV (HBsAg), HCV and

syphilis respectively. In this study higher seroprevalence of HIV was observed in age groups of 20-29 (13%) and 30-39 (12.1%) years [41].

In a study performed in rural hospital of Southern Ethiopia to determine seroprevalence of Human immunodeficiency virus type 1 (HIV-1), hepatitis B virus (HBV), human T-cell lymphotropic virus type 1 (HTLV-1) and *Treponema pallidum* infections among 165 pregnant women shows with a prevalence of HIV-1 with 1.8% and HBV (HBsAg seropositivity) 6.1% . In this study Co-infection with HIV-1 and HBV was detected in one patient (prevalence: 0.6%). Moreover; no cases of HTLV-1 infection and syphilis were found [42].

In a cross sectional study conducted in Bahir Dar city, Northwest Ethiopia Sero prevalence and risk factors of HBV and HIV infection among pregnant women were carried out. As a result the study sought a prevalence of HIV with 6.6% and HBsAg 3.8% as well as co-infection of HIV-HBV was observed in 4(19%) pregnant women. Moreover; in this study identified risk factors that have statistical association with HIV were previous history of piercing with sharp materials, history of abortion and that of with HBV was previous history of blood transfusion, body tattooing, history of surgery and unsafe injection [43].

In Addis Ababa a five year study were conducted to assess the prevalence of HIV and evaluate its trend in pregnant women who are attending antenatal care by Henok Sileshi, showed with decreasing its trend from 11.15% to 5.69% where the prevalence of HIV in each year were 11.15%, 7.88%, 8.66%, 6.70%, and 5.69% in 2006, 2007, 2008, 2009, and 2010 respectively. In this study the highest rates of prevalence were observed in the age categories of 20 to 29 [44].

The prevalence of HBsAg among pregnant women was conducted in different parts of Ethiopia. In a study done by Walle et al, 2008 in DebreTabor Hospital, Northwest Ethiopia, the overall prevalence of HBsAg among pregnant women was 5.3%. In this study, history of using sharp materials, hypodermic needles and tattoo for cosmetics had statistically significant association with HBsAg seropositivity [45]. A similar study conducted in Jimma, Southwest Ethiopia by Awole et al, 2005 showed with a prevalence of 3.7% where it ranges from 1.4% to 6.4%. In this study high positivity rate was among illiterate individuals (61%), low economic income <500

Birr / month (88.9%). In addition among the possible risk factors identified, those pregnant women who have experienced in abortion had higher prevalence of HBsAg (7.3%) [46].

In studies reported in different parts of Ethiopia; the prevalence of syphilis ranging from 1% to 10.9% in a diverse risk groups such as pregnant women, blood donors, street dwellers and elderly people. Moreover; according to the antenatal care (ANC)-based sentinel surveillances, syphilis prevalence increased from 1.8% in the year 2003 to 2.7% in 2005 and then stabilized at 2.3% in 2007 and 2009 [47].

On the other hand; a study conducted in three teaching hospitals of Addis Ababa; to assess the extent of syphilis seropositivity and identifying associated risk factors, 12 (2.9%) were positive for Venereal Disease Research Laboratory (VDRL) [48].

As evidenced above, the prevalence of sexually transmitted infections is high and they are also still the major and serous public health problems all over the world particularly to pregnant women. This problem is more common in developing world where their transmission is high.

3. Objectives

3.1. General Objective

To determine seroprevalence and risk factors of HBV, HIV and syphilis infections among pregnant women attending at Gandhi Memorial Hospital, Addis Ababa, Ethiopia.

3.2. Specific Objectives

- To determine seroprevalence of HIV infection in pregnant women
- To determine seroprevalence of HBV infection in pregnant women
- To determine seroprevalence of syphilis infection in pregnant women
- To identify risk factors for acquiring HIV, HBV and syphilis in pregnant women

4. Methods and Materials

4.1. Study design and study period

- A descriptive-correlational study design was used
- The study was conducted from January – April, 2014.

4.2. Study area

The study was conducted in Gandhi Memorial Hospital, Addis Ababa, Ethiopia, which is referral Hospital for gynecological case and maternity services as well as the hospital serves as a center of excellence for Addis Ababa University for those who are specializing in gynecology and obstetrics. Besides to this it provides other services like ART, VCT, rape clinic and neonatology. Moreover; the Hospital is under Addis Ababa city administration Health bureau and is located in the central part of Addis Ababa, Kirkos sub city in which its average service delivering for about 300 clients per day. The total numbers of staffs in the hospital are 349 (with 209 professional staffs and 140 administrative staffs). Of these, three of them are gynecologists and obstetrician, one pediatrician, three other specialist and medical practitioner, 14 health officer, 21 midwife, 132 nurses, 10 laboratory, 13 pharmacy, 10 anesthetist, one environmental health and one x-ray health professionals.

In addition to participating in quality related issues; the service providing in Gandhi Memorial Hospital laboratory comprises all discipline except culture, molecular techniques and analysis of electrolytes. But Compared to the current technology and as far the laboratory is the only hospital laboratory for gynecological and maternity case in the country; its performance capacity isn't competitive not only in providing integrated service but also in adhering current test methodologies.

4.3. Population

4.3.1. Source population

Pregnant women visiting Gandhi Memorial Hospital during the study period

4.3.2. Study population

Pregnant women referred to the laboratory for investigating hematological tests

4.4. Study variables

4.4.1. Dependent variables

Seroprevalence of HIV, HBV and syphilis among pregnant women

4.4.2. Independent (Explanatory) variables

Socio-demographic variables (Age, Occupation, Level of education, Monthly income, marital status) and other variables (Unsafe injection, habit of multiple sexual partners Ear piercing, tattooing, history of contact with infected person, Blood transfusion ,Tooth extraction, surgery and any gynecological related history).

4.5. Inclusion and exclusion criteria

4.5.1. Inclusion criteria

- All pregnant women refereed to the laboratory for hematological investigations and willing to give informed written consent.

4.5.2. Exclusion criteria

- Who are unable to give consent due to mental problem or coma
- Lipaemic, icteric or hemolized samples were excluded due to their test interference.

4.6. Sample size determination and Sampling

4.6.1. Sample size determination

The sample size is calculated based on single sample size estimation [49] as shown below. The value of p is taken as 50% since there is no data related to the prevalence of these three STIs (HBV, HIV and syphilis) in Ethiopia in pregnant women.

$$n= \frac{\left(z_{\frac{-}{2}}\right)2\ pq}{d2}$$

Where *n* = sample size, *Z* = Z statistic for a level of confidence (95%), *P* = expected prevalence or proportion (P = 0.5), q=1-p=0.5 and *d* = precision or degree of error (d = 0.05) Z = *Z* statistic: For the level of confidence of 95%, which is conventional, *Z* value is 1.96.

$$n= \frac{(1.96)2\ 0.5\times0.5}{(0.05)2} = 384$$

By considering 5% non- response rate the total sample size was 403.

4.6.2. Sampling technique

Facility based consecutive sampling was employed to select study participants who were attending at Gandhi Memorial Hospital.

4.6.3. Sample collection techniques and laboratory diagnosis

I. Data and Sample collection

The clinical examinations were performed by the clinician (gynecologist) and written informed consent was obtained from the participants by the selected nurse during the study period. All study participants have informed concerning the study verbally and a write-up given to each one of them to ensure that they had all the necessary information needed to make an informed choice. This includes a complete description of the aims of the study; infectious agents that were being screened; potential benefit and risks; blood collection procedures and assurance of confidentiality of any information given as well as test results. The pre designed interview questionnaire contains socio-demographic characteristics (like age, marital status, level of education, occupation, income) and history of risk factors (unsafe injection, habit of multiple sexual partners , Ear piercing, tattooing, history of contact with infected person, Blood transfusion ,Tooth extraction, surgery and any gynecological related history). A trained nurse was collecting the data after one day training have provided by the principal investigator.

Moreover; those respondents having agreed to give informed written consent was sent to the laboratory with a requisition form in order to give blood sample for further detection of antibody to HIV, *Treponema palladium* and antigen to HBV. As a result blood samples comprising four milliliters of whole blood were drawn by a sterile needle from a vein in the arm of each participant by the laboratory technologist or technician. The test tube used for blood collection was red top serum separator. The specimens were labelled appropriately using the patient identification number (for future references). Hospitals routine standard operating procedures for sample collection were strictly followed.

II. Work flow chart for the test procedure

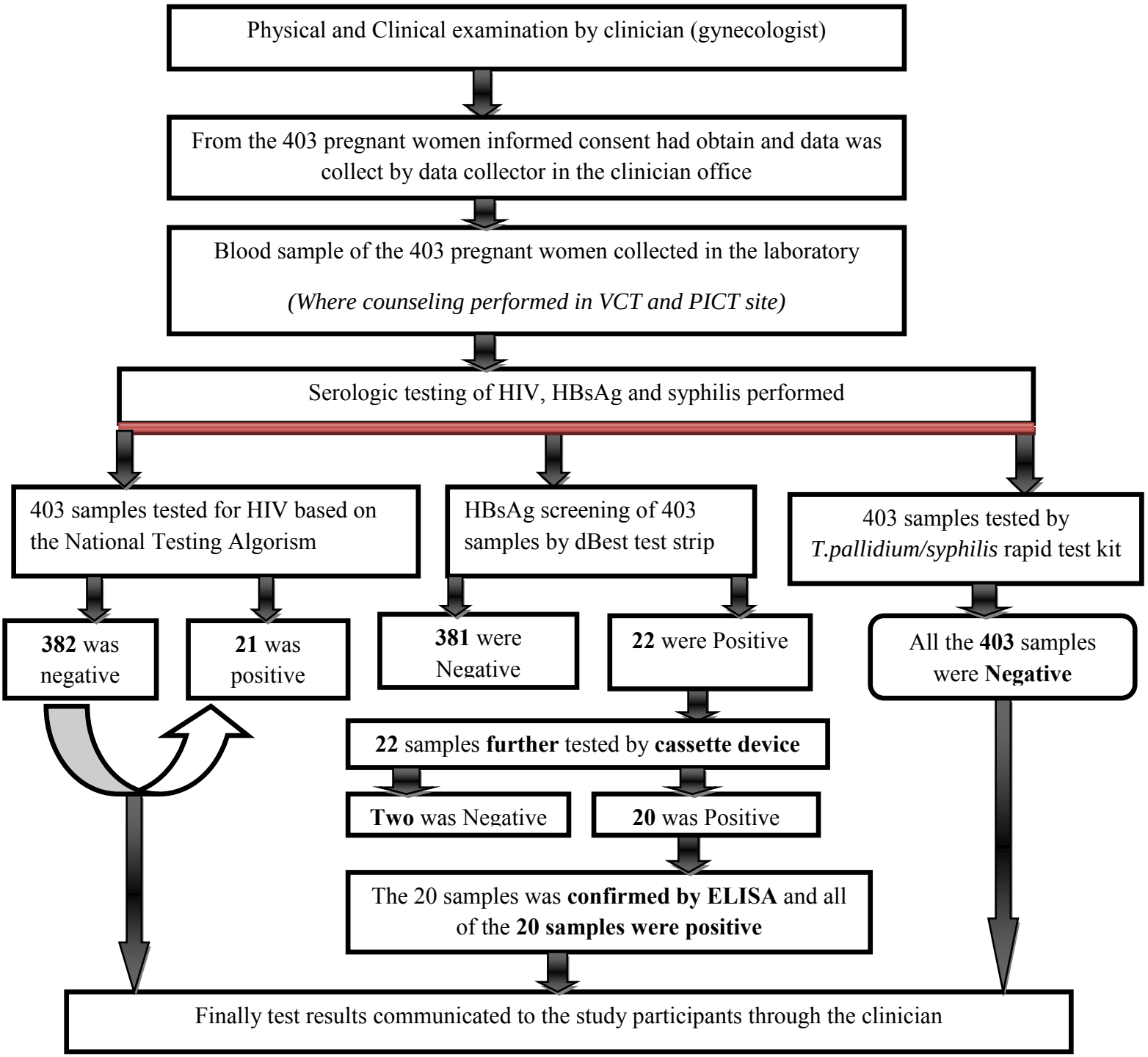

Figure-1: Work flow chart of the overall test procedure

III. Laboratory procedures

1. HIV

Currently the national testing algorism of HIV follows with PICT (provider initiative for counseling and testing) at any corner site and VCT (voluntary counseling and testing). Therefore; assuring the voluntariness of respondents and counseling carried out by the nurse and testing performed in the laboratory. As a result testing follows the national testing algorism of HIV where Sera were first tested for the qualitative detection of HIV-1/2 antibodies using a solid phase colloidal gold immunochromatographic technique (KHB). Nonreactive samples reported as HIV negative where as reactive samples tested using a more specific secondary Chembio HIV1/2 STATPAK test kit. Any discordant results tested using ti-breaker Uni-Gold. Finally the reactive samples reported as HIV-positive and non-reactive results reported as negative. To assure confidentiality, test results have never given for the pregnant women rather for the requesting physicians or nurse.

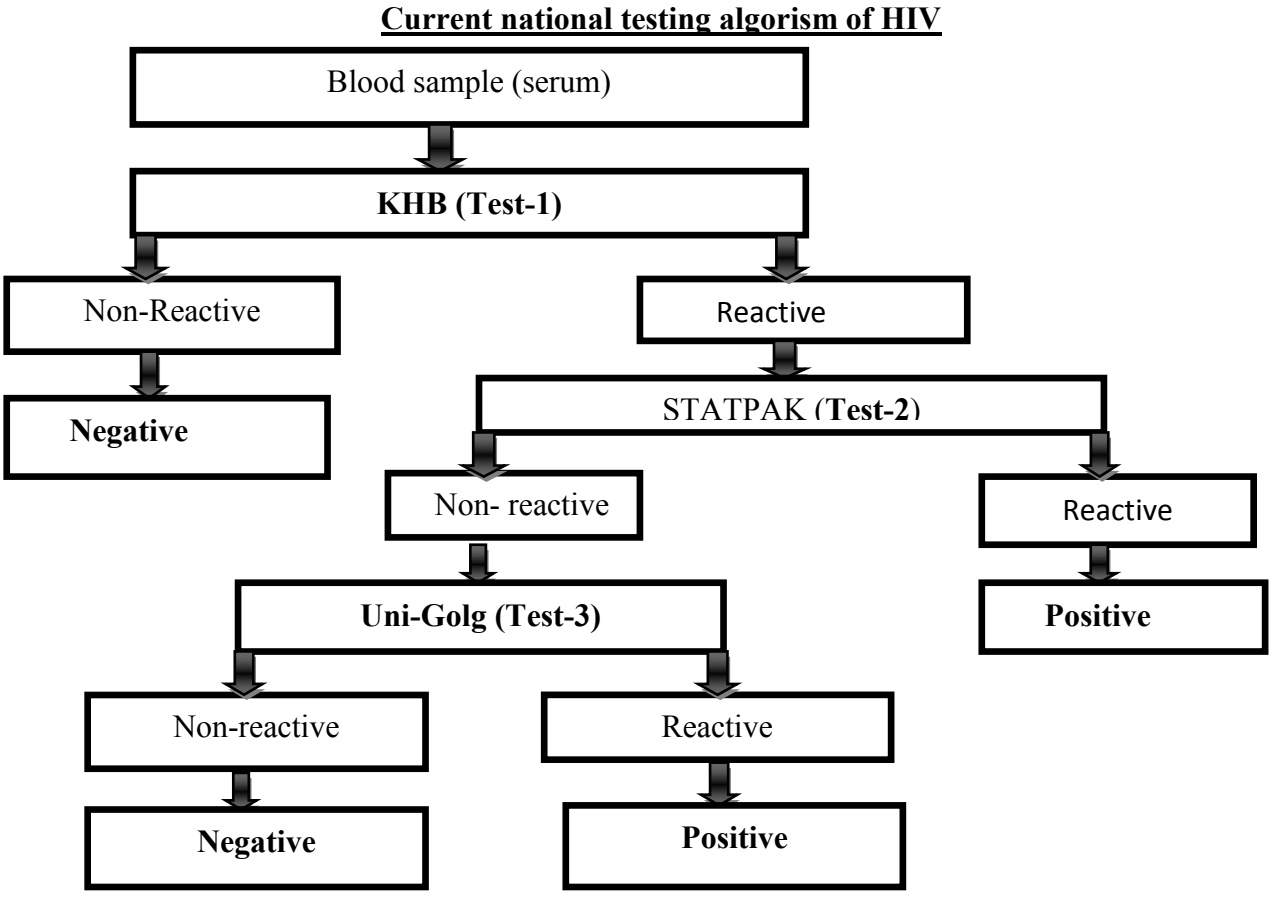

Figure-2: The current national testing algorism of HIV

2. Hepatitis B virus (HBV) testing

Primarily sera of 403 pregnant women screened using dBest one step HBsAg test strip which is highly sensitive (99.8%) but less specific (97.9%) to detect antigen of HBV and from the total 403 screened samples 22 were positive for HBsAg. Further screening of the positive samples was also performed using GENEDIA® HBsAg rapid device (manufactured by GREEN CROSS MEDICAL SCIENCE, KOREA) where its sensitivity and specificity was 99%, 100% respectively. Of the 22 positive samples; two (2) of them were showing discordant results where as the rest 20 were positive for HBsAg screened using the GENEDIA® HBsAg rapid device. The reason why we have carried out using the rapid test kit and device was due to the financial problem we had and to minimize unwanted wastage through systematic economis. Moreover; these testing devices were never miss positive samples for HBsAg because of their high sensitivity rather a fear of false positivity that may appear. Therefore; to minimize this fear of false positivity rate happened by the sensitive tests a final confirmatory tests (ELISA test) used to confirm the positive samples for HBsAg; as a result all the 20 positive samples were also positive for HBsAg by the confirmatory tests.

Test Procedure for both the rapid device and test strip

- Remove test cassettes from a foil pouch and place it on a flat surface
- Label the test device with patient identification number
- Add 100µ1 of sera (precision pipette) to the sample area
- Interpretation of results at the end of 15 minutes.

To ensure assay validity, a procedural control bar is incorporated in the assay device and test strip as a result it seen in the window labeled “control”. Moreover additional positive and negative in house controls incorporated and performed during opening of new batch.

2.1. Confirmation of HBV using DRG ELISA kit for HBsAg

Test procedure

- The solid phase of multi wells was coated with anti- HBsAg antibodies (primary antibody)

- 50 µl of serum and 50 µl of enzyme (horseradish peroxidase, HRP) conjugated antibody (secondary antibody, HRP-conjugated; "enzyme conjugate") were added to the coated wells
- This followed by a 60 minutes incubation period that allowed a complex to form between the primary antibody (anti-HBsAg), the antigen (HBsAg) and the HRP-conjugated antibody
- After that five times washing was performed to remove unbound components
- 50 µl of the substrate was added to the wells and incubated for a period of 30 min in a dark place that resulted in the formation of a colored product (blue color)
- After the addition of stop solution a yellow colored product was formed. The presence of HBsAg was then detected at 450nm in an ELISA reader.

To assure the quality of the procedure three negative and two positive controls were run simultaneously to the test procedures according the manufacturer instruction.

3. Syphilis testing using The Quick Test™ Syphilis Serum/ Plasma/Whole Blood Strip

Though the testing algorism mostly go through non-specific to more specific testes; excluding false positivity rate which could created by the non-specific tests may minimize through adhering tests that have better specificity. Therefore; all the 403 samples analyzed using Syphilis Rapid Test Strip (Quick Test™ Syphilis Serum/ Plasma/Whole Blood Strip) to detect antibodies produced against *Trepollema palladium* in the serum of pregnant women. Interpretation of results performed based on the manufacturer instruction and all quality related issues addressed to validate the performance of the method and storage condition of the test kit simultaneously.

Test Procedure

- Bring all reagents and specimen to room temperature
- Remove the test strip from the foil pouch and place on a clean dry surface
- Apply 60ul of specimen on the sample pad
- Interpret test result at 15 minutes based on the manufacturers instruction

To ensure assay validity, a procedural control bar is incorporated in the test strip as a result it was seen in the window labeled "control".

4.7. Quality Assurance and Quality control

Standard operating procedures (SOPs) were strictly followed and internal quality control materials included from the test kit and performed based on the manufacturers' instruction. The questionnaire preparation was also check by the adviser and pre-tested before the detailed work started. Data collectors trained prior to data collection time. In addition, there were a daily follow up by the principal investigator and supervisors.

4.7.1. Pre-analytical phase

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

4.7.2. Analytical phase

To assure controlled performance of our testing procedure primarily we had find out in house control for both negative and positive control samples in addition to controls provided from the manufacturers. As a result the reagents and the test method have assessed with those known positive and negative control materials in order to evaluate the storage conditions of reagents and performance capability of the method. Moreover; all manufacturer instructions that are necessary have performed and blood samples analyzed basically. Positive samples confirmed by specific test procedures and analyzed separately. In addition; during conformation of HBsAg three negative and two positive controls used simultaneously with the sample. Here, the standard laboratory procedures that address the quality of our methods also strictly followed and the results checked by the supervisors.

4.7.3. Post-analytical phase

The results recorded with the unique patient identification number and errors of data entry have avoided through repeatedly checking and finally test results given to the hospital.

4.8. Statistical analysis & interpretation

The data entered in to EpiInfo 5.3.1soft ware and double checked before analysis and exported to SPSS version 16 for analysis. The descriptive statistics (means, percentages or frequency) have calculated & the bi-variant logistic regression analysis used to see the relation between dependent variable and independent variables. The association has assessed using x^2 –test. Variables that had a significant association have selected for further analysis using multiple logistic regression models with a p-value ≤ 0.05 considered as statistically significant. The strength of the association measured using an odd ratio and interpreted using the 95% confidence interval method. Finally, the results presented on graphs and tables.

4.9. Dissemination of results

The results of the study would be submitted to the Department of Medical Laboratory Sciences**,** School of Allied Health Sciences, Collage of Health Sciences, Addis Ababa University as well as to the study sites. The principal investigator is planning to present the study abstract in local associations (like EMA, EPHA and EMLA) and other international associations for a continuous medical educational events or conferences. The summary of the thesis would be submitted to the international or national peer reviewed journal for publication.

4.10. Ethical clearance

The study approved by "Departmental Research and Ethical Review Committee" of the Department of Medical Laboratory Science**;** after being approved, a written letter obtained from the department before the actual work started. In addition Permission letter was also obtained from Addis Ababa City Administration Health Bureau Ethical Reviewing Committee. Those pregnant women who gave written consent selected and enrolled as a study subjects for testing of HBsAg and syphilis but for HIV after being assured their voluntariness and counseled by the nurses in the VCT and PICT site; testing done according to the current national testing algorism in the laboratory. Pregnant women positive for HIV were post-counseled and referred to Anti-Retroviral treatment (ART) for further management and positive for HBsAg were also managed based on the protocol. Moreover; Confidentiality was the first concern and all information treated as strictly confidential and used for this study purpose only.

5. Results

During the study period a total of 403 pregnant women recruited to submit their blood and all necessary information regarding the risk factors for acquisition of HIV, HBV and syphilis infections.

5.1. Socio-Demographic Data

Table 1:- The socio demographic characteristics of pregnant women attending at GMH, January to April, 2014, Addis Ababa, Ethiopia.

	N	Percentage
1. Marital status		
• Married	348	86.4 %
• Unmarried	34	8.4 %
• Separated	21	5.2 %
2. Educational level		
• No formal education	47	11.7 %
• Primary	115	28.5 %
• Secondary	141	35 %
• Tertiary	100	24.8%
3. Occupational status		
• Self employed	37	9.2 %
• Government employed	69	17.1 %
• Private employed	102	25.3 %
• Not employed	195	48.4 %
4. Average monthly income (Birr)		
• <1000	212	52.6%
• 1001-2000	79	19.6%
• 2001-3000	65	16.1%
• >3000	47	11.7%

Of the 403 responding pregnant women majority of them have married 348 (86.4%) and 21(5.2%) separated (divorced or widowed), moreover; 141(35%) attained secondary education and 47(11.7 %) didn't have formal education. Concerning their occupational status 195(48.4%) weren't employee and 37(9.2%) were self employed individuals. Moreover; majority of their average monthly income of the respondents were <1000 (52.6%) birr.

Regarding to age group distribution of the respondents; majority of the pregnant women were between 20-29 (51.1 %) years of age and followed by 30-39 (43.4%) years of age. Fewer individuals were below 20 years (2%) and above 40 years (3.5%). In addition the mean age of the study subjects were 24.8 (SD±5.99).

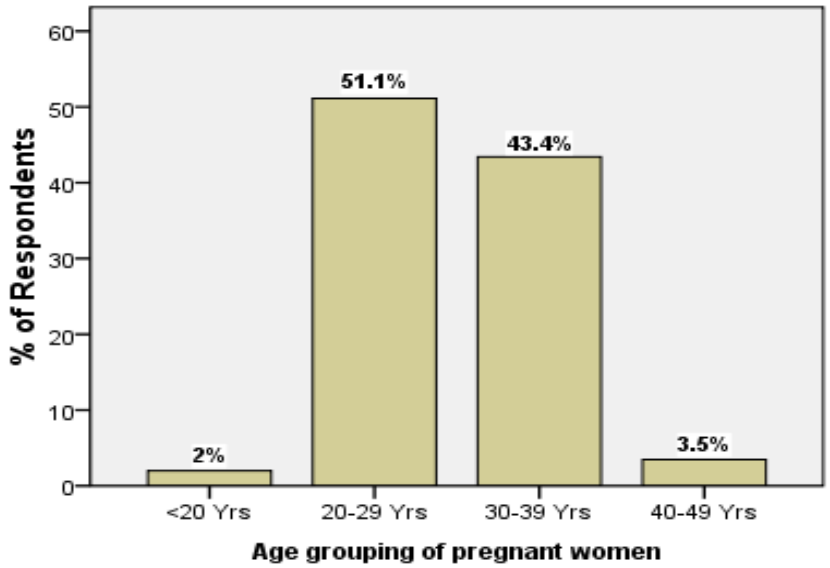

Figure-3: Distribution of the sampled pregnant women in relation to their age group attending at GMH, January to April, 2014, Addis Ababa, Ethiopia.

5.2. Prevalence

The overall prevalence of HIV, HBsAg and syphilis were 5.2% (21/403), 5 %(20/403) and 0% respectively. Of the 5.2% HIV positive respondents 3% (12/403) of them had aware of their previous status whereas; 2.2% (9/403) were newly infected.

5.3. Socio-Demographic Data related to HIV and HBV infection

Table- 2: Association of socio demographic characteristics related to HIV infection in pregnant women attending at GMH, January to April, 2014, Addis Ababa, Ethiopia.

Variables	**HIV infection status**			
	Pos	Neg	p-value	AOR(95%CI)
1. Age in group				
• <20 years	0(0%)	8(100%)	0.533	0.485-0.582
• 20-29 years	13(6.3%)	193(93.7%)		
• 30-39 years	8(4.6%)	164(95.4%)		
• 40-49 years	0(0%)	14(100%)		
2. Marital status				
• Married	17(4.9%)	331(95.1%)	0.673	0.609-0.701
• Unmarried	2(5.9%)	32(94.1%)		
• Separated	2(9.5%)	19(90.5%)		
3. Educational level				
• No formal education	2(4.3%)	45(95.7%)	0.538	0.409-0.587
• Primary	9 (7.8%)	106 (92.2%)		
• Secondary	6(4.3%)	135(95.7%)		
• Tertiary	4(4%)	96(96%)		
4. Occupational status				
• Self employed	2(5.4%)	35(94.6%)	0.945	0.923-0.968
• Government employed	4(5.8%)	65(94.2%)		
• Private employed	4(3.9%)	98(96.1%)		
• Not employed	11(5.6%)	184(94.4%)		
5. Average monthly income (Birr)				
• <1000	17(8.0%)	195(92.0%)	0.045	1.023-1.062
• 1001-2000	3(3.8%)	76(96.2%)		
• 2001-3000	1(1.5%)	64(98.5%)		
• >3000	0(0.0%)	47(100%)		

Among the socio demographic data of pregnant women related to HIV positivity rate; average monthly income level of respondents were statistically associated with positivity of HIV infection ($p<0.05$) where respondents with monthly income level of <1000 birr had higher frequency. Whereas; age group, educational level, marital and occupational status have statistical difference related to HIV infectivity rate.

Table- 3: Association of socio demographic characteristics related to HBV infection in pregnant women attending at GMH, January to April, 2014, Addis Ababa, Ethiopia.

Variables	**HBV infection**			
	Pos	Neg	p-value	AOR(95%CI)
1. Age in group				
• <20 years	1(12.5%)	7(87.5%)	0.583	0.535-0.631
• 20-29 years	11(5.3%)	195(94.7%)		
• 30-39 years	8(4.6%)	167(95.4%)		
• 40-49 years	0(0%)	14(100%)		
2. Marital status				
• Married	18(5.2%)	330(94.8%)	0.806	0.768-0.845
• Unmarried	2(5.9%)	32(94.1%)		
• Separated	0(0.0%)	21(100%)		
3. Educational level				
• No formal education	2(4.3%)	45(95.7%)	0.744	0.702-0.787
• Primary	4(3.5%)	111(96.5)		
• Secondary	7(5%)	134(95%)		
• Tertiary	7(7%)	93(93%)		
4. Occupational status				
• Self employed	2(5.4%)	35(94.6%)	0.342	0.296-0.389
• Government employed	4(5.8%)	65(94.2%)		
• Private employed	8(7.8%)	94(92.2%)		
• Not employed	6(3.1%)	189(96.9%)		

5. Average monthly income (Birr)				
• <1000	6(2.8%)	206(97.2%)	0.000	1.002-1.007
• 1001-2000	7(8.9%)	72(91.1%)		
• 2001-3000	7(10.8%)	58(89.2%)		
• >3000	0(0.0%)	47(100%)		

In respect to the socio demographic data of pregnant women and HBV infection; average monthly income level of respondents were statistically associated with positivity rate of HBV infection ($p<0.05$) where pregnant women with their monthly income level of 2001-3000 and1000-2000 birr were 10.8% and 8.9% respectively. On the other hand; age group, marital status, occupational back ground, level of education had statistical difference with HBV infection ($p>0.05$).

5.4. Risk Factors for acquisition of HIV and HBV

Table- 4: Association of possible risk factors related to HIV infection in pregnant women attending at GMH, January to April, 2014, Addis Ababa, Ethiopia.

Variables		**HIV Infection**			
		Pos N (%)	Neg N (%)	p-value	AOR (95% CI)
1.Multiple sexual practices	Yes	8(10.7)	67(89.3)	0.039	1.154-7.255
	No	13(4)	315(96)		
2.Use of sharp material	Yes	2(28.6)	5(71.4)	0.046	1.45-43.59
	No	19(4.8)	377(95.2)		
3.Ear piercing	Yes	19(5.8)	310(94.2)	0.392	0.103-1.99
	No	2(2.7)	72(97.3)		
4.Tattooing	Yes	4(5.1)	74(94.9)	1.000	0.334-3.124
	No	17(5.2)	308(94.8)		
5.Contact history with infected person	Yes	7(20)	28(80)	0.000	2.36-16.94
	No	14(3.8)	354(96.2)		
6.Blood transfusion	Yes	4(12.9)	27(87.1)	0.068	0.102-1.028
	No	17(4.6)	355(95.4)		
7.Hospital admission	Yes	5(5.4)	88(94.6)	1.000	0.341-2.688
	No	16(5.2)	294(94.8)		
8.Tooth extraction	Yes	10(5.6)	170(94.4)	0.957	0.366-2.126
	No	11(4.9)	212(95.1)		
9.Surgery	Yes	8(8.2)	90(91.8)	0.211	0.201-1.247
	No	13(4.3)	292(95.7)		
10.Catheterization	Yes	3(7.7)	36(92.3)	0.443	0.175-2.222
	No	18(4.9)	346(95.1)		

Among the risk factors for acquisition of HIV; habit of multiple sexual practices, use of sharp material and contact history with infected person have significantly associated with HIV infection ($p<0.05$); particularly contact history with infected person had strongly associated with

HIV infection (where p=0.000 which is much lower than p=0.05).Whereas; Blood transfusion, hospital admission, tooth extraction, surgery, catheterization, ear piercing and tattooing didn't show statistical association (p>0.05) with positivity rate of HIV infection.

Table- 5: Association of risk factors related to HBV infection in pregnant women attending at GMH, January to April, 2014, Addis Ababa, Ethiopia.

Variables		**HBV infection**			
		Pos N (%)	Neg N (%)	p-value	AOR (95 % CI)
1.Multiple sexual practices	Yes	11(14.7)	64(85.3)	0.000	2.426-15.301
	No	9(2.7)	319(97.3)		
2.Use of sharp material	Yes	2(28.6)	5(71.4)	0.062	0.022-0.656
	No	18(4.5)	378(95.5)		
3.Ear piercing	Yes	20(6.1)	309(93.9)	0.033	1.036-1.094
	No	0	74(100)		
4.Tattooing	Yes	4(5.1)	74(94.9)	1.000	0.311-2.95
	No	16(4.9)	309(95.1)		
5.Contact history with infected person	Yes	1(2.9)	34(97.1)	0.713	0.24-14.256
	No	19(5.2)	349(94.8)		
6.Blood transfusion	Yes	8(25.8)	23(74.2)	0.000	3.881-28.05
	No	12(3.2)	360(96.8)		
7.Hospital admission	Yes	4(4.3)	89(95.7)	1.000	0.395-3.715
	No	16(5.2)	294(94.8)		
8.Tooth extraction	Yes	13(7.2)	167(92.8)	1.00	0.162-1.067
	No	7(3.1)	216(96.9)		
9.Surgery	Yes	7(7.1)	91(92.9)	0.382	0.224-1.494
	No	13(4.3)	292(95.7)		
10.Catheterization	Yes	1(2.6)	3897.4)	0.708	0.273-16.072
	No	19(5.2)	245(94.8)		

- Fisher Exact was used for value < 5 in the cells

In this study positivity of HBV infection had significantly associated ($p<0.05$) with habit of multiple sexual practices and Ear piercing. Moreover; history of receiving blood through transfusion from other people was strongly associated with positivity of HBV ($p<0.05$). In contrast; even if the statistical association between HBV infection and tooth extraction wasn't significant; its frequency of positivity is higher in pregnant women that had history of tooth extraction followed to history of ear piercing.

5.5. Health or Gynecological Related

Concerning pregnancy status; of the total 403 responding pregnant women about 36.7% (148) were having first pregnancy, 34.2% (138) second pregnancy, 20.1% (81) third and the rest 8.9% (36) were in a pregnancy status of fourth or above.

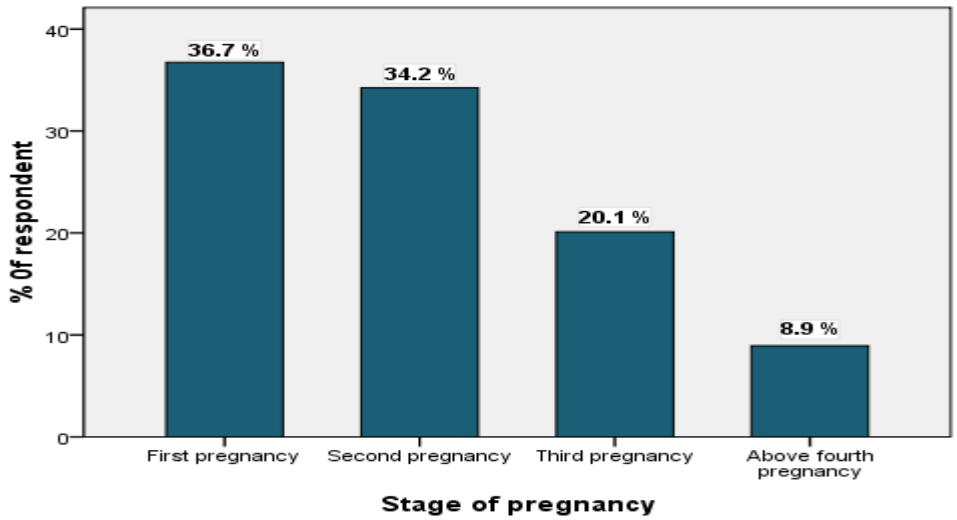

Figure-4:-Frequency distribution of pregnant women Vs their pregnancy status attending at GMH, January to April, 2014, Addis Ababa, Ethiopia.

Regarding to the status of sexual partners of the responding pregnant women 82.9% (334) were free of any related sexual transmitted infections whereas; 13.2 %(53) and 3.7 %(15) had unknown status and having history of exposure to either of these STIs respectively.

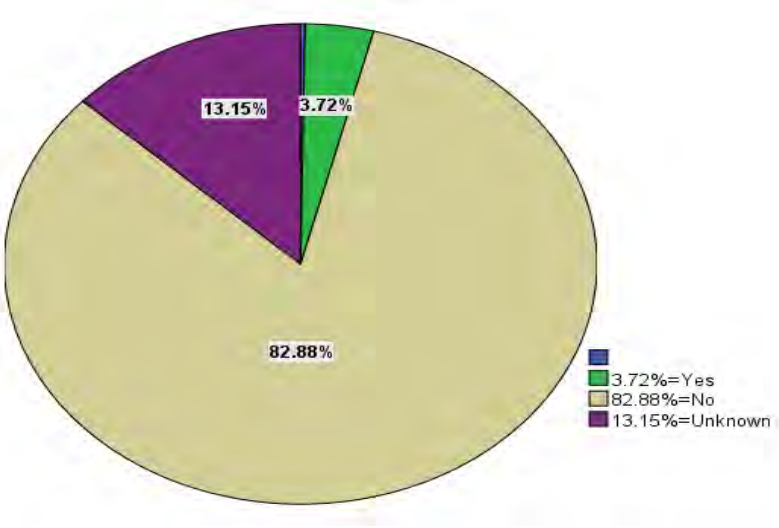

Figure-5:- Status of sexual partners of pregnant women attending at GMH, January to April, 2014, Addis Ababa, Ethiopia.

Table-6:- Association of HIV infection Vs their health related status of pregnant women attending at GMH, January to April, 2014, Addis Ababa, Ethiopia.

Variables	HIV Infection				
		Positive	Negative	p-value	AOR (95 % CI)
1.History of STI	Yes	12(50%)	12(50%)	0.000	14.56-116.08
	No	9(2.4%)	370(97.6%)		
2.Pregnancy related problems	Abortion	5(7%)	66(93%)	0.880	0.373-2.168
	others	5(6.4%)	73(93.6%)		

- **Others** include ectopic pregnancy ,Still birth, Obstructed labour

Here there is also strong statistical association between HIV infection and having exposure of sexually transmitted infections ($p<0.05$). On the other hand infection of HIV shows statistical difference with any of the pregnancy related problems like abortion, ectopic pregnancy ,Still birth and Obstructed labour ($p>0.05$).

Table-7:- Association of HBV infection Vs their health related status of pregnant women attending at GMH, January to April, 2014, Addis Ababa, Ethiopia.

Variables	HBV Infection				
		Positive	Negative	p-value	AOR (95 % CI)
1.History of STI	Yes	5(20.8%)	19(79.2%)	0.001	2.1-19.42
	No	15(4%)	364(96%)		
2.pregnancy related problems	Abortion	13(18.3%)	58(81.7%)	0.011	1.39-12.905
	others	3(5.4%)	53(94.6%)		
4.HbV vaccination status	Yes	0(0%)	4(100%)	1.000	0.929-0.972
	No	20(5%)	379(95%)		

- **Others** include ectopic pregnancy, caesarian section and edema.
- Fisher Exact was used for value < 5 in the cells

Not only pregnant women having history of any related sexually transmitted infections but also history of abortion was strongly associated with positivity of HBsAg ($p<0.05$). On the one side; out of the total respondents only 1% (4) were vaccinated with HBV and the rest 99 %(399) were non-vaccinated.

5.6. CO-INFECTION OF HIV AND HBV

Table-8:- Co-infection of HIV and HBV among pregnant women attending at GMH, January to April, 2014, Addis Ababa, Ethiopia.

	HBsAg Positive	**HBsAg Negative**	**total**
HIV Positive	2 (9.5%)	19 (90.5%)	21(100%)
HIV Negative	18 (4.7%)	364 (95.3%)	382 (100%)

From the sampled pregnant women only 2 (9.5%) study subjects have co-infection of both HIV and HBV.

6. DISCUSSION

This study sought the seroprevalence of HIV, HBV and Syphilis infections and identifying the possible risk factors among pregnant women attending at Gandhi Memorial Hospital from January to April, 2014, Addis Ababa, Ethiopia. Dependinglly; the study demonstrated a prevalence of HIV with 5.2% (3% was previously positive and 2.2% newly HIV infected) and HBsAg was 5% but there weren't any case that are positive for Syphilis.

6.1. HIV INFECTION

In this study, the overall prevalence of HIV among pregnant women attending in Gandhi Memorial Hospital (5.2%) was higher not only compared to the adult (1.3%) but also the women national HIV prevalence (1.8%) [16] .Moreover it shows higher prevalence relative to a study done on similar study subjects of rural hospitals of southern Ethiopia (1.8%) [42]. But it was in line with Addis Ababa HIV prevalence (5.2%) [17].

The variation of this finding compared to the national HIV prevalence may be as result of difference in sample size, study area coverage and study population. Where; the national prevalence done on the total population and covers all over the nation whereas our finding has focused only in one segment of the population and limited to one study area. But compared to the study conducted in rural hospitals of southern Ethiopia the variation may be due to difference in living conditions where living condition of urban people are more crowded than rural setting this facilitates the risk of HIV transmission.

In contrast to this; the finding is lower than previous studies in Kazanchis Health Center on ANC attendants in Addis Ababa, where the overall seroprevalence was 7.68% [44]. Moreover, the current research shows lower prevalence of HIV infection among pregnant women than reports in Gonder Teaching Hospital and Gonder Health Center which was 9.6% and 11.9% respectively [40,41]. The higher prevalence of HIV in studies conducted in Gonder may be due to socio-cultural difference where early marriage and tattooing can be considered as a main contributor for the variation.

Furthermore; lower and higher prevalence rates were also observed compared to similar study populations conducted in different parts of the world. Where; higher prevalence observed in Brazil 8% [29], South Africa 14% [34], Northern Tanzania 7.6% [35] and lower were in Cameroon 4.2% [37], Niger Delta Nigeria 4.1% [13] respectively.

Even though the association of age group of pregnant women and HIV positivity wasn't significant; this study observes higher HIV prevalence rates in the age groups of 20 to 29 followed by 30 to 39 which was 13(6.3%) and 8(4.6%) respectively. This age specific prevalence of HIV among pregnant women is supported by results of a research conducted in Gonder health center in which higher seroprevalence of HIV observed in age groups of 20 to 29(13%) and 30 to 39(12.1%) years [41]. More over in a study conducted in Addis Ababa suggests that HIV prevalence tends to be higher in pregnant women of young adult age groups (20 to 29) years [44]. Similar finding has been also reported in Nigeria in which women aged 20 to 29 years had more than 4 fold increased risk of HIV and prevalence of HIV was 10.9% among women aged 20 to 29 years of age [38].

There are certain reasons why the prevalence rate is high in this age group. The increased risk of HIV among relatively young women has been associated with increased biological vulnerability and relatively prevalent asymptomatic and untreated STIs. In addition, young women tend to have sexual relationships with relatively older men who have been exposed to the risk of HIV for many years [44].

This finding tries to sought statistical association between HIV positivity and average monthly income level ($p<0.05$) of pregnant women where respondents having <1000 birr per month had higher frequency (8.0%). whereas; educational level, occupational and marital status did not show any correlation with HIV seropositivity ($P>0.05$). But frequency of HIV infectivity is higher in having primary education (7.8%) followed by secondary education (4.3%) and those didn't have formal education (4.3%). This suggests that possession of formal education among women is not a main risk factor rather than sexual preference of active young adults as risk factors. Regarding occupational back ground pregnant women employing in governmental office was more infected (5.8%). In relation to marital status, HIV seropositivity was higher among

separated pregnant women (9.5%) in this study. This variation may imply that marital status can be considered as a risk factor for HIV infection.

Among the risk factors identified; habit of multiple sexual practices, participating in different sharp materials, contact history with infected person and having pre-exposure to STIs had significant association with HIV infection. In line to this study; a cross sectional study conducted in Bangladesh; shows that husband not living at home or suspected of being unfaithful were identified as a risk factor [33]. Similarly in a study carried out in Bahir Dar on pregnant women have find out the association of HIV infection with previous history of piercing with sharp materials [43].

6.2. HEPATITIS B VIRUS INFECTION

The results from this study (5%) were in agreement with the investigation done among pregnant women in Cameron 5.4% [36], Nigeria 5% [13] and in Ethiopia at Debre Tabor Hospital 5.3% [45]. Lower and higher prevalence rates were also observed in similar study populations in different parts of the world. Studies with lower prevalence compared to our finding includes; in Brazil with 1.1% [28], Spain 0.4% [30], India 2.4% [31] and in Jimma southwest Ethiopia with a prevalence of 3.7% [46]. On the other hand; higher prevalence was reported among a similar study population in Bangladesh 7.6% [33], Gonder Health Center Ethiopia with a prevalence of 7.3% [41] and rural Hospitals of Southern Ethiopia with a prevalence of 6.1% [42]. The variations observed compared to this study could be due to the methods used, socio-cultural variations, sample size and the study design.

In relation to the seropositivity rate of HBV infection and socio-demographic data; average monthly income level of respondents were statistically associated ($p<0.05$). This was supported by a research conducted in Jimma, Southwest Ethiopia where pregnant women having low economic status were more infected [46]. On the other hand; marital status, educational level and occupational back ground shows statistical difference with positivity of HBV infection. But pregnant women with tertiary (7%) or secondary (5%) level of education were found to possess the highest HBV infection compared to those with no formal education (4.3%). The reason is not

clear but some study suggests that women whose husbands had finished university education were more likely to have HBsAg, while women whose husbands had no formal education were less likely to have HBsAg in their serum [13]. All occupational groups were affected except the public servants and not employed had the highest seroprevalence; indicating a possibility of sharing common routes of infection, for example; sexual contact and clustered living conditions. This study revealed that the highest prevalence for HBsAg was observed in the age group of <20 years and 20-29 years with 12.5% and 5.3%respectively.

Risk factors, including surgery, dental and surgical procedures, tattooing, Catheterization and habit of sharing sharp materials were not associated ($p>0.05$) with HBV infection in this study. But pregnant women that have history of multiple sexual practices, receiving of blood through donation, ear piercing and pre-exposure to STI were significantly associated with positivity of HBV infection. In line to this a study conducted in Brazil [28] and Bahir Dar city, Northwest Ethiopia [43], shows with previous history of blood transfusion have identified as a risk factor. Moreover; this study revealed an association between HBsAg positivity and history of abortion. In line to this in a study conducted in Jimma, Southwest Ethiopia among the possible risk factors identified, pregnant women who have experienced in abortion had higher prevalence of HBsAg (7.3%) [46].

6.3. HBV and HIV Co-infection

Human immunodeficiency virus (HIV) and HBV infections are two major viral infections worldwide, though information on the actual role of HBV in HIV infection is not well established [4]. Co-infection with HBV and HIV is becoming common and a growing public health concern due to their similar transmission routes. In HIV infected individuals, HBV infection prevalence is approximately ten times higher than in the general population. In addition individuals infected with HIV who contract acute hepatitis B are more likely to develop chronic hepatitis B than individuals who contract acute hepatitis B without HIV [4].

In this study only two pregnant women had having both HIV and HBV infection. The 0.5% prevalence of co-infection of HIV/HBV in the sampled pregnant women was in line with a similar study subjects (0.7%) from report conducted on, Nigeria [38]. In addition to this in a study conducted in rural hospital of Southern Ethiopia co-infection of HIV/HBV was observed in only one pregnant woman with a prevalence of 0.6% [42] which is almost similar with our finding. In comparison to these findings in a study carried out in Bahir Dar city, Northwest Ethiopia co-infection of HIV/HBV was higher where four pregnant women were positive for both infections with a prevalence of 19% [43].

Although a number of prevalence studies on HIV/HBV co-infection have been performed within HIV infected study subjects, there is no enough data done in pregnant women in around Addis Ababa. Unfortunately to date, data on co-infection even from Africa is very limited and consequently the scope and impact of the problem as well as priorities for intervention are poorly understood.

6.4. SYPHILIS INFECTION

Syphilis in pregnant women attending in GMH in this study was very low; different studies suggest that syphilis prevalence varies widely depending on the type of population being studied and associated risk factors. But the reason behind why the prevalence was low in this study may be as a result of delivering integrated services for pregnant women through early screening of syphilis and effective treatment of symptomatic individuals.

The finding of syphilis in this study was in line with similar study conducted in India byJindal Neerja *et al* in 2012 [31] and Spain that shows with antibodies against *T. pallidum* were not detected in any case [30]. Furthermore; in a study performed in rural hospital of Southern Ethiopia to determine seroprevalence of HIV-1, HBV, human T-cell lymphotropic virus type 1 (HTLV-1) and *Treponema pallidum* infections also shows with similar findings where no cases of syphilis were found among the pregnant women [42].

However; the finding was much lower compared to studies conducted in Brazil (3%) [28], in Bangladesh (2.9%) [33], in Annaba (Algeria) (0.26%) [39], in Ethiopia at Gonder University Teaching Hospital (1%) [40], Gonder Health center (2.3%) [41], in three teaching hospitals conducted in Addis Ababa (2.9%) [48]. Extremely high prevalence (17.4%) was also reported in Cameroon [37].In addition; due to the absence of case detected for syphilis in this study no risk factors were identified which was statistically associated.

Generally in the sampled pregnant women, there was no sample that tested positive for all the three infections (HIV, Syphilis and HBsAg). Infection of the pregnant women by HIV was not significantly associated to infection by HBV though co-infection was observed in two pregnant women.

7. Limitation of the Study

- Though the seroprevalence for HIV and HBsAg was figurative compared to the other studies; this study was done in one health institution. Whenever the study areas increases the exact figure may realize and representativeness of the data also increased. But due to financial and other related problems we were forced to confine in one study area. However; we still feel that our findings sought the reality.
- Moreover this study includes only those clients who have get access to the hospital during the data collection period. It doesn't include those who didn't have coming to the hospital during the study period that may underestimate findings.

Conclusion

This study showed that HIV infection was similar with Addis Ababa HIV prevalence whereas; HBV infection was intermediate according to WHO classification. Syphilis is non-existing and HBV-HIV co-infection was very low. In relation to the risk factors; history of sex with multiple sexual partners, pre-exposure to STI and low level of monthly income were found to be significantly associated with both HBV and HIV, while each infection were found to have additional different risk factors; these include: receiving of blood through donation, ear piercing and history of abortion for HBV infection and participating in different sharp materials and contact history with infected person for HIV infection alone.

Recommendations

Based on the findings the following recommendations are given:

- Further studies on syphilis that includes different study sites are recommended
- Promote routine Screening of all pregnant women for both HIV and HBV infection during antenatal care
- There should be management protocol for HBV positive pregnant women
- Health education about identified risk factors for HIV-HBV and prevention methods should be given to expectant mothers.

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Annex

1. English version of participant information sheet, consent and questionnaire

1.1.Participant information sheet

Department of Medical Laboratory Sciences, School of Allied Health Sciences, Collage of Health Sciences, Addis Ababa University, Addis Ababa, Ethiopia.

Title: To determine seroprevalence of HBV, HIV and syphilis infections and identifying the possible risk factors among pregnant women attending Gandhi Memorial Hospital, Addis Ababa, Ethiopia.

First of all I would like to say thanks in advance to your cooperation and consent in participating in this study. More over; I were request you politely to read or listen attentively in order to provide relevant information about the study and if there is any confusable information or question which isn't clear regarding the study please don't hesitate to ask freely.

Background information

Sexually transmitted infections (STIs) like HIV, HBV and syphilis are major public health problems worldwide with a highly prevalent in developing countries like Asia and Sub-Saharan Africa. All of these common sexually transmitted infections share similar mode of transmission which is horizontal and perinatal (vertical) transmission. Their complication isn't only restricted to the pregnant women but also to their newborn infants.

Objective: The aim of the study was undertake to determine the sero-prevalence of HBV, HIV and syphilis infections and to identify the possible risk factors for acquisition of the infection in pregnant women attending Gandhi Memorial Hospital, Addis Ababa, Ethiopia.

Benefits for participants

Study participants were not having any financial incentives or other inducements being participated in this study. However, those positives for either of these common STIs (HIV, HBsAg or syphilis) were contact with the physician in the hospital for treatment, immunization and follow up. Furthermore, this study were provide a base line information or data for nationwide to develop health programmes for health policy and pave a strategic means of prevention for such sexually transmitted infections.

Risks and complication

Other than minor bleeding from the site of venipuncture when they give sample, there are no considerable risks to the study subjects being they are participated in the study. Venipuncture is a routine clinical practice for blood sample collection and can stop by pressing cotton on the site of puncture within a short period of time. Additionally the amounts of blood collected were too little which is 5 ml (1 to 2 tea spoon) blood only.

Confidentiality

In order to maintain the confidentiality of participants' information, their name was not given and the samples had coded. Participants were not prohibited to stop or withdraw at any time from the study. Only interested participants can retrieve their own laboratory results using their code number and the information can only be accessed through the physician. The physician was responsible for the interpretation of the results and providing treatment. No personnel information have disclosed to third party or were not appear in any report from this study.

Assurance of Principal Investigator

I put my signature below to confirm you that I take over the responsibility for the scientific ethical and technical conduct of the research project and for provision of progress reports for all stakeholders of the research project.

Kinfe Fissehatsion (PI)

Signature: _____ Date: _____

Note: If you have any questions about this study, you should feel free to ask now or at anytime throughout the study by contacting:

PI Address: Kinfe Fissehatsion: Department of Medical Laboratory Sciences, School of Allied Health Sciences, Collage of Health Sciences, Addis Ababa University, Addis Ababa, Ethiopia.

E-mail: kfissehatsion@gmail.com; Tel.: +251 9 10 21 38 69

1.2.Informed consent

I, (Full Name) ……...……

Here by voluntarily authorize the researcher to interview me and consent to him in order to obtain information that is relevant to his research topic on "seroprevalence of HBV , HIV and syphilis infections and identifying the possible risk factors among pregnant women attending Gandhi Memorial Hospital, Addis Ababa, Ethiopia, 2014". I understand that as a participant, my privacy was maintained and the information obtained in this research was used in a manner that protects with guaranteed confidentiality, respect and personal rights.

I am aware that participating in this study is voluntary and I haven't obligated to answer every question asked for me and that I may withdraw my consent at any time without disadvantage to myself or others. I am informed that information collected in this study was strictly confidential and for this study only.

Signature of Participant …… Date……..

Signature of data collector……. Date……

1.3. Questionnaire in English version

Questionnaire to "determine seroprevalence of HBV , HIV and syphilis infections and identifying the possible risk factors among pregnant women attending Gandhi Memorial Hospital, Addis Ababa, Ethiopia 2014"

- Facility name _____ Year _____
- Participant code _____ Ward_____
- Participants address (Sub city) _____Telephone _____ signature_____
- Data collector name_____date_____signature_____

A. Socio-demographic information (TICK) of Study participants.

1. Age (in years) _____
2. Marital status ;(circle)

a) Married b) Unmarried c) Divorced 4) Widowed

3. Your level of education ;(circle)

(a) Illiterate (b) Primary (c) Secondary (d) Tertiary

4. What is your occupational status? (Circle)

a) Self employed
b) Government employed
c) Private employed
d) Not employed

5. Your monthly income (in Ethiopian birr) _____

B. **Risk factors for Acquiring HIV, HBV and syphilis in the study subjects**

6. Did you involved in any of the following practices? (Circle)

- Unsafe injection (intravenous drugs user) a) NO b) YES
- Multiple sexual partners a) NO b) YES
- Ear piercing a) NO b) YES
- Tattooing a) NO b) YES
- History of contact with infected person a) NO b) YES

7. Did you receive any of the following treatment? **(Circle)**

- Blood transfusion a) NO b) YES
- Hospital admission a) NO b) YES
- Tooth extraction a) NO b) YES
- Surgery a) NO b) YES
- Catheterization a) NO b) YES

C.**Health and pregnant/gynecological history (Circle)**

8. Pregnancy status;
   a) First pregnancy
   b) Second pregnancy
   c) Third pregnancy
   d) Above Four pregnancy

9. Did you have history of sexually transmitted diseases? (Circle)

   a) Yes b)No

10. If **yes** for question number "9" what type of STI?

    a) HIV b) HBV c) syphilis d) others

11. What about your sexual partner? a) Yes b) No c) unknown

12. Have you experienced yourself any pregnant related problems? **(Circle)**

    a) Yes b) No

13. If **yes** for question number "12" what pregnant problems? **(Circle)**

A) Abortion/miscarriage B) Still birth C) Obstructed labour

D) Ectopic pregnancy E) Caesarian section/ Vacuum

F) Death of a baby while in uterus G) Edema H) others (mention) ……

14. If you have experienced with either of the above pregnancy problems, for how many……

15. Vaccination status of Hepatitis B virus (circle)

    a) Yes b) No

16. HIV status a) positive b) negative c) unknown

D. Final Serology result (Laboratory findings) (circle)**;**

1. HIV-1/2
    a) Positive
    b) Negative
2. Hepatitis B Virus (HBsAg)
    a) Positive
    b) Negative
3. Syphilis; Anti-Treponema pallidum/syphilis test
    a) Positive
    b) Negative

Annex -II: Amharic Version of the participant information sheet, Consent and questionnaire

2.1. የተሳታፊወች መረጃ ቅጽ

አዲስ አበባ ዩኒቨርሲቲ ፤የጤና ሳይንስ ኮሌጅ ፤የአላይድ ጤና ሳይንስ ት/ቤት፤የህክምና ላቦራቶሪ ሳይንስ ዲፓርትመንት

አርእስት፡ በአዲስ አባባ ከተማ ፣ የጋንዲ መታሠቢያ ሆስፒታል ፤ የኤች አይቪ ቫይረስ፤ የሔፓታይተስ ቢ ቫይረስና የአባላዘር በሽታ ስርጭት በወላድ እናቶች ብሎም ዋና ዋና ለስረጭቱ ምክንያት የሆኑ መተላለፍያ ዘዴዎቻቸውን ለማወቅ ና ለማጥናት የታሰበ ጥናት፡፡

አጠቃላይ መረጃ

በጥናቱ በመሳተፍዎ ከልብ እያመሰገን ከመወሰንዎ በፊት፡- ይህንን ቅጽ በትክክል አንብበው ተረድተውት ወይም ሲያነቡም ሆነ ሲያደምጡ ግልፅ ያልሆነልዎትን ነገር በሙሉ ነፃነት መጠየቅ ይችላሉ፡፡

መግቢያ

በግብረ ሥጋ ግንኙነት የሚተላለፉ በሽታዎች እንደነ ኤች አይቪ ቫይረስ፤ ሔፓታይተስ ቢ ቫይረስ (የጉበት ቫይረስ) ና የአባላዘር በሽታ በአሁኑ ግዜ በዓለማችን በተለይም እንደነ አፍሪካና ኤስያ የመሳሰሉ ታዳጊ አገሮች ከፍተኛ ጉዳት እያደረሱ ያሉ በሽታዎች ሲሆኑ መተላለፍያ ዘዴዎቻቸውም ከእናት ወደ ልጅ ወይም ልቅ በሆነ ግብረ ሥጋ ግንኙነት ከሰው ወደ ሰው ስርጭታቸው እየጨመረ እንዲሄድ አድርግዋል፡፡እነዚህ በሽታዎች ከከፍተኛ የስርጭት ዓቅማቸው በተጨማሪ በወላድ እናቶች የሚያደርሱቱን ጉዳት መጠኑ ከፍ ያለ ከመሆኑ የተነሳ ከማህተጸን ውጭ እርግዝና፤የጉበት ካንሰር፤ውርጃና የመሳሰሉ ችግሮች የሚያስከትሉ በሽታዎች ናቸው፡፡በመሆኑም የእነዚህን በሽታዎች በእናቶች ላይ ያለውን ስርጭት ማወቅ ታካሚዎችን በአግባቡ ለማከም ና ለመከታተል ብሎም የህገርንም ሆነ የግለሰቦችን ያለአግባብ ወጭ ና የሞት መጠን ለመቀነስ በሚደረገው ርብርብ የበኩሉን ከማበርከት በተጨማሪ በሐገር አቀፍ ደረጃ ወደፊቱ ለሚካሔዱ ጥናቶች መሰረት ለመጣል ወሳኝ የሆነ ሚና አለው፡፡ በዚህም መሰረት ይህን ጥናት በማድረግ ላይ እንገኛለን፡፡

የጥናቱ አላማ

የጥናቱ አላማ በግብረ ሥጋ ግንኙነት የሚተላለፉ በሽታዎች ፤የኤች አይቪ ቫይረስ፤የሔፓታይተስ ቢ ቫይረስና የአባላዘር በሽታ በወላድ እናቶች ያለው ስርጭት ብሎም ዋና ዋና ለስረጭቱ ምክንያት የሆኑ መተላለፍያ ዘዴዎቻቸውን ለማወቅ ና ለማጥናት የታሰበ ነው፡፡

ለጥናቱ ተሳታፊዎች ያለው ልዩ ጥቅም

በጥናቱ ለሚሳተፉ ፍቃደኛ ተሳታፊዎች ምንም አይነት የገንዘብ ክፍያ የለውም:: ነገር ግን የጥናቱ ውጤት ለሆስፒታሉ ስለሚሰጥ በሽታው የተገኘባቸው ተሳታፊዎች ከሚመለከታቸው የጤና ተቋሙ ባለሙያዎች ጋር በመነጋገር አስፈላጊ የሆነ ህክምና፣ ክትባት እና ክትትል ማድረግ ይቻላሉ፡፡

በጥናቱ ተሳታፊዎች ላይ ያለው ጉዳት እና ተዛማጅ ችግር

በዚህ ጥናት ላይ በመሳተፍዎ ሊደርስብዎ የሚችል አንድም ጉዳት አይኖርም፡፡ ለዚህ ጥናት የምንጠቀምበት የደም ናሙና ከክንድዎ የሚወሰድ ሲሆን መጠኑም ከ 5 ሚሊ ሊትር (ከ 1 -2 የሻይ ማንኪያ) ያልበለጠ ነው፡፡ ይህም ከመጠነኛ ስሜት በስተቀር በጤናዎ ላይ ምንም አይነት ጉዳት አያደርስም፡፡

የመረጃ ሚስጥራዊ አጠባበቅ

የሚሰጡት መረጃ በጥናቱ ወቅትም ሆነ ከዛ በኃላ ባሉት ጊዜያት ሙሉ በሙሉ ሚስጥራዊነቱ የሚጠበቅና መረጃውም የሚያዘዉ በስም ሳይሆን በ መለያ ቁጥር ነው፡፡ በጥናቱ ላይ እያሉ በፈለጉት ጊዜ የማቆም ወይም የማቋረጥ መብት አለዎት፡፡ ይህ መረጃ በጥንቃቄ የሚያዝና መረጃውን በፈለጉ ጊዜ ሊያገኙ የሚችሉ ይሆናል፡፡ በመጨረሻም የደም ናሙናው ውጤትም ለ ተቋሙ ተልኮ ተገቢውን ህክምና የሚያደርጉ ና የጥናቱም ውጤት ለሚመለከተው አካል ለጥናቱ አላማ ብቻ የሚገለፅ ይሆናል፡፡

ያስታውሱ: ስለዚህ በጥናት ማንኛውም ጥያቄ ካልዎት በማንኛውም ጊዜ ከዚህ በታች በተጠቀሱት አድራሻዎች መጠየቅ ይችላሉ፡፡

የዋና ተመራማሪው አድራሻ

ከንፈ ፍስሀጽዮን፤ አዲስ አበባ ዩኒቨርሲቲ ፤የጤና ሳይንስ ኮሌጅ ፤የአላይድ ጤና ሳይንስ ት/ቤት፤የህክምና ላቦራቶሪ ሳይንስ ዲፓርትመንት ፣ አዲስ አበባ፣ ኢትዮጵያ

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2.2.የፈቃደኝነት ማረጋገጫ ቅፅ

‘‘በግብረ ሥጋ ግንኙነት የሚተላለፉ በሽታዎች ፤የኤች አይቪ ቫይረስ፤የሔፓታይተስ ቢ ቫይረስ (የጉበት ቫይረስ) ና የአባላዘር በሽታ በወላድ እናቶች ያለው ስርጭት ብሎም ዋና ዋና ለስረጭቱ ምክንያት የሆኑ መተላለፍያ ዘዴዎቻቸውን ማጥናት’’ በሚል ርዕስ ለይ በተመለከተ በሚደረገዉ ጥናት ላይ ለመሳተፍ መሆኑ የጥናቱ ዓላማና ጥቅም ተገልጾልኛል፡፡ በመጠይቁ ላይ የምሰጠዉ የእኔ ሙሉ መረጃም ሆነ የደም ናሙና ውጤቴ በሚስጥር እንደሚያዝ ተነግሮኛል፡፡ በተጨማሪም በጥናቱ ዉስጥ ያለመሳተፍ መብቴ እደሆነና በማንኛዉም ጊዜ ከጥናቱ በራሴ ዉሳኔ መዉጣት እንደምችል፤ በዚህም ምክንያት ምንም አይነት መጉላላት እንደማይደርስብኝ በሚገባ ተረድቻለሁ፡፡

ስለዚህ ሁኔታዉን በሚገባ በማጤን በፈቃደኝነት በምርምሩ ላይ ለመሳተፍ ለተመራማሪዉ ፈቃደኝነቴን ሰጥቻለሁ፡፡ በተጨማሪም የምሰጠዉ የደም ናሙና ለኤች አይቪ ቫይረስ፤ የሔፓታይተስ ቢ ቫይረስ (የጉበት በሽታ) ና የአባላዘር በሽታ ምርመራዎች ብቻ እንደሚዉል ተነግሮኝ ተስማምቻለሁ፡፡ ማንኛዉንም ያልገባኝን ነገር የመጠየቅ ዕድል ተሰጥቶኝ በሚገባ ቋንቋ መልስ አግኝቻለሁ፡፡ በተጨማሪም የሁሉም የላብራቶሪ ምርመራ ዉጤቶች ለተቋሞች እንደሚሰጥና ዉጤቴን ማወቅ ከፈለኩ ማግኘት እንደምችል ተነግሮኛል፡፡

እኔ _____ የተባልኩ ግለሰብ ይህን ሁሉ በማገናዘብ ምርምሩ ላይ ስለኔ መረጃ እና የደም ናሙና ለመስጠት ተስማምቻለሁ፡፡

ፊርማ _____ ቀን _____

መረጃዉን ያስረዳዉ አካል _____ ፊርማ_____

2.3. መጠይቅ:

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

ይሕ መጠየቅ ለተሳታፊዎች (ስለ ወላድ እናቶች) አጠቃላይ መረጃ ና ለበሽታው (የኤች አይቪ ቫይረስ ፤ የሔፓታይተስ ቢ ቫይረስና የአባላዘር በሽታ) ስርጭት መንስኤ የሆኑትን ምክንያቶች ለማወቅ የሚደረግ መጠይቅ ነው፡፡ መጠይቁን የሚያስሞላው በጥናቱ ጊዜ የተመረጠው(ችው) ነርስ ሲሆን መጠይቁ የሚሞላው ጥናቱ በሚከሄድበት ቦታ ነው፡፡ እባክዎን ለጥናቱ መሳካት ያግዘን ዘንድ ጥያቄዎችን በጥንቃቄ እንዲሞሉልን በትህትና እንጠይቃለን፡፡

የተቋሙ ስም---------- ዓ.ም. ---------- የተሳታፊው መለያ ቁጥር---------- ዋርድ----------

አድራሻ (ክፍለ-ከተማ)----------

የዳታ ሰብሳቢው ስም---------- ቀን---------- ፊርማ----------

I. የተሳታፊውን አጠቃላይ መረጃ በተመለከተ የሚሞላ

እባክዎን ትክክለኛውን መልስ ይምረጡ

1. እድሜ --------

2. የጋብቻ ሁኔታ ሀ) ያገባች ለ) ያላገባች ሐ) የተፋታች መ) ባሏ የሞተባት

3. የትምህርት ደረጃ. ሀ) ያልተማረች ለ)1ኛ ደረጃ ሐ) 2ኛ ደረጃ መ) 3ኛ ደረጃ

4. የስራ ሁኔታ ሀ) የመንግስት ስራ ለ) የግል ስራ ሐ) የግል ተቀጣሪ መ) ስራ የሌለው

ሠ) ሌላ ካለ(ይጥቀሱ)----------

5. ወርሀዊ ገቢ (በብር) ----------

II. በኤች አይቪ ቫይረስ ፤ ሔፓታይተስ ቢ ቫይረስና በአባላዘር በሽታ ለመያዝ ዋናና ወሳኝ የሆኑት ምክንያቶች

6. ከሚከተሉት ውስጥ ለየትኞቹ ተጋልጠው ያውቃሉ? (ከአንድ በላይ መልስ መመለስ ይቻላል)

ሀ) ስለታም ነገሮች መጠቀም (መዋስ) ለ) ከአንድ በላይ የትዳር ወይም የፍቅር ጓደኛ ሐ) ጀሮ መበሳት
መ) ንቅሳት (ጥቁራት) ሠ) በበሽታው ከተያዘ ሰው ጋር መነካካት

7. ከሚከተሉት ሕክምናዎች የትኞቹ ተጠቅመው ያውቃሉ? (ከአንድ በላይ መልስ መመለስ ይቻላል)

ሀ) ከሌላ አካል በደም ልገሳ ደም መቀበል ለ) ተኝቶ መታከም ሐ) የጥርስ ሕክምና መ) ቀዶ ጥገና ሕክምና

ሠ) በመሳርያ መሸናት

III.ከጤናና ከእርግዝና (ከማህጸን) የተያያዙ ጥያቄዎች

8.የእርግዝና ሁኔታ

ሀ) የመጀመርያ እርግዝና ለ)ሁለተኛ እርግዝና ሐ)ሦስተኛ እርግዝና መ)ከአራት በላይ እርግዝና

9.በግብረ- ሥጋ ግንኙነት ከሚተላለፉ በሽታዎች ተጋልጠው ያውቃሉ; ሀ) አዎ ለ) አላውቅም

10.በተራ ቁጥር 9 መልስዎ አዎ ከሆነ ለምን ዓይነት የግብረ-ሥጋ ግንኙነት በሽታ ተጋልጠው ያውቃሉ;

ሀ)ለኤች አይቪ ቫይረስ ለ) ለሔፓታይተስ ቢ ቫይረስ (የጉበት ቫይረስ) ሐ) የአባላዘር በሽታ መ) ለሌላ

11.የትዳር አጋርዎስ ለግብረ-ሥጋ ግንኙነት በሽታ ተጋልጠው ያውቃሉ; ሀ) አዎ ለ) አያውቅም

12. ከእርግዝና የተያያዙ ችግሮች አጋጥመዎት ያውቃሉ; ሀ) አዎ ለ) አያውቅም

13.በታራ ቁጥር 12 መልስዎ አዎ ከሆነ ከሚከተሉት የትኞቹ አጋጥመውት ያውቃሉ?

ሀ) ውርጃ ለ) የሞተ ልጅ መውለድ ሐ) Obstructed labour መ) ከማህጸን ውጭ እርግዝና

ሠ) በቀዶ ጥገና መውለድ ሰ) በማህጸን ውስጥ የሞተ ልጅ ረ) እብጠት ሸ) ሌላ ……

14.በተራ ቁጥር 13 ያጋጠመዎት ችግር ካለ ለስነት ግዜ (ቁጥሩን ያስቀምጡ) ----------

15.የክትባት ሁኔታ

- ሔፓታይተስ ቢ ቫይረስ ተከትበው ያውቃሉ? ሀ) አዎ ለ) አላውቅም

16.የኤች አይቪ ቫይረስ ሁኔታ በደም ውስጥ ሀ) አለ ለ)የለም ሐ) አላውቅም

Annex-III: Standard Operating Procedures (SOPs) for HIV, HBV and syphilis

3.1. Sample collection, storage and transportation

1) Label tubes with the client's identification number (Labeling can also be done immediately after the specimen is obtained).

2) Explain the blood drawing procedure to the client and reassure her.

3) Wear the rubber gloves and make the client in a comfortable position.

4) Prepare the Vacutainer tube and needle

5) Tie the tourniquet around the arm of the client just above the bend in the elbow. The tourniquet should be positioned 7.5cm to 10cm above the puncture site.

6) Tell the client to clench her fist

7) Using the tip of the index finger examine the phlebotomy site , feel the vein, and decide exactly where to place the puncture

8) Disinfect the phlebotomy site by swabbing the skin in small outward circles with alcohol swab or cotton wool soaked in isopropyl alcohol. Do not touch the prepared puncture site with your fingers after disinfecting the skin.

9) Insert the needle directly into the vein and withdraw peripheral blood of approximately 4 ml in the EDTA Vacutainer tube of syringe

10) Tell the client to open his/her clenched fist

11) Release the tourniquet

12) Withdraw the needle from the vein and cover the puncture site cotton swab and hold (or have the subject hold) pressure at the puncture site for 3 minutes or until adequate haemostasis is visible.

13) Properly discard the used materials in a safe container and tell the subject to do so if handled the cotton swabs to stop the bleeding.

14) Shipment of the samples from the collection site to the area of analysis should strictly adhered with proper packaging, labeling and maintaining at 2-8 °C by using cold chain.

15) Keep the samples in the refrigerator at 2-8°C or frozen at -20 °C according to the storage time required (i.e. if the processing time is within 48 hours & longer than this time respectively).

3.2. Safety Precautions

Adhering universal persecution for all blood born infections is better (gloves, lab coat, washing hands) when handling infectious materials that are referred in the national health and safety guideline for standard safety procedure.

3.3. Procedures (standard operating procedure) and principles for HIV testing Algorism

A.KHB

Principle

The gold-gp160 conjugate and gold-gp36 conjugate are coated to the conjugate pad in advance. The test line (HIV type I+II antigens) and the control line (monoclonal antibody against gp160) are pre-coated on the surface of NC membrane. When the sample that added to the sample pad migrate through the conjugate pad, it reconstitutes and mixes with colloidal gold-antigen conjugates. The mixture continues to migrate through the NC membrane to the pre-coated antigens or antibody present on the membrane. A purple red test line was visible in the strip if there are enough antibodies to HIV-1/HIV-2 in the sample. If antibodies to HIV-1/ HIV-2 are absent, then no color was appearing in the test line. The control line purple red is used as quality control only and does not affect the result of the test.

Procedure

1) Remove a test cassette from a foil pouch and place it on a flat surface
2) Label the test device with patient name or identification number
3) Use the sample either serum/ whole blood and add 40µl of sample (precision pipette) to the sample area
4) Add one drop (~40µl) of sample diluents to the same area
5) Positive result can be seen within 2-3 minutes but do not interpret the test results after 30 minutes.

Result Interpretation

a) **Positive result (two bands)**
   - A reddish-purple band appears both on control line (C-line) and test line (T-line) of the cassette.

b) **Negative result (one band)**
   - A reddish-purple band appears only at the control line (C-line) of the cassette.

c) **Invalid result**
   - No reddish-purple band appears neither at the control line nor the test line of the cassette
   - If there is no distinct pink/purple line visible in the CONTROL (C) area
   - Any lines that appear outside of the Control (C) Area or Test (T) Area

B.STATPAK

Principle

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, the sample continues to migrate along the membrane and produces a pink/purple line in the CONTROL (C) area containing immunoglobulin G antigens.

Procedure

1) Remove the Chembio HIV 1/2 STATPAK test device from its pouch and place it on a flat surface
2) Label the test device with patient name or identification number
3) Touch the 5 µL sample loop provided to the specimen, allowing the opening of the loop to fill with the liquid
4) Holding the sample loop vertically, touch it to the sample pad in the center of the sample (S) well of the device to dispense ~5 µL of sample (serum or whole blood) onto the sample pad.

5) Add 3 drops (~ 10^5 µL) of buffer slowly, drop wise, into the sample well.
6) Read the Test Result within 20 minutes after the addition of the Running Buffer

Result Interpretation

1. **Positive result (two bands)**

- A reddish-purple band appears both on control line (C-line) and test line (T- line) of the cassette.

2. **Negative result (one band)**

- A reddish-purple band appears only at the control line (C-line) of the cassette.

3. **Invalid result**

- No reddish-purple band appears neither at the control line nor the test line of the cassette
- If there is no distinct pink/purple line visible in the CONTROL (C) area
- Any lines that appear outside of the Control (C) Area or Test (T) Area

C.UNI-GOLD

Principle

During testing two drops of serum, plasma or whole blood is applied to the sample port, followed by two drops of wash buffer and allowed to react. Antibodies of any immunoglobulin class, specific to recombinant HIV-1 or HIV-2 proteins will react with colloidal gold linked antigen. The antibody protein-colloidal gold complex moves chromatographically along the membrane to the test and control region.

Procedure

1) Allow the kit to reach room temperature
2) Lay the devices on a clean flat surface and Label each device with the appropriate client information / ID.
3) Add two drops of sample (approx. 60 ul)
4) Add two drops (approx. 60 ul) of wash reagent to sample port
5) Allow 10 min. from the time of wash reagent addition for the reaction to occur, the result should be read immediately after the end of 10 minutes incubation time but is stable for further 10 minutes
6) Don't read the result after 20 minutes

Result Interpretation

NEGATIVE: A line in the control region only indicates a negative test result.
POSITIVE: A line of any intensity forming in the test region, plus a line in the control region, indicates a positive result.
INCONCLUSIVE: No line appears in the control region. The test should be repeated

3.4. Procedures (standard operating procedure) and principles for HBsAg

3.4.1. Principle dBest one step HBsAg test strip

Serum specimen is added directly to the sample pad. As the test sample flows through the sample pad, the labeled antibody-dye conjugate binds to HBsAg forming an antibody-antigen complex. The pad is in contact with a chromatographic test strip which contains a region of immobilized polyclonal anti-HBsAg antibody in the test line. The antibody-antigen complex moves by capillary action along the strip forming a line of immobilized complex by the zone of antibody in the test line, indicating the presence of HBsAg in the sample (pink line). If no antigen is present, the test line was remaining clear. The appearance of a pink line in the control line shows that the test has been carried out correctly.

3.4.2. Principle of GENEDIA® HBsAg rapid device

An Enzyme immunoassay procedure was carried out for the detection of HBsAg in the samples using the GENEDIA® HBsAg rapid device with a sensitive of 99% and specific of 100%. The test strip is composed of nitrocellulose 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 region. During the addition of 100ul of serum, 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 bind to the antigen forming an antigen-antibody-colloidal gold complex. The complex then migrates 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 the control band region. For a positive result a colored band pink with the complex was form in the test band region on the test membrane but absence of this colored band

in the test region suggests a negative result. To serve as a procedural control, a colored band at control region always appears in the test area.

Storage and stability

The test kit can be stored at Room temperature .The test strip and device is stable through the expiration date printed on the sealed pouch and must remain in the sealed pouch until the expiration date.

Quality Control

To ensure assay validity, a procedural control bar is incorporated in the assay device and is seen in the window labeled "CONTROL"

Procedure

1) Remove a test cassettes from a foil pouch, and place it on a flat surface
2) Label the test device with patient identification number
3) Add 100µ1 of sera (precision pipette) to the sample area
4) Strong positive samples may give results within 2-3 minutes.
5) Result should interpret at the end of 15 minutes.

Result Interpretation

Negative: One pink line appears in control line but no line in the test region

Positive: when there is pink color in the control line and testing region.

Invalid: when there is a total absence of color in both regions, in such case the test should be repeated using a new strip.

3.4.3. CONFIRMATORY ELISA TEST FOR HBsAg

Principles

For detection of HBsAg, the method uses antibody "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) and directed against a different epitope of HBsAg. This conjugate incubated and then 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 a wavelength of 450 nm and it is proportional to the amount of antigen captured in the wells and to the sample.

Procedures

Summary of the assay procedures	Amount to be added or required
Add the sample	50 ul
Add HRP-conjugate	50 ul
Incubate	60 minutes
Wash	5 times
Coloring	50 ul A + 50 ul B
Incubate	15 minutes
Stop the reaction	50 ul stopping solution (sulpheric acid)
Read the absorbance	450nm

3.5. Procedures (standard operating procedure) and principles for syphilis

Test Principle

The Syphilis Rapid Test Strip (Quick Test™ Syphilis Serum/ Plasma/Whole Blood Strip) was used to determine the presence of syphilis antibodies. This is a qualitative membrane strip based immunoassay for the detection of *Treponema pallidum* antibodies (IgG and IgM) in whole blood serum or plasma. In this test procedure, recombinant syphilis antigen was immobilized in the test line region of the strip. After a specimen (serum) was added to the specimen pad; it reacted with syphilis antigen coated particles that had been applied to the specimen pad. The mixture migrated chromatographically along the length of the test strip and interacted with the immobilized syphilis antigen. This double antigen test format can detect both IgG and IgM in the

specimens. For specimens containing antibodies to *Treponema pallidum* a red line appeared in the test line region indicating a positive result; those without *Treponema pallidum* did not have a red line appearing in this region indicating a negative result. To serve as a procedural control, a colored band at control region always appears in the test area.

Procedure

1) Bring all reagents and specimen to room temperature
2) Remove the test strip from the foil pouch and place on a clean dry surface
3) Identify the test strip for each specimen or control
4) Apply 60ul of specimen on the sample pad
5) Interpret test result at 15 minutes
6) Do not interpret test result after 20 minute

Result Interpretation

Negative: One pink line appears in control line but no line in the test region

Positive: when there is pink color in the control line and testing region.

Invalid: when there is a total absence of color in both regions, in such case the test should be repeated using a new strip.

Annex- IV: Declaration

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

Name: Kinfe Fissehatsion Berhe

Signature: _____

Place: Addis Ababa University

Date of submission: May, 26/2014

This thesis has been submitted for examination with my approval as University Advisor and external adviser.

Name: Dr.Ibrahim Ali (PHD)

Signature: _____

Place: Dean of School of Allied Health Sciences, Addis Ababa University

Name: Dr. Ashebir Getachew

Signature: _____

Place: Senior Consultant Obstetrician and Gynecologist in Gandhi Memorial Hospital, Addis Ababa.