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Prevalence of Hepatitis B Virus, Human immune Deficiency Virus and Associated Risk Factors among Individuals with Presumptive Pulmonary Tuberculosis Attending at St. Peter`s Specialized Hospital, Addis Ababa, Ethiopia.

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This is to certify that the thesis prepared by Kahasit G/hiwet, entitled:

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List of Abbreviations

AIDS:	Acquired Immunodeficiency Syndrome
CD 4:	Cluster of differentiation 4
DNA:	Deoxyribonucleic acid
EDHS:	Ethiopian Demographic Health Survey
ELISA:	Enzyme linked immuno sorbent assay
EPHI:	Ethiopia public health institute
FM:	Fluorescent Microscopy
HBV:	Hepatitis B Virus
HIV:	Human immuno deficiency virus
MDR:	Multi drug resistance
MTB:	Mycobacterium Tuberculosis
NTM:	Non tuberculosis mycobacteria
PTB:	Pulmonary Tuberculosis
RIF:	Rifampicin
SOP:	Standard Operating Procedure
SPSH:	St. Peter`s specialized Hospital
TB:	Tuberculosis
UNAIDS:	Joint United Nations Programme on HIV/AIDS
WHO:	World Health Organization
ZN:	Ziehl Neelsen

Abstract

Back ground: Hepatitis B virus (HBV), Human immunodeficiency virus (HIV) & M.Tuberculosis are the causes of widely spread infectious disease around the world, especially in resource limited countries. On the other hand the magnitude of HBV infection among Pulmonary tuberculosis (PTB) suspected individuals was not well addressed.

Objective: To assess the prevalence of HBV, HIV & their associated risk factors and the magnitude of TB among individuals with presumptive pulmonary tuberculosis (PTB) attending at St. Peter`s Specialized hospital, Addis Ababa, Ethiopia.

Methods: A cross sectional study was conducted among 387 individuals with presumptive PTB from October to December 2019. A convenient sampling technique was employed. A standard questionnaire was used to collect socio-demographic data & associated risk factors from the study subjects. Sputum & whole blood samples were collected. Sputum samples were analyzed by Gene Xpert, Florescent Microscopy & Zehl Neelson`s staining technique. HBsAg ELISA test was done by Murex Version 3 ELISA test kit from serum/Plasma samples , HIV testing was performed as per the testing algorithm recommended by national guideline and data was analyzed using SPSS version 23.

Results:- Of 387 study participants 214 (55.3%) males & 173 (44.7%) females were included in this study. Overall 14 (3.6%), 28 (7.2%) & 37 (9.6%) of the TB suspected were positive for HBV, HIV & TB respectively. HBV- HIV co-infection was found to be 1 (0.3%). The TB -HIV co-infection was identified in 6 (1.6%). There was no HBV-HIV-TB triple infection. In the multivariate analysis having partner separated, Alcohol consumption, body piercing & having multiple sexual partner were significantly associated with HBV infection. Having partner separated, widowed, sharing scissors, alcohol consumption & contact with multiple sexual partner also significantly associated with HIV infection.

Conclusion: This study shows that HBV, HIV& TB are still a public health issues which needs awareness and health education on the risky behaviors & transmission of HBV ,HIV &TB among individuals with presumptive TB. Further studies also required.

Key words: HBV, HIV, Presumptive TB, Gene Xpert , ZN, FM

1. Introduction

1.1 Background

Hepatitis B virus (HBV) is an enveloped DNA (Deoxyribo nucleic acid) virus that infects the liver and causes liver disease which is life threatening due to cirrhosis, failure and cancer of the liver. HBV infection can be either acute or chronic and may range from asymptomatic infection or mild disease to severe hepatitis [1,2]. Studies show that HBV is global public health problem with about one third of the world's population infected, amongst which 400 million have a chronic infection and 350 million remaining asymptomatic carriers [3,4].

HBV is transmitted through contact with the blood or other body fluids of an infected person, from infected mother to child & unsafe sex with infected individuals. Since most infected individuals have no symptoms and remain chronic carries of HBV they are not aware as have the infection and this increases transmission of the virus. The development of chronic infection is common when an individual is infected from infected mother or in under 5 years age [2,5].Conditions of sexual partner, surgical procedures, blood transfusion, intravenous drug use, tattooing and body piercing with unsterilized materials, using sharp materials in common and contacts to HBV infected individuals also considered as risk factors in transmission of the virus [6,7].

The human immunodeficiency virus (HIV) is a type of retrovirus, which can infect humans when it comes in contact with tissues that line the vagina, anal area, mouth, eyes, or through a break in the skin & it can also transmitted from mother to a child vertically .HIV is a virus that causes Acquired Immunodeficiency Syndrome (AIDS) if not managed early. HIV infects CD4 lymphocytes & causes immuno suppression due to gradual depletion of these cells. Due to this consequence as the disease progresses the body`s immunity decline and patients disclose to many infections such as TB, HBV and other bacterial and fungal agents [8,9].

Due to sharing similar route of transmission of HBV & HIV, an individual with HIV is more likely to be co-infected with HBV. In co-infection, the presence of one virus impacts the natural history of the other virus. HIV accelerates the natural course of HBV infection and facilitates faster progression of liver disease to cirrhosis and hepatocellular carcinoma. Disease progression

to cirrhosis in HIV positive patients is almost three-times faster as compared to HIV negative patients [10].

Mycobacterium is a rod shaped, obligate aerobic, non-spore forming & acid fast bacilli that causes a contagious bacterial disease called Tuberculosis (TB) . The genus Mycobacterium has two main groups: *M. tuberculosis* complex and environmental Mycobacteria. The *M.tuberculosis* complex comprises the closely related species *M. tuberculosis, M.bovis, M. africanum, M. microti* and *M.canettii*. These species are the causative agents of TB in humans and animals. *M. tuberculosis* is the major cause of human TB globally .*M.tuberculosis* infection occurs through inhaling an aerosol droplet infectious nuclei that is expelled when patient with Pulmonary TB coughs, talks, sneezes, spits and sings [11,12].

Tuberculosis is the most common opportunistic infection in HIV-positive people worldwide, high mortality has been reported among HIV-positive people treated for active TB (HIV/TB patients), particularly in patients with advanced disease. Deaths among HIV/TB patients may result from a number of causes, depending on the degree of immuno suppression and the availability of adequate TB treatment and Combination antiretroviral therapy. Liver failure due to hepatic toxicity of anti-TB and anti-HIV drugs may also play a role in patients with pre-existing liver impairment due to hepatiti s B virus [13].

HBV, HIV& TB are widely spread infectious disease worldwide, especially in resource limited countries. Studies show that chronic liver disease raises a risk of hepatotoxicity during anti-tuberculosis treatment, fourteen fold increase in the risk of anti-TB hepatotoxicity has been also reported in HIV and viral hepatitis co-infected patients .Thus multi infection with HIV, TB & HBV has increased the risk of hepatotoxicity which could be challenged in patient management and resulted in treatment failure, relapse & drug resistance [14-16].

This study was aimed to screening of individuals with presumptive TB for HBV & HIV, since these viruses share similar route of transmission. So, screening individuals with presumptive TB is useful to prevent liver failure due to hepatitis B virus & hepatic toxicity from anti TB & anti HIV drugs which leads to treatment interruption and drug resistance.

1.2 Statement of the Problem

Hepatitis-B viral (HBV), Human immunodeficiency virus (HIV) & Tuberculosis (TB) infections are common public health problem worldwide. Around half of the world population lives in HBV endemic area & around 2 billion individuals infected with HBV globally. Of these, an estimated of 257 million people remain chronically infected and become carriers of the virus in 2015 & each year one million people died of HBV related liver failure , cirrhosis & hepato cellular carcinoma[17].

Likewise HIV & TB are also the leading causes of death globally. According to UNAIDS 2016 global HIV statistics 36.7 million people are living with HIV and one million people are died in ADIS related diseases worldwide at the same time ,there was an estimated 10.4 million people infected with TB of those , People living with HIV accounted for 1.2 million (11%) of all new TB cases and of the 1.7 million died from the disease 0.4 million death was among people with HIV. Over 95% of TB deaths occur in low- and middle-income countries [18-21].

Although the infectious rate a of HBV is multiple of HIV & TB, & the death caused by HBV is a number comparable to HIV & TB but it pays low attention & becomes a silent killer across the globe due to this studies showed that Hepatitis B virus infection is anticipated to be the cause of 30% of cirrhosis and 53% of liver cancer worldwide. Around 15-40% of patients with chronic HBV will develop cirrhosis, end-stage liver failure or hepatocellular carcinoma (HCC) in their lifetime [22,23].

In Africa, around 75 million people are affected by the HBV, this range from about 13.6% of the population in Nigeria to 11% in Senegal and 5.7% in Ethiopia. The prevalence of HBV infection in Africa is on average more than 10% [24,25]. A meta-analysis study conducted in Ethiopia showed that the overall pooled prevalence of hepatitis B virus (HBV) was 7.4%. The pooled prevalence among subgroups showed 5.2% in human immunodeficiency virus (HIV) infected individuals, 8% in community based studies, 8.4% in blood donors, 11% in immigrants and 6.9% in other groups [26].

An estimation of 25.5 million individuals living with HIV are also located in sub Saharan Africa & three in four new infections among the productive age group 15–19 years are in girls. Women within the range of 15–24 years are twice as likely to be living with HIV than men [27,28].

In 2013 there were about 793,700 people living with HIV in Ethiopia & 45,200 ADIS related deaths. According to the 2011 EDHS (Ethiopian demographic health survey), HIV prevalence is 1.9% for women and 1.0% for men with an overall prevalence of 1.5%, as the HIV/AIDS estimates and Projections in Ethiopia, 2011-2016 showed, but, in 2016 it declines to a total of 671,941 of individuals living with HIV, 256,319 male & 415,622 females, with 1.1% over all prevalence, 0.7% in males & 1.4% in females [29-32].

The global prevalence of HBV infection in HIV-infected persons is 7.4% and the chronic HBV infection affects an estimated 5–20% of people living with HIV [3,33].

TB is also the leading cause of death among people with HIV, resulting in 400 000 deaths annually. HIV positive individuals are 20 to 30 times more likely to develop active TB disease than people without HIV. HIV and TB form a fatal combination, each speeding the other's progress. In 2016 about 0.4 million people died of HIV associated TB. About 40% of deaths among HIV positive people were due to TB. In the same year there were an estimated 1.4 million new cases of TB amongst people who were HIV-positive, 74% of who were living in Africa. The presence of HBV among TB patients also increases the risk of hepatotoxicity [21,33,34].

WHO report shows though there has been a major decline on the incidence and TB associated death rates in Ethiopia, there was 164/100,000 population TB incidence rate (TB+HIV) & 24/100,000 TB related mortality rate (TB+HIV) in 2017. However, Ethiopia is among 30 high TB, TB/HIV and DR-TB Burden Countries. TB still predominates in the younger population, 70% of notified cases are within the age group of 15-54 years. People living with HIV are more likely than others to become sick with TB [35].

Another study conducted in west Arsi, Ethiopia also showed that the prevalence of TB-HIV co-infection was found to be 14.97% among which 8.92% hepatitis B virus co-infected [36].

Currently in Ethiopia there is a limited data present on the prevalence of HBV and HIV among individuals diagnosed for TB, but at this time HBV becomes a silent killer because of limited public awareness, the infection could be asymptomatic, due to lack of awareness individuals not diagnosed early, there is also limited access to diagnosis & treatment. So, this study will be help full to have current data on the prevalence of HBV and HIV among TB suspected individuals and to show the magnitude of HBV, HIV, TB & their infections in the study site.

1.3 Significance of the Study

Even though the prevalence of HBV and HIV co-infection is high due to common route of transmission of these viruses , individuals with presumptive TB do not screening for HBV this makes difficult management of patients who have already an infected liver with HBV. So, the study could benefit the patients to screen HBV and HIV prior to receiving anti TB and HIV treatment to reduce drug induced hepatotoxicity and treatment discontinuation. It is also significant for clinicians in drug selection, regular follow up of the patient`s liver function and prolong the use of first line TB drugs for patient management. It is also significant for policy makers to set a policy on screening TB suspected individuals for HBV as they already screening for HIV since these viruses have similar mode of transmission presence of the one can favor risk of getting the other .The study also gave a current data on prevalence of HBV, HIV & TB, HBV-TB, HBV-HIV & TB-HIV co-infection in individuals with Presumptive TB in the study site.

2. Literature Review:

HBV, HIV& TB are widely spread infectious disease worldwide, especially in resource limited countries. Thus multi infection with HIV, TB & HBV has increased the risk of hepatotoxicity which could be challenged in patient management and resulted in treatment failure, relapse & drug resistance [16].

A study of 6200 Iranian prisoners showed that Prevalence of HCV exposure was 9.48% and prevalence of HBV was 2.48% in the general prison population. The most important risk factor for HBV was a history of drug use in lifetime [37].

A study carried out in the Great Tehran Prison showed that among 85 HIV positive patients, five persons (5.9%) had TB. Also, 56 new HIV -infected patients checked for hepatitis B surface antigen and hepatitis C virus antibody. There were three hepatitis B surface antigen (5.4%) and 50 hepatitis C virus antibody (89.3%) results [38]. A similar study in Tehran, Iran , among 593 homeless individuals, The prevalence of HIV, HBV, HCV and latent tuberculosis was 3.4%, 2.6%, 23.3% and 46.7%, respectively. Active pulmonary tuberculosis was found in 7 persons (1.2%). Injection drug use was an independent risk factor for latent HIV, HCV and HBV infections [39].

A hospital based prospective cross-sectional study that carried out on 1215 study participants in Ghatampur, north India showed that Seroprevalence of HIV 1.48% (18/1215) & HBV reactivity was found to be 2.96% (36/1215). 1.5% of the males and 1.1% females were HIV-positive where as 2.7% males and 3.7% females were reactive for HBV [40]. Another cross-sectional study in total 38,247 participants in Brazil estimated HBsAg and anti-HCV prevalence rates were 0.22% and 0.28%, respectively. History of STIs, higher number of partners, inconsistent use of condoms, and luck of awareness of routes of transmission were significantly associated with HBV & HCV infections [41].

Cross-sectional survey in Romania among 17,600 individuals the overall prevalence rate of HBV was 4.4%. The personal history of blood or blood product transfusion, surgical interventions, tattooing, and alcohol consumption were risk factors associated with both anti-HBcAb and HBsAg seropositivity[42].

Another cross-sectional survey in North-Central Nigeria to assess Sexual behaviour and risk factors for HIV infection among 4,302 participants showed that risk factors for HIV infection with significant association were female sex, older age group, residence in an urban area, having multiple sexual partners and being in a polygamous marriage [43]. Another study by Djibo et al.that examined 1,157 randomly selected soldiers from the Republic of Sierra Leone Armed Forces to identify risk factors for HIV and syphilis. The sero prevalence of HIV and syphilis were 3.3% and 7.3% respectively. HIV infection was associated with female gender, unintended sex after alcohol use, condom use at last sex, having multiple sexual partnerships in the same week and HIV testing outside of military facilities [44].

A cross sectional study conducted in adult DM patients in South Africa, among 440 DM patients screened, the active TB prevalence was 3.0% . Of the 13 prevalent TB cases, 53.9% had no TB symptoms, and 61.5% were HIV–1 co-infected [45]. Another study in South Africa among male foreign migrants over all HIV prevalence was 8.7 %. HIV sero-positivity was positively associated with older age ($p = 0.001$), completing high school ($p = 0.025$), not having enough money for food ($p = 0.036$), alcohol use ($p = 0.049$), and engaging in transactional sex ($p = 0.022$) [46].

A cross-sectional survey included 1,149 randomly selected soldiers from thirteen Sudan People's Liberation Army (SPLA) from a total of 1,058 participants included the overall HIV prevalence was 5.0%. High-risk behaviours: multiple or concurrent sexual partners, heavy alcohol use, low condom use were identified among SPLA members [47].

In a study conducted in West Arsi Zone, Ethiopia among 374 TB patients showed that TB-HIV co-infection was found to be 56 (14.97%) among which 5 (8.92%) hepatitis B and 4 (7.14%) were hepatitis C triple infected. The overall TB-HIV-Hepatitis triple infection prevalence was 2.4% (9/374). This study identified being urban dweller, marital status (partners separated), and being illiterate were associated with increased seropositivity of HIV [36]. In another study in Jimma University specialized hospital Blood Bank among 6,063 donors. The prevalence rate of Hepatitis B Virus, Hepatitis C Virus, Human Immunodeficiency Virus and syphilis infection were 2.1%, 0.2%, 2.1% and 0.7%, respectively [48]. In a study that was performed at Goba General Hospital the prevalence of HBsAg & HIV in this study group was 26 (7.4%) & 21 respectively. Risk factors like multiple sexual partners and being positive for HIV infection were

the only significantly associated with Hepatitis B Virus [49]. Another study conducted in three government hospitals, southern Ethiopia, by Amsalu et al.among A total of 152 medical waste handlers (MWH) and 82 non-medical waste handlers (NMWH) to assess the exposure rate to hepatitis B and C viruses, results showed that prevalence of HBsAg, anti-HBc and anti-HCV was 1.3%, 39.4%, and 0.7% in MWH, compared to 2.4%, 17.1%, and 1.2%, respectively, in NMWH [50].

A study carried out on 207 randomly selected diabetic patients in Hawassa Adare Hospital, Southern Ethiopia, the prevalence of pulmonary tuberculosis among diabetics was 5.3% [51].

A study in Deder Hospital, Eastern Ethiopia that conducted among pregnant women who attended antenatal care clinic. The prevalence of HBV infection was 6.9%. Interestingly, the history of abortion, nose piercing, surgical procedure and history of multiple sexual partners were significant predictors of HBV infection [52].

A study conducted at Mekelle hospital, Tigray, Northern Ethiopia on sero-prevalence of HBV and associated risk factors among HIV-positive adult individuals showed that the sero prevalence of HBsAg among HIV positives was 5.9% and this study also associated male sex, multiple sexual partners, surgical history, unsafe injection, and CD4 count <200 cells/µl as risk factors for HBV positivity [7].

Another study conducted at university of Gondar Hospital on 332 health professionals showed that the prevalence of hepatitis B was found to be 4.52% (95%CI:2.4,6.5). Hepatitis B infection was more common among males (P value=0.0299) [53].

A cross-sectional study conducted in government health institutions at Gondar town. To determine the prevalence of hepatitis B and/or C viruses and associated risk factors among 100 medical waste handlers and 100 non-clinical waste handlers, HBV was detected in 6 (6.0%) and 1 (1.0%) and HCV in 1 (1.0%) and 0 (0.0%) of medical waste handlers and non-clinical waste handlers, respectively [54].

A study conducted among 385 pregnant women attending University of Gondar teaching hospital, Northwest Ethiopia, reactive syphilis was noted in 11/385 (2.9%) and seroprevalence of

HIV was 43/385 (11.2%). The prevalence of syphilis and HIV co-infection was 2/385(0.5%) [55].

A cross-sectional study in Northwest Ethiopia, among 462 Visceral Leishmaniasis infected patients. HIV and Visceral Leishmaniasis co-infection was found to be 17.75% [56]. Another study done in four prisons in North Gondar Administrative Zone to estimate the prevalence of smear positive pulmonary tuberculosis on 282 prison inmates suspected of PTB, the overall prevalence of smear positive PTB infection was 5.3 % (15/282), but none of the smear positive TB cases were resistant to rifampicin. The prevalence of HIV infection among TB suspected prisoners and smear positive PTB cases was 6 and 27 %, respectively [57].

A study carried out in adult population in Gojjam zones, northwest Ethiopia. On the detection of Hepatitis B surface antigen (HBsAg) and anti-HCV, Results showed that of a total 481 adults 7.5% of adult population were infected either with HBV, HCV and HIV. The prevalence of HBV was 15 (3.1%) and for HIV was 16 (3.3%). The Seroprevalence of HCV was five (1.0%). HIV-HCV co-infection was found to be two (0.4%) [58]. Another study conducted in Bahir Dar city, Northwest Ethiopia, by zenebe et al. to assess the sero-prevalence & risk factors of HIV and HBV infection among 318 pregnant women. Results showed that Overall, 21/318 (6.6%) and 12 /318 (3.8%) of the pregnant women were positive for HIV and HBsAg respectively. HIV/HBV co-infection rate was 4 (19.0%). Previous history of blood transfusion, body tattooing, history of surgery and unsafe injection were significantly associated with HBV infection. Previous history of piercing with sharp materials and history of abortion were also statistically significant for HIV infection [59].

A study to determined the sero-prevalence of HBV infection and associated factors among health care workers and medical waste handlers in primary hospitals of North-west Ethiopia from a total of 388 study participants included in this study, 268 (69%) were health care workers and 120 (31%) were medical waste handlers. Hepatitis B virus surface antigen (HBsAg) was detected in 2.6% health care workers and 2.5% medical waste handlers and the overall hepatitis B virus infection was 10 (2.6%). History of contact with HBV infected case and history of jaundice were statistically associated factors for HBV infection [60].

A cross sectional study conducted in three referral hospitals and the regional laboratory in Addis Ababa city to studied the prevalence of rifampicin resistance of Mycobacterium tuberculosis (MTB) among presumptive TB patients, Samples were processed by Gene Xpert MTB/ RIF assay. A total of 12,414 presumptive TB patients were included in the study. The overall prevalence of TB was 15.11% (1876/12414) in all age groups. The prevalence of rifampicin resistant TB among new and previously treated was 7.6 and 27.4%, respectively [61].

A study conducted to assess the seroprevalence of HBV and HIV co-infection and associated risk factors among pregnant women in a selected hospital facility around Addis Ababa, Ethiopia, Results showed that the overall prevalence of hepatitis B virus infection was 13 (6 %). History of abortion (p = 0.003), history of surgery (p = 0.0.022), and tattooing (p = 0.033) were significantly associated with HBV infection. A total of 9 (4.2 %) women were found to be HIV seropositive, of whom 2 (22.2 %) were co-infected with HBV [62]. Another study conducted among 252 medical and non-medical waste handlers working in three Government hospitals of Addis Ababa, of the 126 Medical Waste Handlers and 126 Non Medical Waste Handler, HBsAg was detected in 8 (6.3%) and 1 (0.8%), and anti-HBcAg in 60 (47.6%) and 40 (31.7%), respectively[63].

All the above related studies showed that there were strong association of the HBV & HIV viruses due to sharing similar mode of transmission, these viruses also co related with TB due to loss of immunity, so most of these studies suggested that there should be a policy to regular screening & treating of HBV in HIV as well as TB patients & create awareness on the these individuals to reduce transmission. Our study was generated a timely data on the prevalence of HBV, HIV & TB also assessed the co-infections of HBV-TB, HBV-HIV & TB-HIV among TB suspected individuals & suggest policy makers to set policy on regular screening of HBV among TB suspected individuals, to increase HBV diagnosis, reduce liver inffilamation associated to HBV & treatments, also to reduce transmission in the community.

2.1. Conceptual Frame Work

To obtain a better understanding of the context and interactions between multiple proximal and distal risk factors that influence sexual risk behaviors and HIV& hepatitis B infections.

There are numerous adaptations of the health beliefs and behavior change conceptual frameworks; however, the modified socio-ecologic model(MSEM) provides the best framework for this study [64]. The MSEM modifies the social ecological model by modifying the levels of risk individual level risks are necessary for the spread of disease, the modified social-ecologic model is a flexible model that allows the examination of individual risk factors within the context of sexual network, community, and the wider public policy environment. This study examined the interplay of individual, behavioral, health facility based & policy that predispose individuals to HBV/HIV infection among individuals with presumptive TB. For this study, risk factors at different levels: Individual level factors included:-age, marital status, Educational, surgery, injecting drug use, history of STIs, body piercing, Tattooing, History of contact with HBV, HIV infected person & Alcohol abuse. At the social and sexual network levels, number of sex partners, partner characteristics. Health facility related: Hospital admission, surgical procedures, surgery, blood transfusion, tooth extraction were examined.

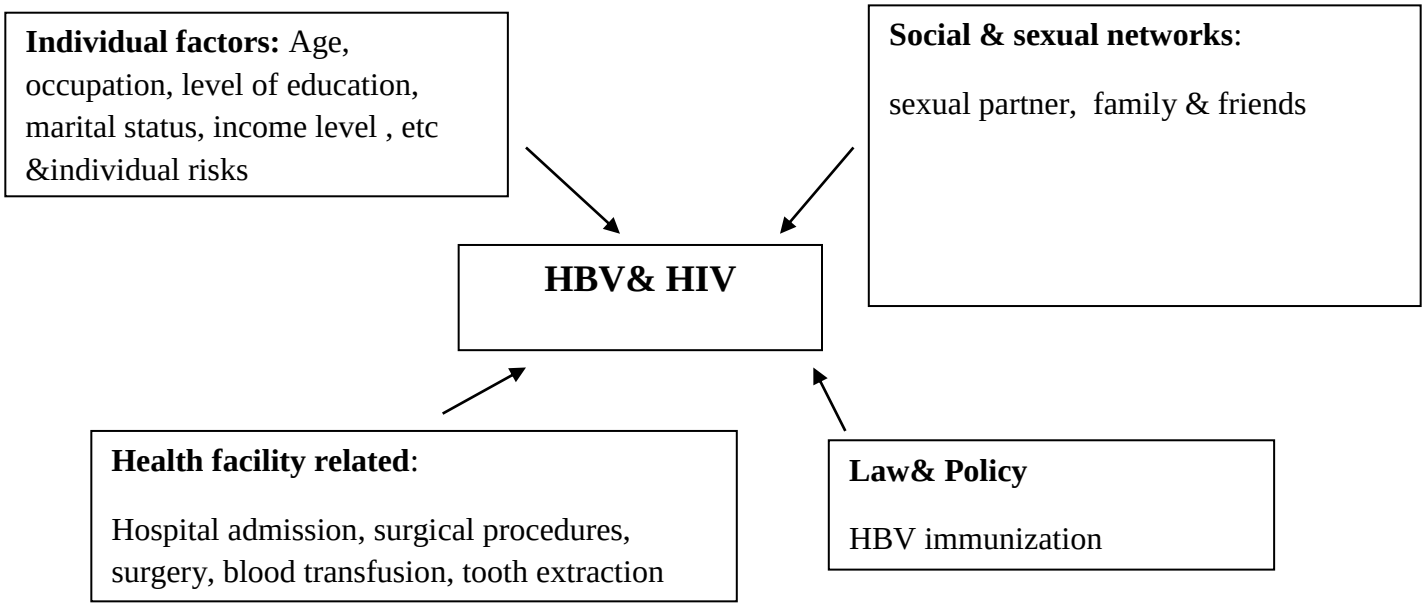


Figure 1. Modified social ecological model for HBV & HIV Exposure to individuals with presumptive TB

3. Objectives

3.1. General Objective

To determine the prevalence of HBV, HIV & their associated risk factors and the magnitude of TB among individuals with presumptive TB attending at St. Peter`s Specialized hospital, Addis Ababa, Ethiopia.

3.2. Specific Objectives

- To determine the magnitude of HBV, HIV and TB among individuals with presumptive TB
- To assess the HBV-HIV, HBV-TB & HIV-TB co-infections among individuals with presumptive TB
- To describe the HBV, HIV & TB triple infections among individuals with presumptive TB
- To assess associated risk factors related to HBV and HIV infections among individuals with presumptive TB

4. Hypothesis

There is no significant difference on the prevalence of HBV & HIV among individuals with Presumptive TB done in other studies.

5. Materials and Methods

5.1. Study Area

The study was conducted in Addis Ababa which is the capital city of Ethiopia. Located in the foothills of the Entoto Mountains and standing 7,726 feet (2,355 meters) above sea level, it is the third highest capital in the world. With a population of 3,627,934, at St. Peter`s Specialized Hospital where found in Gulele sub city, Woreda 1, Addis Ababa, Ethiopia. St. Peter`s Specialized Hospital was established by Emperor Haileslassie on 25 January 1955 E.C. By the time the clinical service on TB has been given. The hospital has formerly been known only by TB & HIV /AIDS but starting from 2004 E. C create clean and comfortable health institution currently named St. Peter`s Specialized Hospital. Now St. Peter`s Specialized Hospital gives more than 25 clinical service & including training center. This Hospital has more than 706 total workers & give services for 80,519 OPD patients, 3,273 ward patients & 7,188 emergency patients in general this hospital serves a total of 90,980 patients including 2,130 patients in the ART service & around 630 patients visit the TB laboratory monthly.

5.2. Study Design and Period

A cross sectional study was conducted from October to December 2019.

5.3. Population

5.3.1. Source Population

The source populations was individuals who have sign and symptoms of TB & visit St. Peter`s Specialized Hospital during the study period.

5.3.2. Study Population

The study population was individuals with presumptive TB & visit the St. Peter`s Specialized Hospital cougher OPD during the study period and fulfilling the inclusion criteria.

5.4. Inclusion and Exclusion Criteria

5.4.1. Inclusion Criteria

- Individuals with presumptive TB previously not on ART & or anti HBV treatment.

5.4.2. Exclusion Criteria

- Individuals with previously confirmed pulmonary tuberculosis cases who are on treatment or relapse.
- Children under Eighteen years old

5.5. Study Variables

5.5.1. Dependent variables

- Serological status for HBV
- Serological status for HIV
- Prevalence of TB
- Magnitude of HBV - TB co-infection
- Magnitude of HBV-HIV Co-infection
- Magnitude of HIV - TB co-infection

5.5.2. Independent variable

Socio-demographic (age, sex, religion, residence, occupation, education and marital status).

Risk factors (Sharing scissor with others, Body piercing, tattooing, unsafe injection, regular sexual partner, multiple sexual partner, history of abortion, history of blood transfusion, had surgery, history of STI, history of chronic infections, contact with infected person, hospital admission, alcohol consumption , Injection drug users).

5.6. Sample Size Calculation and Sampling Method

5.6.1. Sample size

Since there is no study conducted on the prevalence of HBV & HIV among individuals with presumptive TB we use the prevalence of HBV among TB-HIV co-infected 8.92% from a related study conducted in West Arsi zone by Mengesha et al. with 95% confidence interval (CI), the minimum sample size was estimated using single population formula; significance level calculated at 95%CI

Margin of error tolerated is 5 %(0.05)

$$n = \frac{Z^2_{1-\alpha/2} P(1-P)}{d2}$$

Where: n is n minimum required sample

Z is Z-score at 95% CI (1.96)

P is 8.92%, taken from Mengesha, et al. The prevalence of HBV in TB-HIV co-infected done in west Arsi zone, Ethiopia [36].

d is margins of error (0.05)

$$n = \frac{(1.96)^2 (0.0892)(1-0.0892)}{(0.05)^2}$$

$$n = \frac{(3.84)(0.0892)(0.9108)}{0.0025}$$

n =124.8

It is 125 and by considering the non-response rate 10%

N=125 + 10%(125) =138

Though the minimum sample size calculated was 138 to increase the representativeness of the study participants a triple of the minimum sample size was included in this study. i. e. 388 participants were used.

5.6.2. Sampling Method

A convenient sampling technique was employed to draw the study subjects who meet the inclusion criteria until the needed sample size was achieved.

5.7. Measurement and Data Collection

5.7.1. Data Collection Procedure

A standard questionnaire was used to collect information regarding the socio-demographic status and associated risk factors of the study participants. Data collectors (Nurses) were identified, trained and informed to collect the data as per the pre-structured questionnaire. A written informed consent was obtained from participants using Nurses during the study period. The purpose of the study as well as any related harm and benefit was explained to the study participants accordingly. HIV test was done in the cougher OPD. Participants who gave their

consent to participate in this study were sent to the cougher OPD sample collection site to give blood for HBV antigen test and then sent to the TB laboratory to give sputum samples for TB diagnosis.

5.7.2. Laboratory Analysis

5.7.2.1. Sample Collection and Processing

5.7.2.1.1. Whole Blood Sample Collection and Processing

Five ml of whole blood sample was collected from each participants by vein puncture using sterile syringes with needles or a vacutainer system after disinfection the collection site with 70% alcohol & placed in to a sterile serum separator tube (SST)/EDTA tube, after the blood was allowed to clot & centrifuged for 10-15 minutes at 3000 rpm then serum/Plasma sample was separated to another tube & stored at -20°C until being tested for ELISA HBsAg test.

5.7.2.1.2. Sputum Sample Collection and Processing

Sputum samples for Gene Xpert, acid fast bacilli (AFB) microscopy & florescent microscopy was collected as per sputum collection SOP of St. Peter's Specialized hospital TB laboratory in a clean, un cracked, wide mouthed, transparent with a tight-fitted lid sputum container. Gene Xpert, AFB & florescent microscopy was done in St. Peter's specialized hospital TB laboratory.

5.7.2.2. HBsAg ELISA test principle

The sample is incubated in microwell strips pre-coated with monoclonal antibodies specific to HBsAg. A secondary antibody conjugated with horseradish peroxidase (HRP) is then added to the sample in the well. During the two incubation steps any HBsAg present in the sample is bound to the well in an antibody-antigen-antibody-enzyme complex. In the absence of HBsAg no conjugate will be bound. After washing to remove sample and unbound Conjugate, a solution containing tetramethylbenzidine (TMB) and hydrogen peroxide is added to the wells.

Wells which contains HBsAg and hence bound Conjugate will develop a purple colour which is converted to orange when the enzyme reaction is terminated with sulphuric acid .Within 15 minutes the absorbance of each well was read at 450 nm using 620 to 690 nm as the reference wave length [65].

5.7.2.4. HIV test

HIV testing was performed following the current national testing algorithm.

5.7.2.4.1. HIV 1/2 STAT-PAK™ ASSAY

Principles of the test

The Chembio HIV 1/2 STAT-PAK™ Assay employs a unique combination of a specific antibody binding protein which is conjugated to colloidal gold dye particles and HIV-1/2 antigens which are bound to the solid phase membrane. The venous or capillary whole blood, serum or plasma is applied to the sample (S) well of test device followed by the addition of Running Buffer. The specimen/buffer mixture migrates along the test strip by capillary action, reconstituting the conjugate. If present, the antibodies bind to the colloidal gold conjugated antibody binding protein. In a reactive sample, the dye conjugated-immune complex migrates on the nitrocellulose membrane and is captured by the antigens immobilized in the test (T) area producing a pink/purple line. In the absence of HIV-1 and HIV-2 antibodies, there is no pink/purple line in the test (T) area. The sample continues to migrate along the membrane and produces a pink/purple line in the control (C) area containing immunoglobulin G antigens. This procedural control serves to demonstrate that specimen and reagents have been properly applied and have migrated through the device [66].

5.7.2.4.2. ABON HIV 1/2/O Tri-Line Human Immunodeficiency Virus Rapid Test Device

Principle of the test:

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

2, the specimen will bind to the T2 test line. As liquid continues to migrate down the test strip, the control line will appear. If the control line is present, in addition to either or both test lines, then the test is reactive for HIV1/2 antibodies. If the specimen does not contain HIV-1 or HIV-2 antibodies, no colored lines will appear for either of the test lines region indicating a non-reactive result [67].

5.7.2.4.3. SD BIOLINE HIV-1/2

SD BIOLINE HIV 1/2 kit is a rapid, qualitative test for the detection of antibodies to certain isotypes (IgG, IgM, IgA) specific to HIV-1 including Subtype-O and HIV-2 simultaneously in human serum, plasma or whole blood.

Principle of the test

SD BIOLINE HIV-1/2 contains a membrane strip, which is pre-coated with recombinant HIV-1 capture antigen (gp41, p24) on test line "1" region and with recombinant HIV-2 capture antigen (gp36) on test line "2" region, respectively. The mixture (recombinant HIV1/2 antigen (gp41, p24 and gp36) - colloid gold conjugate and the specimen moves upward on the membrane chromatographically to the test region (T) and form a visible line as the antigen antibody-antigen gold particle complex forms with high degree of sensitivity and specificity. This test device has the letter of 1, 2 and C as Test Line 1 (HIV-1), Test Line 2 (HIV-2) and Control Line on the surface of the device [68].

5.7.2.4. GeneXpert MTB/RIF Assay

GeneXpert testing performed according to the manufacturer's instructions. Sputum is treated with sample reagent (SR) containing NaOH and isopropanol at a ratio of 2:1, manually agitated and kept for 10 minutes at room temperature, then shaken again and kept for 5 minutes; 2 ml of the inactivated material transferred to the test cartridge and inserted into the test platform then after completed the PCR cycles results are reported [69].

5.7.2.5. Ziehl Neelsen staining (ZN staining)

The Ziehl-Neelsen method uses a carbol fuchsin stain, acid alcohol decolorizer, and methylene blue counter stain. Acid-fast organisms stain red, while the background of debris stains blue. The ZN stain confims the acid-fast property of Mycobacteria [70].

2-3 cm size sputum smear was done in a microscopic slide, air dry, fix with cotton misted with Alcohol, after cooling flooded with Carbolfuchsin reagent and flamed underneath of the slide with cotton misted with Alcohol until vapor rises but not boil then kept for 5 minutes, after washed with tap water acetone with alcohol was added for Decolorization and kept for 3 minute, washed and counter stained with 1 % Methylene blue for one minute washed and placed the stained smear on a slide rack & air dried at clean dust free area then examined microscopically & reported as No AFB seen, Scanty,+1, +2, +3 per 100 fields[70].

5.7.2.6. Auramine Staining

The property of acid-fastness of mycobacteria is based on the presence of mycolic acid in their cell wall. With auramine O stain, organisms fluoresce bright yellow, non-specific debris stains pale yellow, and the background is almost black. 2-3 cm size sputum smear was done in a microscopic slide, air dry, fix with cotton misted with Alcohol, Placed the slides on the staining rack over a sink and kept distance between every slide at least 1cm then covered the smears completely with 0.1% auramine solution & leaved for 20 minutes, after washed with tape water, 0.5% acid alcohol was poured over them & allowed to act for 3 minutes, slides were rinsed with tape water, covered with 0.5% KMnO4 (Potassium paramagnet) solution & leaved for 1 minute ,Washed with running water, the slides were tilted to drain excess water & Placed on the slide rack upright to dry in the air out of strong light. Acid-fast bacilli appear bright yellow against the dark background material were examined using High power field (HPF) & reported as No AFB seen, Scanty,+1, +2, +3 per HPF [70].

5.8. Data Quality Assurance

Data quality was ensured through use of standardized data collection materials, pretesting of the questionnaires, proper training was given for data collectors before the start of data collection and intensive supervision during data collection by the principal investigator. Data collection

was conducted after the participant informed the purpose of the study and when gave their consent. Data was collected by trained nurses and Standard operational procedures of the St. Peter`s hospital TB laboratory for sputum & whole blood sample collection was used to ensure the reliability and validity of test result. Questionnaire used to collect demographic data & risk factors which was not be incompletely filled was discarded.

5.8.1. Pre Analytical

Sputum specimens was collected in clean, wide mouth, unbreakable, leak proofed ,preferably sterile containers with a tight-fitted lid or cap by informing the participants to clean their mouth and produce a purulent Sputum sample not saliva ,with a volume of about 3-5 ml each and labeled with participants identification & date of collection. Samples which was not possibly analyzed at the same day was Stored in the 2-8°C overnight and placed in sample racks in the same order as registered in the worksheet and request forms.

For whole blood samples after 5ml of whole blood sample was collected by trained laboratory personal from each participants using sterile syringes with needles or a vacutainer system in to a sterile serum separator tube (SST)/ EDTA tube & labeled with participants identification & date of collection, the blood was allowed to clot & centrifuged then serum sample was separated to another tube & stored at -20°C to be used for ELISA HBsAg test & transported by using ice pack box to EPHI HIV laboratory.

5.8.2 Analytical

All laboratory tests were performed by well-trained laboratory personnel. Standard operational procedures of the laboratory were used to ensure the reliability and validity of test result. All Samples was tested as soon as possible. Refrigerated samples & reagents was bring to room temperature before testing. During testing of samples, preparing new reagents a positive & negative control samples was run as samples at the same time to ensure quality of test results.

5.8.3 Post Analytical

The results were recorded with the unique patient identification number and errors of data entry were avoided through repeated checking & remaining Samples were Stored at -20°C.

5.9. Data Analysis and Interpretation

Data entry and analysis of the pre coded & checked data was done using SPSS (Statistical Package for Social Sciences) statistical software version 23. During the analysis frequencies of the different variables was determined, descriptive statistics was used to compare frequencies & to describe the study participants in relation to relevant variables. Statistical analysis such, as chi-square and logistic regression were calculated at 95% of confidence interval to see the relation between dependent and independent variables. The Odds Ratios (ORs) and their respective 95% CI were calculated. All Factors were entered to logistic regression to control the effect of confounding variables and adjusted odds ratio was calculated. In all cases P-value less than 0.05 was considered as statistically significant. Finally, the results were presented on words, graphs and tables.

5.10. Ethical Considerations

Before starting the research work, ethical clearance was obtained from the Departmental Research and Ethics Review Committee (DRERC) of Addis Ababa University College of Health Sciences, Department of Laboratory Sciences. Then a letter informing to St. Peter's specialized hospital permission was obtained from St. Peter's specialized hospital Research and Ethics Review Committee to access data from study population. All eligible subjects were informed as their participation was voluntary and as the aim of this study was only to collect necessary information which was helpful to assess the prevalence of HBV& HIV among individuals with presumptive TB. All the information obtained from the study subjects was coded to maintain confidentiality & participants with positive test results were linked to their physicians.

5.11. Dissemination of the Result

After the research was conducted, the results of the study were submitted to Addis Ababa University, College of Health Sciences, School of Allied Health Sciences, Department of Laboratory Sciences. In addition, the finding of this study will be given to St. Peter's specialized hospital. The finding of the study will also be presented to the annual conferences of professional societies and manuscript will be submitted to peer reviewed journals for publication.

5.12. Operational Definition

Presumptive TB: an individual who have cough for 3 weeks & above but not bacteriologically confirmed for TB.

HBV infected: Detection of Hepatitis surface Antigen in a blood.

Chronic infections: History of any chronic infection during three years

HIV/HBV Co-infection: An individual with HIV become positive for HBV

HIV/TB Co-infection: An individual with HIV become positive for TB.

Alcohol: Who took 4 beers or related alcohol types more than one per a week.

Blood transfusion: History of any blood transfusion during life time .

Household contact: An individual who have contact with chronically ill persons at home.

Hospital Admission: An individual who had admitted at least ones.

Injection drug users: People, who inject (usually illicit) drugs such as heroin, cocaine, steroids into a vein, muscle, or under their skin.

Multiple Sexual Partners: A person having sexual partner more than one .

Regular sexual partners: A person having only one regular sexual partner.

6. Results

6.1. Socio-demographic Characteristics of Participants

From a total 388 study subjects participated in this study, 387 were included & making an overall response rate 99.7%. One was excluded as a result of incomplete data. Among the participant, male were dominant accounting 214 (55.3%) and female were 173(44.7%) giving male to female ratio of 1.2:1 (Table 1).

The mean age was 44.19 years with a standard deviation of 15.177, majority of them 93 (24.1%) were among the age group of 35-44 years followed by 86 (22.3%) between 45-54 years. Three hundred and ten (80.1%) of the participants were urban residents while the remaining 77 (19.9%) were rural residents. Majority of the study participants 209 (54.0%) were married followed by 77 (19.9%) single and 264 (68.22%) had monthly income of less than 1000 birr (Table 1).

Table 1. Socio-demographic characteristics of study participants among individuals with presumptive pulmonary tuberculosis attending at St. Peter's Specialized Hospital, Addis Ababa, Ethiopia 2019.

Variables		Frequency(n=387)	Percent (%)
Sex	Male	214	55.3
	Female	173	44.7
Age (in years)	15 - 24	38	9.8
	25 - 34	70	18.1
	35 - 44	93	24.1
	45 - 54	86	22.2
	55 - 64	55	14.2
	>64	45	11.6
Residence	Urban	310	80.1
	Rural	77	19.9
Current occupational status-o0n	Driver	12	3.1
	Merchant	23	5.9
	Government Employee	48	12.4
	Jobless	27	7.0
	Student	23	5.9
	Farmer	61	15.8
	Other	193	49.9
Educational status	Can't write and read	67	17.3
	No formal education	65	16.8
	Grade 1-4	41	10.6
	Grade 5-8	81	20.9
	Grade 9-10	57	14.7
	Grade 11-12	38	9.8
	college	20	5.2
	University	18	4.7
Marital status	Single	77	19.9
	Married	209	54.0
	Widowed	59	15.2
	Divorced	38	9.8
	Separated	4	1.0
Monthly Income	<1000 birr	264	68.2
	1001 - 2500 birr	78	20.2
	2501 - 3999 birr	27	7.0
	4000 birr and above	18	4.7

6.2. Prevalence of HBV among the study participants

The overall prevalence rate of HBV positive was 3.6% [95% CI, 1.8 – 5.7] (14/387). The prevalence of HBV was higher in males 12 (5.6%) as compared with females which composed of 2 (1.2%) from the total female participants. The sero positivity of Hepatitis B virus was 4.7% among the age groups of 45-54 years followed by 4.3% among age groups of 25-34 & 35-44

years. The prevalence of HBV was 5.2% & 3.2% among the Rural & Urban residents respectively (Table 2).

6.3. Prevalence of HIV among study participants

The overall prevalence of HIV in this study was 7.2% [95% CI, 4.7 – 9.8] (28/387). The prevalence of HIV among male & female participates was 18 (8.4%) & 10 (5.8%) respectively.

The prevalence of HIV among the age groups 25-34 years was 11.4%, 14.0% between 35-44 years & none of the participants between the age group of 15-24 years were positive for HIV. Urban residences accounted 8.4% HIV prevalence followed by rural residences 2.6%. (Table2).

Table 2: Socio-demographic characteristics of study subjects versus HBV, HIV & HBV-HIV co-infection among individuals with presumptive pulmonary tuberculosis attending at St. Peter's Specialized Hospital, Addis Ababa, Ethiopia, 2019.

Variables		HBV status		HIV status		HBV-HIV co-infection	
		Negative (%)	Positive (%)	Negative(%)	Positive (%)	Negative (%)	Positive (%)
Sex	Male	202(94.4)	12(5.6)	196(91.6)	18(8.4)	213(99.5)	1(0.5)
	Female	171(98.8)	2(1.2)	163(94.2)	10(5.8)	173(100.0)	0(0.0)
Age Group (in year)	15 - 24	38(100.0)	0(0.0)	38(100.0)	0(0.0)	38(100.0)	0(0.0)
	25 - 34	66(94.3)	4(5.7)	62(88.6)	8(11.4)	70(100.0)	0(0.0)
	35 - 44	89(95.7)	4(4.3)	80(86.0)	13(14.0)	92(98.9)	1(1.1)
	45 - 54	82(95.3)	4(4.7)	79(91.9)	7(8.1)	86(100.0)	0(0.0)
	55 - 64	54(98.2)	1(1.8)	55(100.0)	0(0.0)	55(100.0)	0(0.0)
	>64	44(97.8)	1(2.2)	45(100.0)	0(0.0)	45(100.0)	0(0.0)
Residence	Urban	300(96.8)	10(3.2)	284(91.6)	26(8.4)	309(99.7)	1(0.3)
	Rural	73(94.8)	4(5.2)	75(97.4)	2(2.6)	77(100.0)	0(0.0)
Current occupational status	Driver	11(91.7)	1(8.3)	9(75.0)	3(25.0)	12(100.0)	0(0.0)
	Merchant	22(95.7)	1(4.3)	21(91.3)	2(8.7)	23(100.0)	0(0.0)
	Gov.Employee	45(93.8)	3(6.2)	44(91.7)	4(8.3)	48(100.0)	0(0.0)
	Jobless	26(96.3)	1(3.7)	24(88.9)	3(11.1)	27(100.0)	0(0.0)
	Student	23(100.0)	0(0.0)	23(100.0)	0(0.0)	23(100.0)	0(0.0)
	Farmer	57(93.4)	4(6.6)	59(96.7)	2(3.3)	61(100.0)	0(0.0)
	Other	189(97.9)	4(2.1)	179(92.7)	14(7.3)	192(99.5)	1(0.5)
Educational status	Can't write & read	64(95.5)	3(4.5)	66(98.5)	1(1.5)	67(100.0)	0(0.0)
	No formal education	63(96.9)	2(3.1)	63(96.9)	2(3.1)	65(100.0)	0(0.0)
	Grade 1-4	40(97.6)	1(2.4)	38(92.7)	3(7.3)	41(100.0)	0(0.0)
	Grade 5-8	79(97.5)	2(2.5)	72(88.9)	9(11.1)	80(98.8)	1(1.2)
	Grade 9-10	56(98.2)	1(1.8)	52(91.2)	5(8.8)	57(100.0)	0(0.0)
	Grade 11-12	38(100.0)	0(0.0)	33(86.8)	5(13.2)	38(100.0)	0(0.0)
	collage	17(85.0)	3(15.0)	17(85.0)	3(15.0)	20(100.0)	0(0.0)
	University	16(88.9)	2(11.1)	18(100.0)	0(0.0)	18(100.0)	0(0.0)
Marital status	Single	76(98.7)	1(1.3)	73(94.80)	4(5.2)	77(100.0)	0(0.0)
	Married	198(94.7)	11(5.3)	196(93.8)	13(6.2)	208(99.5)	1(0.5)
	Widowed	59(100.0)	0(0.0)	55(93.2)	4(6.8)	591(0.4)	0(0.0)
	Divorced	37(97.4)	1(2.6)	34(89.5)	4(10.5)	38(100.0)	0(0.0)
	Separated	3(75.0)	1(25.0)	1(25.0)	3(75.0)	4(100.0)	0(0.0)
Monthly Income in birr	<1000 birr	258(97.7)	6(2.3)	249(94.3)	15(5.7)	263(99.6)	1(0.4))
	1001 - 2500	73(93.6)	5(6.4)	70(89.7)	8(10.3)	78(100.0)	0(0.0)
	2501 - 3999	25(92.6)	2(7.4)	22(81.5)	5(18.5)	27(100.0)	0(0.0)
	4000 and above	17(94.4)	1(5.6)	18(100.0)	0(0.0)	18(100.0)	0(0.0)

6.4. Magnitude of HBV -HIV co infection

HBV & HIV co-infection was 0.3% [95% CI, 0.0 – 0.8] (1/387) (Figure 2).

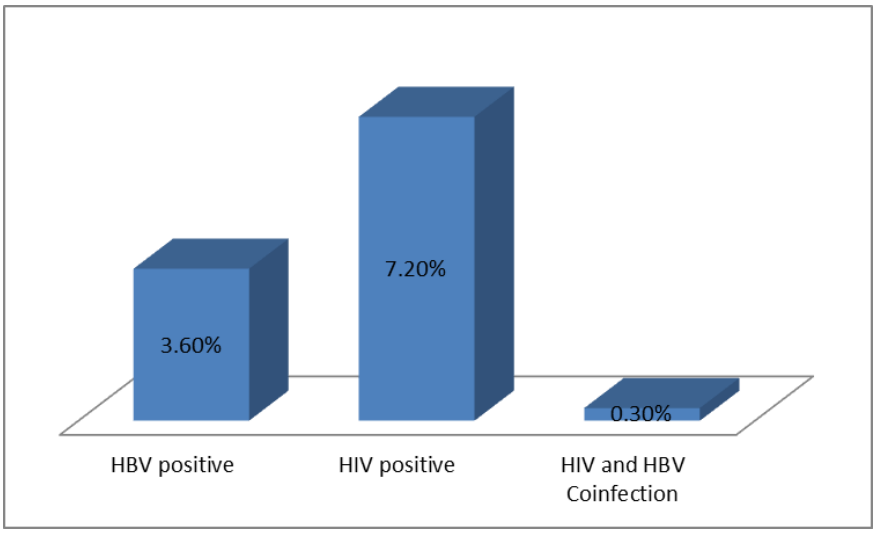
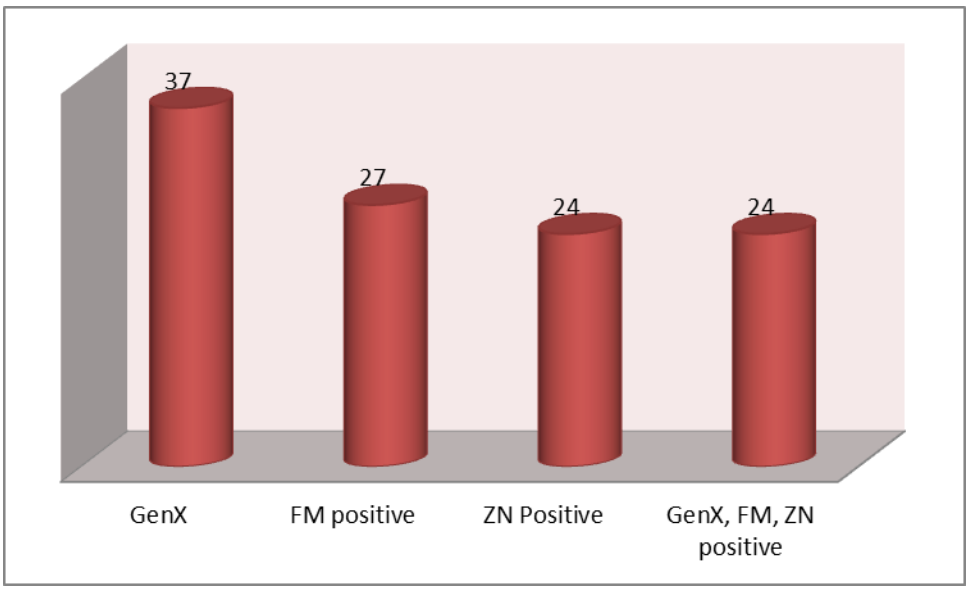


Figure 2**. Prevalence of HBV, HIV & HBV/HIV co-infection among individuals with presumptive pulmonary tuberculosis attending at St. Peter's Specialized Hospital, Addis Ababa, Ethiopia 2019.**

6.5. Prevalence of TB among Study Participants

The overall prevalence of TB was 9.6% [95% CI, 6.7 – 12.4] (37/387). The prevalence of TB among male & female participants was 11.7% & 6.9 % respectively. Rural residents accounted 11.7% TB prevalence while urban residents accounted 9.0% (Table 3).

In this study the presence of TB was diagnosed by using GeneXpert, Fluorescent microscopy & Ziehl Neelson methods. Among 387 sputum samples analyzed with Gene Xpert 350 (90.4%) was MTB not detected, 34 cases (8.8%) as MTB detected and among these Rifampicine Resistance (RR) not detected, MTB & Rifampicine Resistance(RR) detected was identified in 3 cases (0.8%). With auramine stainig (Fluorescent microscopy) method 360 (93%) was negative & detected 27 (7%) cases as TB positive & in Ziehl Neelson staining method 363 (93.8%) was negative & detected 24 (6.2%) cases as TB positive. The following figure also shows that the prevalence of TB based on diagnosis methods (Figure 3**).**



GenX=GeneXpert, FM= Fluorescent microscopy, ZN=Zeheel Neelson

Figure 3. **Tuberculosis positivity by Gene Xpert, FM & ZN methods among individuals with presumptive pulmonary tuberculosis attending at St. Peter's Specialized Hospital, Addis Ababa, Ethiopia 2019.**

6.6. Magnitude of TB-HIV co-infection among Study Participants

The TB -HIV co-infection was 1.6% [95% CI, 0.5 -2.8] (6/387). The TB-HIV co-infection was 1.9% in males & 1.2 % in females. All of the infected individuals were urban dwellers (1.9%). The TB-HIV co-infection was relatively high among the age groups 35-44 & 45-54 years, 3.2% & 2.3% respectively. None of the co-infected individuals was resistance to Rifampcilin (Figure 4 & Table 3).

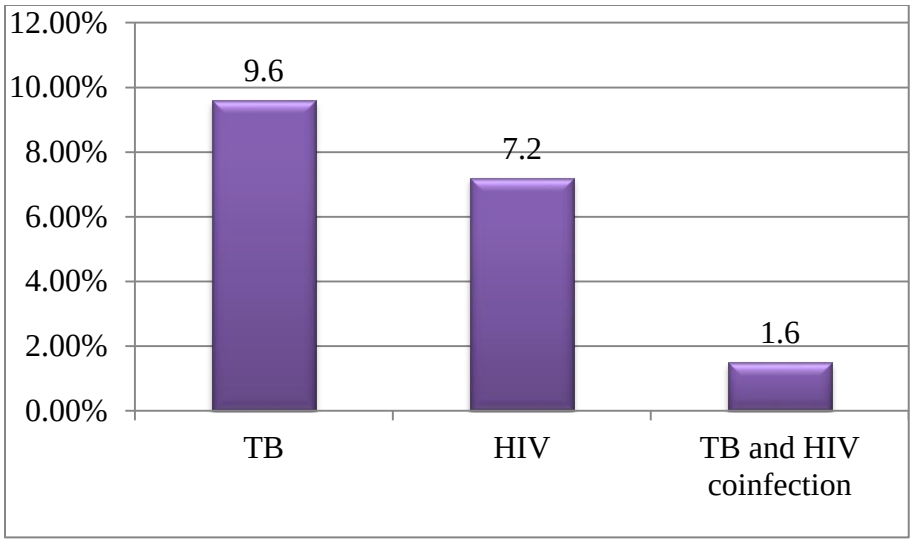


Figure 4. **Prevalence of TB, HIV & TB-HIV co-infection among individuals with presumptive pulmonary tuberculosis attending at St. Peter's Specialized Hospital, Addis Ababa, Ethiopia 2019.**

6.7. TB-HBV co-infection among Study Participants

The TB -HBV co-infection was 0.3% [95% CI,0.0-0.8] 1(1/387). The TB-HBV co-infection was shown in male (0.5%), among the age groups 35-44 years (1.1%), whose occupation was driver (8.3%), completed up to collage & with income level 1001-2500 birr. This co-infected individual was also resistant to Refampicin (Figure 5 & Table 3).

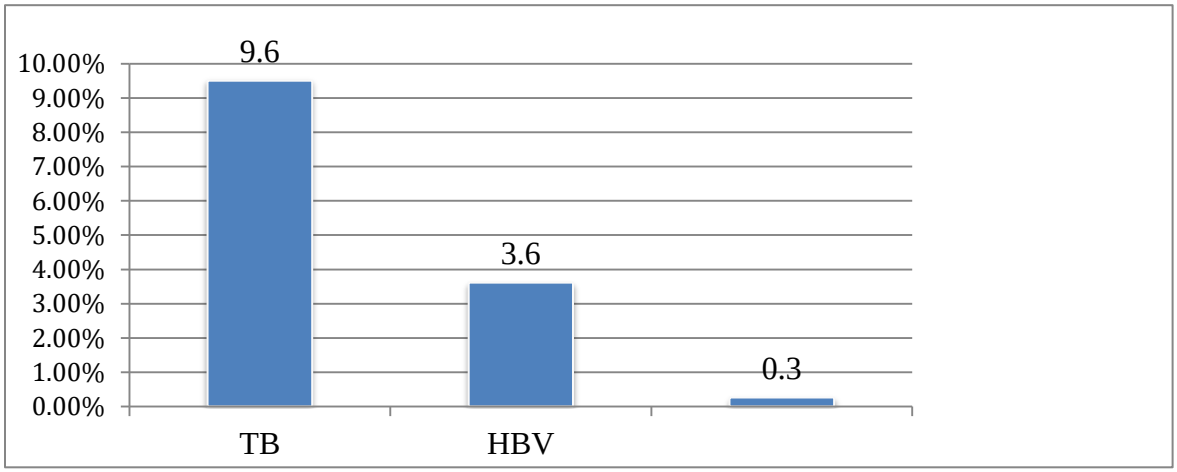


Figure 5. **Prevalence of TB, HBV & TB-HBV co-infection among individuals with presumptive pulmonary tuberculosis attending at St. Peter's Specialized Hospital, Addis Ababa, Ethiopia 2019.**

The table below shows TB, TB- HIV & TB- HBV co-infections among study participants. TB-HIV co infection (1.6%) was relatively high but the TB-HBV co infection (0.3%) was low & none of the participants had TB-HBV-HIV triple infection.

Table 3: Socio-demographic characteristics of study subjects versus TB, TB- HIV & TB-HBV co-infections among individuals with presumptive pulmonary tuberculosis attending at St. Peter's Specialized Hospital, Addis Ababa, Ethiopia ,2019.

Variables		TB status		TB-HIV Co infection		TB-HBV Co infection	
		Negative (%)	Positive (%)	Negative (%)	Positive (%)	Negative (%)	Positive (%)
Sex	Male	189(88.3)	25(11.7)	210(98.1)	4(1.9)	213(99.5)	1(0.5)
	Female	161(93.1)	12(6.9)	171(98.8)	2(1.2)	173(100.0)	0(0.0)
Age Group (in years)	15 - 24	30(78.9)	8(21.1)	38(100.0)	0(0.0)	38(100.0)	0(0.0)
	25 - 34	60(87.0)	9(13.0)	69(98.6)	1(1.4)	70(100.0)	0(0.0)
	35 - 44	83(89.2)	10(10.8)	90(96.8)	3(3.2)	92(98.9)	1(1.1)
	45 - 54	77(89.5)	9(10.5)	84(97.7)	2(2.3)	86(100.0)	0(0.0)
	55 - 64	55(100.0)	0(0.0)	55(100.0)	0(0.0)	55(100.0)	0(0.0)
	>64 Years	44(97.8)	1(2.2)	45(100.0)	0(0.0)	45(100.0)	0(0.0)
Residence	Urban	282(91.0)	28(9.0)	304(98.1)	6(1.9)	309(99.7)	1(0.3)
	Rural	68(88.3)	9(11.7)	77(100.0)	0(0.0)	77(100.0)	0(0.0)
Current occupational status	Driver	10(83.3)	2(16.7)	12(100.0)	0(0.0)	11(91.7)	1(8.3)
	Merchant	21(91.3)	2(8.7)	23(100.0)	0(0.0)	23(100.0)	0(0.0)
	Gov. Employee	43(89.6)	5(10.4)	46(95.8)	2(4.2)	48(100.0)	0(0.0)
	Jobless	27(100.0)	0(0.0)	26(96.3)	1(3.7)	27(100.0)	0(0.0)
	Student	19(82.6)	4(17.4)	23(100.0)	0(0.0)	23(100.0)	0(0.0)
	Farmer	56(91.8)	5(8.2)	61(100.0)	0(0.0)	61(100.0)	0(0.0)
	Other	174(90.2)	19(9.8)	190(98.4)	3(1.6)	193 (100)	0(0.0)
Educational status	Can't write and read	62(92.5)	5(7.5)	65(100.0)	0(0.0)	65(100.0)	0(0.0)
	No formal education	62(95.4)	3(4.6)	41(100.0)	0(0.0)	41(100.0)	0(0.0)
	Grade 1-4	38(92.7)	3(7.3)	78(96.3)	3(3.7)	81(100.0)	0(0.0)
	Grade 5-8	73(90.1)	8(9.9)	57(100.0)	0(0.0)	57(100.0)	0(0.0)
	Grade 9-10	47(82.5)	10(17.5)	36(94.7)	2(5.3)	38(100.0)	0(0.0)
	Grade 11-12	34(89.5)	4(10.5)	19(95.0)	1(5.0)	19(95.0)	1(5.0)
	collage	17(85.0)	3(15.0)	18(100.0)	0(0.0)	18(100.0)	0(0.0)
	University	17(94.4)	1(5.6)	76(98.7)	1(1.3)	77(100.0)	0(0.0)
Marital status	Single	63(81.8)	14(18.2)	207(99.0)	2(1.0)	208(99.5)	1(0.5)
	Married	192(91.9)	17(8.1)	59(100.0)	0(0.0)	59(100.0)	0(0.0)
	Widowed	58(98.3)	1(1.7)	36(94.7)	2(5.3)	38(100.0)	0(0.0)
	Divorced	34(89.5)	4(10.5)	3(75.0)	1(25.0)	4(100.0)	0(0.0)
	Separated	3(75.0)	1(25.0)	261(98.9)	3(1.1)	264(100.0)	0(0.0)
Monthly Income Category	<1000 birr	238(90.2)	26(9.8)	77(98.7)	1(1.3)	77(98.7)	1(1.3)
	1001 - 2500 birr	71(91.0)	7(9.0)	25(92.6)	2(7.4)	27(100.0)	0(0.0)
	2501 - 3999 birr	24(88.9)	3(11.1)	18(100.0)	0(0.0)	18(100.0)	0(0.0)
	4000 birr and above	17(94.4)	1(5.6)	210(98.1)	4(1.9)	213(99.5)	1(0.5)

6.8. Associated Risk factors for Study Participants

One hundred eight (27.9%) of the study participants have body piercing. One hundred twenty-four (32.0%) participants had circumcision in traditional way. Habits of alcohol consumption had reported by fifty eight (15.0%) of participants. Two hundred nine (54.0%) of study participants had regular sexual partner. Eighty six (22.2%) of participants had multiple sexual partner. None of the study participants had history of intravenous drug use, sharing toothbrushes with others, Organ transplantation, house hold contact with HIV & HBV infected person (Table 5).

6.8.1. Associated risk factors for Hepatitis B Virus Sero Positivity

The prevalence of HBV among male & female participants was 5.6% & 1.2% respectively. The difference was statistically significant males were five times more likely to test positive for HBV compared to females [COR= 5.079; 95% CI, 1.121-23.009; P=0.035].

Separated individuals had twenty five times more likely to test positive for HBV than individuals with single marital status [COR=25. 333; 95% CI, 1.258-510.018; P=0.035).

Even though not statistically significant married individuals had four times more likely to test positive for HBV than individuals with single marital status [COR=4.222 ; 95% CI, 0.536-33.264; P=0.171]. After adjusted for the socio-demographic variables separated individuals found to be independent predictor to test positive for HBV with an adjusted odds ratios [AOR=113.603; 95% CI, 1.556-8293.97; P=0.031] (Table 4).

Table 4: Socio-demographic Variables, bivariate and multivariate analysis of HBV among individuals with presumptive pulmonary tuberculosis attending at St. Peter's Specialized Hospital, Addis Ababa, Ethiopia 2019.

Variables	Total (%)	Number positive (%)	Crude OR (95% CI)	P-value	Adjusted OR	P-value
Sex						
Male	214(55.3%)	12(5.6)	5.079(1.121-23.009)	0.035	1	0.157
Female	173(44.7%)	2(1.2)	1		0.239(0.033-1.737)	
Age in Years						
15 - 24	38(9.8)	0(0.0)	-	-	-	-
25 - 34	70(18.1)	4(5.7)	-	-	-	-
35 - 44	93(24)	4(4.3)	-	-		
45 - 54	86(22.2)	4(4.7)	-	-		
55 - 64	55(14.2)	1(1.8)	-	-	-	-
>64	45(11.6)	1(2.2)	1			
Residence						
Urban	310(80.1)	10(3.2)	1	0.412	1	0.461
Rural	77(19.8)	4(5.2)	1.644(0.501-5.389)		2.877(0.17347.88)	
Occupational status						
Driver	12 (3.1)	1(8.3)	1		1	
Merchant	23(5.9)	1(4.3)	0.500(0.028-8.772)	0.635	0.359(0.00816.24)	0.599
Gov. Employee	48(12.4)	3(6.2)	0.733(0.069-7.745)	0.796	0.146(0.006-3.603)	0.240
Jobless	27(7)	1(3.7)	0.423(0.024-7.388)	0.556	0.372(0.009-15.282)	0.602
Student	23(5.9)	0(0.0)	0.000	0.998	0.00	0.998
Farmer	61(15.8)	4(6.6)	0.772(0.079-7.580)	0.824	0.208(0.004-11.102)	0.439
Other	193(49.9)	4(2.1)	0.233(0.024-2.263)	0.209	0.233(0.013-4.122)	0.320
Edu.status						
Can't write and read	67(17.5)	3(4.5)	1		1	
No formal education	65(16.8)	2(3.1)	0.677(0.109-4.191)	0.675	0.548(0.064-4.702)	0.583
Grade 1-4	41(10.6)	1(2.4)	0.533(0.054-5.306)	0.592	0.760(0.053-10.902)	0.840
Grade 5-8	81(20.9)	2(2.5)	0.540(0.088-3.331)	0.507	0.347(0.037-3.292)	0.357
Grade 9-10	57(14.7)	1(1.8)	0.381(0.039-3.767)	0.409	0.094(0.003-3.468)	0.199
Grade 11-12	38(9.8)	0(0.0)	0.000	0.998	0.000	0.997
Collage	20(5.2)	3(15.0)	3.765(0.697-20.348)	0.124	1.94(0.120-31.425)	0.640
University	18(4.7)	2(11.1)	2.667(0.411-17.323	0.304	2.28(0.091-57.109)	0.617
M.status						
Single	77(19.9)	1(1.3)	1		1	
Married	209(54)	11(5.3)	4.222(0.536-33.264)	0.171	3.06(0.176-53.077)	0.442
Widowed	59(15.2)	0(0.0)	0.000	.997	0.000	0.997
Divorced	38(9.8)	1(2.6)	2.054(0.125-33.762)	0.614	3.78(0.098-145.734)	0.475
Separated	4(1.0)	1(25.0)	25.333(1.258-510.018)	0.035	113.603(1.556-8293.97)	0.031*
M.Income in birr						
<1000	264(68.2)	6(2.3)	1		1	
1001 - 2500	78(20.2)	5(6.4)	2.945(0,874-9.925)	0.081	2.659(0.414-17.091)	0.303
2501 - 3999	27(7.0)	2(7.4)	3.440(0.659-17.950)	0.143	2.360(0.237-23.539)	0.464
4000 and above	18(4.7)	1(5.6)	2.529(0.288-22.223)	0.403	2.405(0.069-83.686)	0.628

The Crude Odd ratio result for the associated risk factors showed that subjects who had needle stick or sharp injury were three times more likely to test positive for HBV than no needle stick or sharp injury [OR = 3.5; 95% CI, 1.055- 11.791; p=0.041], individuals who had history of previous surgery were three times more likely to test positive for HBV than those who had not history of previous surgery [COR =3.287; 95% CI, 0.684-15.801; P=0.137], subjects who had body piercing were 3.6 times more likely to test positive for HBV compared to those had not pierced [OR= 3.640 ; 95% CI, 1.232-10.751 ; P=0.019].

Participants who share scissors with others were 5.9 times more likely to test positive for HBV than who hadn`t shared [OR = 5.911; 95% CI=1.956 – 17.866; p =0.002], subjects who had habits of alcohol consumption more than one a week were 8.6 time more likely to test positive for HBV than those who had not consumed [OR = 8.613; 95% CI, 2.868- 25.866; p =0.000], individuals who had multiple sexual partner were 6.9 times more likely to test positive for HBV compared to those had not [OR = 6.919; 95% CI, 2.254-21.241; p =0.001].

Nevertheless, after adjustment the adjusted odds ratio results showed that Alcohol consumption [AOR=11.100; 95% CI, 2.147-57.383; P=0.004], body piercing [AOR=8.068; 95% CI, 1.725-37.737; P=0.008] & having multiple sexual partner [AOR=4.573; 95% CI, 1.187-17.613; P=0.027] found to be independent predictors to test positive for HBV (Table 5).

Table 5: Associated risk factors, bivariate and multivariate analysis of HBV among individuals with presumptive pulmonary tuberculosis attending at St. Peter's Specialized Hospital, Addis Ababa, Ethiopia 2019.

Variables		Total (%)	HBsAg pos=No (%)	Crude OR (95% CI)	P-value	Adjusted OR	P-value
Needle stick or sharp injury	No	345(89.1)	10(2.9)	1	0.041	1	0.920
	Yes	42(10.9)	4(9.5)	3.53(1.055-11.791)		0.911(0.150-5.520)	
History of surgery	No	367(94.8)	12(3.3)	1	0.137	1	0.254
	Yes	20(5.2)	2(10.0)	3.29(0.684-15.801)		4.27(0.353-51.606)	
Traditional tooth extraction	No	367(94.8)	13(3.5)	1	0.735	1	0.565
	Yes	20(5.2)	1(5.0)	1.43(0.178-11.537)		2.13(0.163-27.780)	
Tattooing	No	370(95.6)	14(3.8)	1	0.999	1	0.998
	Yes	17(4.4)	0(0.0)	0.00		0.00	
Unsafe injection	No	383(99.0)	14(3.6)	1		1	0.999
	Yes	4(1.0)	0(0.0)	0.00	0.999	0.00	
Body piercing	No	279(72.1)	6(2.2)	1	0.019	1	0.008
	Yes	108(27.9)	8(2.4)	3.64(1.232-10.751)		8.07(1.725-37.737)	
Sharing scissors with others	No	339(87.6)	8(2.4)	1	0.002	1	0.114
	Yes	48(12.4)	6(12.5)	5.91(1.956-17.866)		3.58(0.737-17.371)	
unsafe Abortion	No	385(99.5)	14(3.6)	1	0.999	1	0.999
	Yes	2(0.5)	0(0.0)	-		0.00	
Organ transplantation	No	387(100.0)	0(0.0)	-		-	-
	Yes	0(0.0)	0(0.0)	-		-	
Sharing toothbrushes	No	387(100.0)	14(8.3)	1	0.999	-	-
	Yes	0(0.0)	0(0.0)	-		-	
Circumcision	No	263(68.0)	7(2.7)	1	0.152	1	0.875
	Yes	124(32.0)	7(5.6)	2.188(0.750-6.380)		1.139(0.225-5.766)	
History of hospital admission	No	352(91.0)	13(3.7)	1	0.801	1	0.249
	Yes	35(9.0)	1(2.9)	0.767(0.097-6.043)		0.157(0.007-3.654)	
Blood transfusion	No	383(99.0)	14(3.7)	1	0.999	1	0.999
	Yes	4(1.0)	0(0.0)	-		0.00	
STIs	No	339(87.6)	13(3.8)	1	0.549	1	0.242
	Yes	48(12.4)	1(2.1)	0.534(0.068-4.173)		0.209(0.015-2.884)	
History of chronic infections	No	375(96.9)	13(3.5)	1	0.391	1	0.087
	Yes	12(3.1)	1(8.3)	2.53(0.304-21.102)		11.418(0.702-185.718)	
Alcohol Consumption	No	329(85.0)	6(1.8)	1	0.000	1	0.004
	Yes	58(15.0)	8(13.8)	8.61(2.868-25.866)		11.1(2.147-57.383)	
Intra Venus drug use	No	387(100.0)	14(3.6)	-		-	-
	Yes	0(0.0)	0(0.0)	-		-	
contact with HBV infected	No	387(100.0)	12(3.1)	1	0.999	-	-
	Yes	0(0.0)	0(0.0)	-		-	
Regular sexual partner	No	178(46.0)	2(1.1)	1	0.810	1	0.410
	Yes	209(54.0)	12(5.6)	1.141(0.388-3.353)		1.783(0.450-7.061)	
Multiple Sexual partner	No	301(77.8)	5(1.7)	1	0.001	1	0.027
	Yes	86(22.2)	9(10.5)	6.919(2.254-21.241)		4.573(1.187-17.613)	

In general after adjusted for the socio demographic variables & associated risk factors marital status partner separated, alcohol consumption more than one per a week, had multiple sexual partners & body pierced with traditional ways (used un sterilized materials) found to be increase HBV positivity.

6.8.2. Associated risk factors for HIV Sero Positivity

Separated individuals were fifty four times more likely to be tested positive for HIV than individuals with single marital status [OR = 54.75; 95% CI, 4.599- 651.747; p=0.002], even though not statistically significant Participants with income level between 2501 - 3999 birr were three times more likely to test positive for HIV compared to those with income level <1000 birr monthly [OR = 3.77; 95% CI, 1.253 - 11.357; p=0.018] (Table 7).

Table 6: Socio-demographic variables, bivariate and multivariate analysis of HIV among individuals with presumptive pulmonary tuberculosis attending at St. Peter's Specialized Hospital, Addis Ababa, Ethiopia 2019.

Variables	Total (%)	Number positive(%)	Crude OR (95% CI)	P-value	Adjusted OR	P-value
Sex						
Male	214(55.3)	18(8.4)	1		1	
Female	173(44.7)	10(5.8)	1.497 (.672-3.333)	0.323	0.436(.134-1.420)	0.168
Age category *						
15 - 24	38(9.8)	0(0.0)	1		1	
25 - 34	70(18.1)	8(11.4)	-		-	
35 - 44	93(24)	13(14.0)	-		-	
45 - 54	86(22.2)	7(8.1)	-		-	
55 - 64	55(14.2)	0(0.0)	-		-	
>64	45(11.6)	0(0.0)	-		-	
Residence						
Urban	310(80.1)	26(8.4)	1	0.098	1	
Rural	77(19.8)	2(2.6)	0.291(0.061-1.255)		0.284(0.013-1.420)	0.426
Occupational status						
Driver	12 (3.1)	3(25.0)	1		1	
Merchant	23(5.9)	2(8.7)	0.286(0.041-2.013)	0.208	0.478(.048-4.799)	0.530
Gov. Employee	48(12.4)	4(8.3)	0.273(0.052-1.434)	0.125	0.354(.041-6.579)	0.343
Jobless	27(7)	3(11.1)	0.375(0.064-2.211)	0.279	0.520(.041-6.579)	0.613
Student	23(5.9)	0(0.0)	0.000	0.998	-	0.996
Farmer	61(15.8)	2(3.3)	0.102(0.015-0.695)	0.020	0.905(.024-34.708)	0.957
Other	193(49.9)	14(7.3)	0.235(0.057-0.966)	0.045	0.260(.041-1.658)	0.154
Educational status						
Can't write and read	67(17.5)	1(1.5)	1		1	
No formal education	65(16.8)	2(3.1)	2.095 (0.185-23.684)	0.550	2.448(0.159-37.760)	0.521
Grade 1-4	41(10.6)	3(7.3)	5.211 (0.523-51.872)	0.159	8.985(0.580-139.079	0.116
Grade 5-8	81(20.9)	10(12.3)	8.250 (1.018-66.888)	0.048	7.427(0.589-93.702)	0.121
Grade 9-10	57(14.7)	4(7.0)	6.346 (0.719-56.004)	0.096	4.238(0.265-67.756)	0.307
Grade 11-12	38(9.8)	5(13.2)	10.00 (1.122-89.113)	0.039	6.408(0.425-96.700)	0.180
Collage	20(5.2)	3(15.0)	11.647(0.139-119.123)	0.038	4.323(0.191-97.740)	0.358
University	18(4.7)	0(0.0)	0.000	0.999	-	-
Marital status						
Single	77(19.9)	4(5.2)	1		1	
Married	209(54)	13(6.2)	1.210 (0.382-3.832)	0.745	1.518(.316-7.294)	0.602
Widowed	59(15.2)	4(6.8)	1.327 (.318-5.543)	0.698	14.286(1.551-131.584)	0.019*
Divorced	38(9.8)	4(10.5)	2.147 (.506-9.102)	0.300	5.996(.807-44.563)	0.080
Separated	4(1.0)	3(75.0)	54.750 (4.599-651.747)	0.002	49.849(2.200-1129.498)	0.014*
Monthly Income in birr						
<1000 b	264(68.2)	15(5.7)	1		1	
1001 - 2500	78(20.2)	8(10.3)	1.897 (.773-4.657).	0.162	.861(.235-3.160)	0.822
2501 - 3999	27(7.0)	5(18.5)	3.773 (1.253-11.357)	0.018	3.025(.553-16.557)	0.202
4000 and above	18(4.7)	0(0.0)	0.000	0.998	0.000	0.998

Age category *, not valid for association, COR-Crude odds ratio.

The Crude Odd ratio result for the associated risk factors of HIV showed Subjects who have had needle stick or sharp injury were 6.8 times more likely to be HIV positive than individuals who had not [OR = 6.846; 95% CI, 2.946 – 15.908; p=0.000], individuals who consumed alcohol were found to be ten times more likely to be tested HIV positive than those individuals who didn`t consume alcohol [OR = 10.063; 95% CI, 4.456 – 22.729; p=0.000]. Participants who had sharing scissors with others were 5.6 times more likely to be tested positive for HIV compared to those had not [COR=5.631; 95% CI, 2.452-12.930; P=0.000], individuals who had history of unsafe injection were 42 times more likely to be tested positive for HIV compared to those had not [OR=42.960 ; 95% CI, 4.311-428.149; P=0.001].

Subjects who had history of STI & contact with multiple sexual partner were 3.8 & 40.7 times to test positive for HIV compared to those had not [COR=3.887 ; 95% CI, 1.645-9.184; P=0.002] &[COR=40.710; 95% CI, 11.913-139.116; P= 0.000] respectively (Table 8).

Moreover, after adjusted for the socio demographic variables & associated risk factors individuals who were partner separated [AOR = 49.849; 95% CI; 2.200 -1129.498; p=0.014], widowed [AOR = 14.286 ; 95% CI, 1.551-131.584 ; P=0.019], sharing scissors [AOR= 4.416; 95% CI, 1.134 -17.205; p=0.032], alcohol consumption[AOR = 7.323; 95% CI, 2.068-25.934; p=0.002] & contact with multiple sexual partner [AOR = 29.984 ; 95% CI, 7.9212 - 124.667; p=0.000] were found to be independent predictors to test positive for HIV(Table 7).

Table :7 Associated risk factors, bivariate and multivariate analysis of HIV among individuals with presumptive pulmonary tuberculosis attending at St. Peter's Specialized Hospital, Addis Ababa, Ethiopia 2019.

Variables		Total (%)	HIV pos=No (%)	Crude OR (95% CI)	P-value	Adjusted OR	P-value
Needle stick or sharp injury	No	345(89.1)	17(4.9)	1	0.000	1	0.464
	Yes	42(10.9)	11(26.2)	6.846 (2.946-15.908)		1.737(0.397-7.607)	
History of surgery	No	367(94.8)	26(7.1)	1	0.626	1	0.803
	Yes	20(5.2)	2(10.0)	1.457 (0.321-6.625)		0.736(0.066-8.176)	
T.tooth extraction	No	367(94.8)	25(6.8)	1	0.182	1	0.592
	Yes	20(5.2)	3(15.0)	2.414 (0.663-8.795)		1.730(0.233-12.872)	
Tattooing	No	370(95.6)	26(7.0)	1	0.467	1	0.418
	Yes	17(4.4)	2(11.8)	1.764 (0.383-8.133		2.656(0.249-28.282)	
Unsafe injection	No	383(99.0)	25(6.5)	1		1	0.067
	Yes	4(1.0)	3(75.0)	42.96(4.311-428.149)	0.001	34.06(0.78-1492.274)	
Body piercing	No	279(72.1)	18(6.5)	1	0.341	1	0.214
	Yes	108(27.9)	10(9.3)	1.480(0.660-3.316)		2.222(0.630-7.834)	
Sharing scissors with others	No	339(87.6)	17(5.0)	1		1	0.032
	Yes	48(12.4)	11(22.9)	5.631(2.452-12.930)	0.000	4.416(1.134-17.205)	
unsafe Abortion	No	385(99.5)	28(7.3)	1	0.999	1	0.999
	Yes	2(0.5)	0(0.0)	0.00		0.000	
Organ transplantation	No	387(100.0)	28(7.2)	1	-	1	-
	Yes	0(0.0)	0(0.0)	-		-	
Sharing toothbrushes	No	387(100.0)	28(7.2)	1		1	-
	Yes	0(0.0)	0(0.0)	-		-	
Circumcision	No	263(68.0)	16(6.1)	1	0.207	1	0.631
	Yes	124(32.0)	12(9.7)	1.654(0.757-3.612)		0.728(0.199-2.662)	
Hospital admission	No	352(91.0)	24(6.8)	1	0.321	1	0.765
	Yes	35(9.0)	4(11.4)	1.763 (0.575-5.409)		0.743(0.106-5.190)	
Blood transfusion	No	383(99.0)	28(7.3)	1	-	1	0.999
	Yes	4(1.0)	0(0.0)	0.00		0.000	
STIs	No	339(87.6)	19(5.6)	1	0.002	1	0.629
	Yes	48(12.4)	9(18.8)	3.887 (1.645-9.184)		1.411(0.349-5.699)	
History of chronic infections	No	375(96.9)	27(7.2)	1	0.882	1	0.559
	Yes	12(3.1)	1(8.3)	1.172 (0.146-9.419)		2.442(0.122-48.702)	
Alcohol Consumption	No	329(85.0)	12(3.6)	1	0.000	1	0.002
	Yes	58(15.0)	16(27.6)	10.063(4.456-22.729)		7.323(2.068-25.934)	
Intra Venus drug use	No	387(100.0)	28(7.2)	1	0.999	1	-
	Yes	0(0.0)	0(0.0)	0.00		-	
contact with HIV infected	No	387(100.0)	28(7.2)	-	-	-	-
	Yes	0(0.0)	0(0.0)	-		-	
Regular sexual partner	No	178(46.0)	14(7.9)	1	0.659	1	0.340
	Yes	209(54.0)	14(6.7)	0.841(0.390-1.815)		1.780(0.544-5.824)	
Multiple Sexual partner	No	301(77.8)	3(1.0)	1	0.000	1	0.000
	Yes	86(22.2)	25(29.1)	40.71(11.91-139.12)		29.98(7.212-124.667)	

7. Discussion

HBV, HIV& M.TB are the cause of widely spread infectious disease around the world, especially in resource limited countries. Treatments used to treat active TB, HIV and opportunistic infections are often hepatotoxic. Thus multi infection with HIV, TB & HBV has increased the risk of hepatotoxicity which could be challenged in patient management and resulted in treatment failure & drug resistance [16]. So, this study has generated information on the burden of HBV and HIV among individuals with presumptive TB which helps for appropriate management.

The prevalence rate of HBV positive 3.6% (14/387) found in this study is similar to previous studies in Gojjam Zone South west Ethiopia 3.1% and in Bahrdar 3.8% [58,59]. Comparatively less prevalence rate of HBV, 1.3% in three government hospitals Southern Ethiopia [50], 1% in Gondar town health institution [54], 2.1% in Ethiopia among blood donors [48] & 2.6% in Tehran, Iran [39], have been reported. Comparatively higher prevalence rate of HBV than our study have been documented 6% and 6.3% in Addis Ababa [62,63] and 7.4% in Goba general hospital [49]. This difference might be attributable to socio-demographic characteristics, behavioral differences for the risk factors of HBV infection & methodological difference.

In our study the prevalence of HBV was higher in males as compared to females. This could be due to variations to different exposure factors. Males are more likely to have outdoor exposure to risky behaviors that could increase their risk of getting hepatitis B infection than females. This finding is in line with previous study in University of Gondar Hospital, Northwest Ethiopia [53].

The prevalence of HBV was 5.2% & 3.2% in rural & urban residents respectively in this study. This might be rural residents have less awareness about the HBV infection & its transmission. So, they could be use sharp materials in common and contact to infected individuals easily.

In the present study the overall prevalence of HIV is twenty eight (7.2%) [95% CI, 4.7 – 9.8]. Although, the HIV prevention is well from previous times but HIV is still a social problem in developing countries including our country, this could be due to interruption in creating awareness within the community and paying less attention to the infection. This finding is comparable with a study from Bahir Dar (6.6%) [59] but which is higher than the national adult HIV prevalence 1.1% (in 2016) [31] & findings from previous studies in Gojjam, North west,

Ethiopia among adult population 3.3% [58], in Goba general hospital 5.9% [49], in Ethiopia among blood donors 2.1% [48] & Tehran, Iran 3.4% [39].

In contrast, this finding is lower than previous studies from university of Gondar 11.1% [55] & North West Ethiopia 17.5% [56]. These differences might be due to difference in study population, living condition & behavioral differences for the risk factors of HIV infection.

The prevalence of HIV was 18 (8.4%) & 10 (5.8%) in male & female participates respectively in the present study. This is higher than the national male & female HIV prevalence 0.7% & 1.4 % [31] & previous studies in Gojjam, North west, Ethiopia 1.7 % in males & 5.2 % in females [58]& in north India 1.5% of the males and 1.1% females were HIV-positive [40] but lower than a study from West Arsi Zone, Ethiopia 14.3% male and 16.0% female were positive[36]. This difference could be because of differences in study population & risky behaviors associated to HIV. In this study, HIV prevalence is higher in males which is contrasted with the national HIV prevalence 0.7 % in males & 1.4% in females [31] & study in Gojjam,North west, Ethiopia 1.7 % in males & 5.2 % in females [58]. This might be as a result of the risky behaviors like alcohol consumption & having multiple sexual partners which were significantly associated with HIV infection in our study was more related to males than females.

Urban residences accounted 8.4% HIV prevalence followed by rural residences 2.6% in the present study, although, urban residents more aware on the transmission of HIV but the higher prevalence could be due to carelessness & pay less attention for the transmission & prevention mechanisms of HIV. It could be also due to expose to risk factors of HIV easily than rural residents, mainly it could be also due to many of the participants included in our study were urban dwellers.

In this study the magnitude of HBV & HIV co-infection was 0.3% (1/387). In general, the HBV-HIV co-infection in our study is lower than in previous studies in Mekelle hospital, Tigray 5.9% [7] & in Goba general hospital 42.3% [49]. This low HBV-HIV co-infection in this study might be due to intravenous drug users & sex worker which were highly associated with transmission of HBV & HIV were not participated, it could be also due to the difference in study population & exposure of the participants to the risky behaviors that play roll in the transmission of these viruses.

In the present study, the overall prevalence of TB found to be 9.6% (37/387) . Although the prevention & treating of TB is well around the world but it is still a problem in countries with limited resource like Ethiopia, depending the living condition , immunological status of the population, less awareness in early diagnosing & treating of the infection, less awareness in properly using the anti TB drugs, these all makes difficult full control of the infection.

This finding is higher than previous studies from Hawassa Adare hospital, south, Ethiopia 5.3% [51], in North Gondar Administrative Zone 5.3% [57] & cape Town, South Africa 3% [52]. This could be since our study site is a referral site for Tuberculosis related cases highly TB suspected individuals might included in our study. But this finding is lower than studies in Government hospitals in Addis Ababa 15.11% [61]. These difference might be due to differences in study population, rate of exposure of participants for risks related to TB infection & diagnosis methods.

In the study TB -HIV co-infection was 1.6% (6/387). This finding is comparable a previous study in Ghatampur, north India 1.48% [40] .

The TB-HIV co-infection finding in the present study is lower than from other studies, in west Arsi zone, Ethiopia 14.98% [36] & great Tehran prison 5.9% [38] .This might be due to differences in study population, exposure to risk factors for the infections & test methods. Low TB-HIV co-infection in our study indicates there is less TB -HIV collaboration due to individuals could diagnose for TB or HIV early than previous times before loss their immunity.

The TB -HBV co-infection was 0.3% (1/387) in this study. This finding is lower than other study from Ghatampur, north India 2.96% [40]. This could be due to differences in exposure to risk factors for HBV infection.

In the present study there was no HBV-HIV -TB triple infection this differs from a study in West Arsi zone, Ethiopia 8.92% [36]. This might be due to our study is done in individuals with presumptive TB but the study in West Arsi zone have been done in TB patients who could be less immune & differences in exposure for the risk factors of HBV & HIV.

The most important risk factors for HBV identified in our study were having partner separated [AOR=113.603; 95% CI,1.556-8293.973;P=0.031], body piercing [AOR=8.068; 95% CI,1.725-

37.737 ;P= 0.008], alcohol consumption [AOR=11.100; 95% CI, 2.147-57.383 ;P= 0.004] & having multiple sexual partner. This might reflect individual risky behaviors & traditional practices using unsterilized piercing materials contribute important role in the transmission of HBV.

This finding is in line with a studies in Tigray, North west, Ethiopia [7], in Goba, Ethiopia [49] & in Brazil [41] which detected having multiple sexual partner had statically significant association with HBsAg positivity. This finding is also agreed with previous study in Romania[42] that detected alcohol consumption as important risk factor for HBsAg sero-positivity & in Deder hospital, Eastern Ethiopia [52] which detected nose piercing & having multiple sexual partner had significant association with HBsAg positivity. In contrast ,our finding is differ from other studies in Addis Ababa, Ethiopia identified history of abortion, surgery & tattooing as risk factors [62] , in North-west Ethiopia showed history of contact with HBV infected & history of jaundice as risk factors of HBV infection[60] & in Iran detected history of drug use [37]. These differences could be due to differences in study population & exposure rate for risk factors of HBV infection.

Our study identified marital status who were widowed & separated [AOR=14.286 ;95% CI,1.551-131.584; P=0.019] & [AOR=49.849; 95%CI, 2.200-1129.498; P=0.014] respectively, sharing scissor with others [AOR=4.416; 95% CI, 1.134-17.205 ;P= 0.032] , having multiple sexual partner [AOR=29.984; 95% CI, 7.2121-124.667P=0.000] & alcohol consumption [AOR=7.323; 95%CI ,2.068-25.934; P=0.002] as independent predictors for HIV positivity. This shows individual risky behaviors & using sharp objects in common contributes in the transmission of HIV. This finding is in line with a previous study in west Arsi zone, Ethiopia that identified partner separated & having multiple sexual partner as risk factors for HIV positivity [36] & other studies from Nigeria [43] & south Sudan [47] that identified having multiple sexual partner significantly associated to be tested positive for HIV. In our study alcohol consumption had significantly associated with HIV positivity this agreed with findings in, South Sudan [47], South Africa [46] & in Sierra Leone [44]. In contrast our finding is differ from other study in Bahir Dar North west, Ethiopia which indicated history of abortion [59] as HIV risk factors. This could be since participants included in this study were TB suspected individuals of any age group the number of history of abortion was very low.

8. Conclusion

This study shows HBV, HIV & TB are still public health issues in this area. In this study relatively high prevalence of TB was found though it declined than previous reports. The TB-HIV co-infection was relatively high. TB-HBV co-infection was very low & TB-HIV-HBV triple infection not found. Some behavioral and socio-demographic risk factors such as partners being separated, having multiple sexual partners, alcohol consumption & body piercing & being separated & widowed, having multiple sexual partners, alcohol consumption & sharing scissor with others found to be strongly associated with HBV & HIV infections respectively.

9. Recommendation

Based on the findings, the following recommendations we made.

- A national HBV survey is required to know the current national HBV prevalence among PTB/TB patients in Ethiopia.
- In Ethiopia most researches related to HBV & HIV done among pregnant women but there is limited data about these viruses in other areas especially for HBV. So, further large scale research is needed on the prevalence of HBV & HIV among TB suspected individuals.
- Health education should be given in health institutions to create awareness about HBV transmission in the community. There is also call for create awareness due to health education on the risky behaviors of HBV & HIV.
- The well-established infrastructure for HIV counseling and testing in public health programs, should be expanded to include prevention of HBV infection and Health-care providers should be trained to screen actively for risk factors for HIV&HBV to offer health education , counseling & hepatitis B vaccine to clients with risk factors.
- TB is still a public health problem especially in those with low income level & living in rural areas. So, it needs attention on creating continuous awareness on the prevention & diagnosis of the disease.
- Although TB-HIV co-infection is decreased from previous times but it still needs intensive follow up in the diagnosis of these infections in every heath institutions restrictedly.
- In general the current study showed the need for more information on the HBV-HIV co infection & TB-HIV-HBV triple infection in order to support therapeutic decisions and prevention of hepathotoxity & treatment failure among TB suspected individuals. This underlines the need for further epidemiologic and clinical studies to optimize the management of this medical condition.

10. Strength & Limitation of the Study

10.1. Strength of the Study

- Utilizing more sensitive method for detection of Hepatitis B virus (ELISA) .
- Employing triple methods for detection of TB (GeneXpert, FM and ZN microscopy).

10.2. Limitation of the Study

- TB positive samples not confirmed by Culture due to limited resource.
- HBV positive samples not further analyzed due to limited resource.

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12. Annexes

12.1. Annex I: Participant information sheet

My name is Kahasit. I am a laboratory technologist & a postgraduate student at Addis Ababa University. Now I am conducting a study entitled **Prevalence of Hepatitis B Virus , Human immunodeficiency Virus and associated risk factors among individuals with presumptive Pulmonary Tuberculosis attending at St. Peter`s specialized hospital ,Addis Ababa, Ethiopia.**

Introduction

The topic of this study is **Prevalence of Hepatitis B Virus , Human immunodeficiency Virus and associated risk factors among individuals with presumptive Pulmonary Tuberculosis attending at St. Peter`s specialized hospital ,Addis Ababa, Ethiopia.**

Hepatitis B virus (HBV) is a virus that attacks the liver. **HBV** is one of the major health problems in the world including our country due to liver impairment. Human immunodeficiency virus also a virus that attacks the immune system , disclose the body to different infections due to loss of immunity & leads to death as a result acquired immuno deficiency disease (AIDS) & related cases. TB is a bacterial infection which affects the lung. The result of the study can be helpful in planning screening of individuals with presumptive TB for HBV & HIV at the same time to solve the problems of liver cirrhosis, liver cancer & treatment failure due to HBV ,anti HIV & anti TB drugs. Participation in this study is exclusively voluntarily. I would like to ask dear participates to participate in this study to support in order to obtain current data on prevalence of HBV, HIV,TB, their co-infections & risk factors associated to HBV & HIV for health benefits of individuals with presumptive TB & health professionals in management of such individuals.

Expected from participants

As a participant of this study, you are expected to give Sputum & whole blood samples and interviewed for socio-demographic, such as your age, gender, education level, economic level, & risk factors to HBV & HIV such as needle stick injury, history of partnership, blood transfusion, sharing scissor, alcohol consumption, body piercing, tattooing & etc.

Being asked to give sample does not neccessarly mean that you have the disease. When you are found to be positive for the micro-organism, you will be linked to your doctor for advice and to receive proper treatment. But your name, address will not be disclosed rather an identification code will be used in such conditions.

Time required for participating

You will spend 10-20 minutes until the specimen is collected, the questionnaire is filled and the consent is signed.

Risks of participant

Specimen collection will have no effect and you will not get any risk.

Confidentiality

The information in your records is strictly confidential. All information that you give and the results from your specimen will be used for this study only. Only limited numbers of professional will have access to the information. The information will be recorded and stored with locked protection.

Benefits of participation

By participating, you will get no financial benefits. Even though, there is no direct benefit due to participation in this study, the findings of the study is useful for better understanding of the prevalence of HBV,HIV,TB & risk factors of HBV & HIV on TB suspected individuals .

Rights of participants

Your participation is completely voluntary, and you can refuse to participate or withdraw from the study at any time. Refusal to participate will not result in loss of medical care provided or any other benefits. You can get your results of the analysis.

Communication

In case if you have any questions, unclear ideas and doubt about the project, contact the investigater.

Addresses of the Investigator:

Name :Kahasit G/hiwet (BSc, Msc student)

Phone number : +251914127278

Address: St. peter`s specialized hospital, Addis Ababa Ethiopia.

Email- kahasitg@gmail.com

10.2. Annex II: Participant information sheet (Amharic version)

ስሜ ካሓሲት እባላለሁ የላቦራቶሪ ሞያተኛ ስሆን አሁን በአዲስ አበባ ዩኒቨርሲቲ የጤና ሳይንስ ኮሌጅ የሕክምና ላብራቶሪ ሳይንስ ትምህርት ክፍል የሁለተኛ ዲግሪ ተማሪ ነኝ፡ ፡ በመሆኑም በሄፓታይተስ ቢ እና ኤች ኣይ ቪ ቫይስ በቲቢ ተጠርጥረው ለቲቢ ምርመራ በሚላኩ ግለ ሰች ጥናት እያካሄድኩኝ እገኛለሁ፡ ፡

<u>መግቢያ</u>

ይህጥናት “Prevalence of Hepatitis B Virus , Human immunodeficiency Virus and associated risk factors among individuals with presumptive Pulmonary Tuberculosis attending at St. Peter`s specialized hospital ,Addis Ababa, Ethiopia.”**ማለትም የ ሄፓታይተስ ቢ እና ኤች ኣይ ቪ ቫይስ በቲቢ ተጠርጥረው ለቲቢ ምርመራ በሚላኩ ግለሰዎች ላይ ያላቸው ዝርጋታ እና አጋላጭ የሆኑ ነገሮች ለማየት የሚካሄድ ጥናት ነው፡፡ ሄፓታይተስ ቢ ቫይረስ የተባለው የጉበት በሽታ አምጪ ቫይረስ በአለማችን ብሎም በሀገራችን ጉበትን በማጥቃት ከፍተኛ የጤና እክል እያስከተለ ይገኛል፡፡ ኤች ኣይ ቪ ቫይረስ ደግሞ የሰውነትን በሽታ የመከላከል አቅም የሚያዳክም ቫይረስ ሲሆን ለተለያዩ በሽታዎች በማጋልጥ በኤች ኣይ ቪ ኤድስ እና ተያያዥ በሽታዎች እስከሞት የሚያደርስ ችግር እያስከተለ ይገኛል፡፡ ቲቢም ሳንባን የሚያጠቃ በባክተሪያ የሚመጣ በሽታ ሲሆን ከነዚህ ቫይረሶች ባልተናነሰ መልኩ ጉዳትን ያስከትላል፡፡ ስለዚህ ይህ ጥናት በቲቢ ተጠርጥረውነ ለቲቢ ምርመራ በሚላኩ ግለሰዎች የሄፓታይተስ ቢ ቫይረስ እና ኤች ኣይ ቪ ቫይረስ ምርመራ አንድ ላይ እንዲደረግላችው እና በሄፓታይተስ ቢ ቫይረስ ብሎም በፀረ ኤች ኣይ ቪ እና ፀረ ቲቢ መድሃኒቶች የሚመጣው የጉበት በሽታ ለመቀነስ የሚረዳ ጥናት ነው፡፡በዚህ ጥናት የሚሳተፉ በሙሉ ፈቃደኝነት ሲሆን በጥናቱ እንዲሳተፉ በትህትና እየጠየቅኩኝ ጥናቱን አልሞ የተነሳውን በቲቢ ተጠርጥረው ለቲቢ ምርመራ በሚላኩ ግለሰዎች የሄፓታይተስ ቢ ቫይረስ እና ኤች ኣይ ቪ ቫይረስ ስርጭትና ከቲቢ አብሮ የመገኘት ሁኔታ ለማወቅ የሚረዳ ወቅታዊ መረጃ ለማግኘት በሚደረግ ጥናት የበኩልዎን እንዲያግዙ እጠይቃለሁ፡፡**

በዚህ ጥናት ለመሳተ ፈቃደኛ ከሆኑ በጥናቱ ላይ ከእርስዎ የሚጠበቅ እና በጥናቱ ለመሳተፍ ምን መደረግ እዳለበት ከዚህ በታች ተቀምጧል፡-

ከተሳታፊዎች የሚጠበቅ

በጥናቱ ሲሳተፉ ስለ እድሜዎና ፆታዎን መግለፅ ይኖርብዎታል፡፡ለሄፓታይተስ ቢ ቫይረስ እና ኤች ኣይ ቪ ቫይረስ ሊያጋልጡ የሚችሉ ሁኔታዎች እንደንቅሳት፣አልኮሆል የመውሰድ ሁኔታ፣መቀስ ከሌሎች አብሮ የመጠቀም ሁኔታ ፣የትዳር ጓደኛ ሁኔታ፣በተለያየ አጋጣሚ ከሌላ ደም የመቀበል ሁኔታ የመሳሰሉትን መረጃዎች ይጠየቃሉ፤የአክታና የደም ናሙና ይሰጣሉ፤ ይህንን ናሙና ስለሰጡ በሽታው አለብዎት ማለት አይደለም፤ተመርምረው ሄፓታይተስ ቢ ቫይረስ፤ኤች አይ ቪ ቫይረስ፣ቲቢ ቢኖርዎት ከሀኪምዎ እንዲገናኙ እና አስፈላጊው ምክርናህክምና እንዲያገኙ ይደረጋል፡፡

በጥናቱ ለመሳተፍ የሚወስድብዎት ግዜ

በጥናቱ ለመሳተፍ የሚያስፈልግዎት ግዜ ከ10-20 ደቂቃ ሲሆን ይህንንም መጠይቁ እስኪሞላና ናሙና እስከሚሰጡ ይሆናል፡፡

ጥናቱ የጤና ጉዳት የማያስከትል መሆኑ

ናሙና መውሰድ ለጤናዎ ምንም ጉዳት የማያስከትል መሆኑን ማወቅ ይኖርብዎታል፡፡በጥናቱ ያለመሳተፍና እንዲሁም በማንኛዉም ጊዜ የማቋረጥ መብት እንዳለብዎትም አሳውቃለሁ፡፡

በጥናቱ ላይ የእርስዎ መረጃ ሚስጢራውነት በተመለከተ

ማንኛውም እርስዎ በተመለከተ ያሉ መዛግብትና ውጤቶች ሚስጢራዊነታቸው የተጠበቀና የላቦራቶሪ ውጤትዎን የያዙ መዛግብቶች ተቆልፈው የሚቀመጡ መሆኑን ማወቅ ይኖርብዎታል፡፡

በጥናቱ መሳተፍ የሚያስገኘው ጥቅም በተመለከተ

በጥናቱ በመሳተፍዎ የገንዘብ ጥቅም የማያስገኝ መሆኑን ሆኖም ግን በዚህ ጥናት በመሳተፍዎና ይህንን ጥናት በመካሄዱ፤ የሄፓታይተስ ቢ ቫይረስ እና የኤች ኣይ ቪ ቫይረስ ስርጭት ለማወቅና ማንኛውም ለቲቢ ተጠርጥረው የመጡ ግለሰዎች ለሄፓታይተስ ቢ ቫይረስ እና ኤች ኣይ ቪ ቫይረስ መመርመር የሚያስችል ስርአት እንዲዘረጋና በሄፓታይተስ ቢ ቫይረስ፣በፀረ ኤች ኣይ ቪ ቫይረስ እና በፀረ ቲቢ መድሃኒቶች የሚመጣውን የጉበት በሽታ ለመከላከል ያግዛል፡፡

የጥናቱ ባለቤት:- ካሓሲትግ/ህይወት

ስልክቁጥር፡- 0914127178

የስራአድራሻ፡-የቅዱስ ጴጥሮስ ስፔሻላይዝድ ሆስፒታል

12.3. Annex III: Informed consent form

Dear participant, this study will benefit the patients to screen HBV,HIV and receive appropriate anti TB treatment to reduce drug induced hepatotoxicity, treatment discontinuation and development of drug resistance TB strains. So, if you are agreed to take part in this study based on the information given to you, please listen or read this form one by one and tick every box to show your agreement on each points and sign the consent sheets at the end of this form. If there is any unclear point, don't hesitate to ask question until you understand it to make your decision by your own interest.

1. I know the objective and procedure of this study after I have read, or it was read to me, the information sheet concerning this study and I understand what will be required from me if I take part in the study.	□
2. I understand that being participation on this study is voluntary; confidentiality of my personal information is guaranteed.	□
3. I understand that at any time I have the right to withdraw from this study without giving a reason.	□
4. I know the information regarding HBV,HIV &TB disease after he/she explain for me the procedure of data & sample collection.	□
5. The interviewer explain for me as there is no any risk or discomfort	□
6. I understand that as information collected from me are confidential, they will be reported with my approval and results will be reported without my personal information by using code number.	□
7. I know there is no extravagant to me without time taken for interview, counseling and health education.	□
8. I know that, if there are any physical, mental problems due to participating in the study, all responsibilities are come to project manager	□
9. I know that, if there is any disagreement with the procedure of the study I will appear to research ethical clearance board of the Ethics Committee of the Departmental Research and Ethics Review Committee of Addis Ababa University, College of Health Sciences, School of Allied Health Sciences, Department of Laboratory Sciences & St. Peter`s hospital.	□
This informed consent will be filled and signed in two copies, and then one copy will be provided for participant and the other for project manager.	

I understand all the information given above and I agreed to participate in this study by my full interest and I assure my agreement by my official signature.

Signature: ---------- Date: ----------/--------/2019

Participant Phone Address if possible: ----------

I project manager kahasit G/hiwet, agreed on all commitment on this form to fulfill all safety procedure, right and benefit for the participants, and then I assure my agreement by my official signature

Project Manager Name: kahasit G/hiwet

Phone Number:---------- work

Address: St. peter`s specialized hospital, Addis Ababa Ethiopia.

Signature: ---------- Date: -------/------/2019

12.4. Annex IV: Informed consent form (Amharic version)

የተከበራችሁ የጥናቱ ተሳታፊዎች ይህ ጥናት በቲቢ ተጠርጥረው ለቲቢ ምርመራ በሚላኩ ግለሰዎች የሄፓታይተስ ቢ ቫይረስ እና ኤች ኣይ ቪ ቫይረስ ምርመራ አንድ ላይ እንዲደረግላችው እና በ ሄፓታይተስ ቢ ቫይረስ ብሎም በፀረ ኤች ኣይ ቪ እና ፀረ ቲቢ መድሃኒቶች የሚጫው የጉበት በሽታ ለመቀነስ የሚዳ ጥናት ነው ፡ ምክንያቱምፀረ ቲቢና ኤች አይ ቪ ቫይረስ መድሃኒቶች ጉበትን ሊጎዱ ስለሚችሉ ታካሚው ቀደም ብሎ በሄፓታይተስ ቢ ቫይረስ የተጎዳ ከሆነ ጉብት ስራውን በትክክል ስለሚሰራ መድሃኒት የመቋረጥ ብሎምመድሃኒት ለተላመደ ቲቢ ሊጋለጥ ይችላል።ስለዚህ ይህ ጥናት ትክክለኛውን መድሃኒት ለማሚጥና በመድሃኒቶችና በሄፓታይተስ ቢ ቫይረስ በታካሚው የሚደርሰው የጉብት በሽታ ለመቀነስ ይረዳል፡ ፡

<u>በጥናቱ ለማሳተፍ ፈቃደኛ ከሆናችሁ ፎርማን በማ ባ ካነ በባችሁ/</u>

ከተነ በበላችሁ በኋላ በፎርሙላይ የማ ኙ ክፍት ሳጥኖች ላይ ምልክት በማድረግና በፎርሙ ሜጨረሻ በማ ኝ ክፍት ቦታ ላይ በሚረምእንድታረግጡሉኝ እጠይቃለሁኝ፡ ፡ ያልተሩዱት ካለ እባክዎትን ከማከየ ቅ ወደኋላ አይበሉ፡ ፡

1.የ ጥናቱ ዋና አላማ አተገ ባበሩ ካነ በብኩኝ/ከተነ በበልኝ በኋላ በጥናቱ ብሳተፍ ከኔ ምን እንደሚጠበቅ ተረድቻለሁ፡ ፡	□
2. በጥናቱየ ምሳተፍበፈቃዴሲሆን፤ የ ምሳ ጡውሚ ጃበጥብቅእንደሜቋማጥተረድቻለሁ፡ ፡	□
3. በጥናቱ መቀጠል ካልፈለኩኝ በማ ኛውምግዜ ሜቋረጥእንደምቸል ተረድቻለሁ፡ ፡	□
4. የ ሄፓታይተስ ቢ ቫይረስ፡ ኤች ኣይ ቪ ቫይረስ ና የሳንባ ነቀርሳ ምንነትና ለጥናቱ የሚያን ናሜኛ እንዴት እንደምሳጥገለጻ ተደርጎልኛል፡ ፡	□
5. በጥናቱ በማሳተፌየ ሜርስብኝ ጉዳት እንደሌለ ጢያቂዬ አስረድቶኛል፡ ፡	□
6. ያለኔ ፈቃድየ ጥናቱ ውጡት የ ግል ሚጃዎቼን ይፋ እንደሚደረግ ተረድቻለሁ፡ ፡	□
7.በጥናቱ በማሳተፌ ምንም አይነት የገንዘብ ወጪይሁን ገቢ እንደሌለኝ ከጢያቀዬ ተረድቻለሁ	□
8.በአሰራሩ ላይ ችግር ባይበት ለአዲስ አበባ ዩኒቨርሲቲ ጡፍ ሳይንስ ኮሌጅ የሕክምና ላብራቶሪ ሳይንስ ትምህርት ክፍልና የቅዱስ ጴጥርስ ስፔሻላይዝድ ሆስፒታል ማሳወቅ እንዳለብኝ ተረድቻለሁ፡ ፡	□

ከላይ የተዘረዘሩት ሚጃዎች ተረድቼ በጥናቱ ለማሳተፍ የተስማማሁ ሚሆኔን በፊርማዬ አረጋግጣለሁ፡ ፡ ፊርማ ----------ቀን: ----------/--------/2019 ስልክቁጥር / ካለ/: ----------

እኔ ካሓሲት ገ/ህይወት የ ጥናቱ ተሳታፊዎች ደህንነት ማበት የተጠበቀ ሚሆኑን በፊርማዬ አረጋገጣለሁ፡ ፡

ፊርማ.....
የጥናቱባለቤት፡ - ካሓሲትገ /ህይወት
ስልክቁጥር፡ -+251914127178
የስራአድራሻ፡ - የቅዱስ ኤጥርስ ስፔሻላይዝድ ሆስፒታል አዲስ አበባ ኢትዮጲያ
ፊርማ---------- ቀን: -------/------/2019

12.5. Annex V: Questionnaires

Direction

The purpose of this questionnaire is for a research paper to collect Socio demographic data & associated risk factors to determine the prevalence and associated risk factors of hepatitis B virus & Human immuno deficiency virus among individuals with presumptive TB.

For data collectors: please circle on the answer choice or write on the space provided.

Identification Code _____
A. Socio- demographic data

No	Question	Response Option
1	Sex	1. Male 2. Female
2	Age	……
3	Residence	1. Urban 2. Rural
4	Current occupational status	1.Driver 2. Merchant 3. Government employee 4. Jobless 5. Student 6. Farmer 7. Other specify ….
5	Educational status	1. Can't write and read 2. No formal education 3. Grade 1-4 4. Grade 5-8 5. Grade 9-10 6. Grade 11-12 G. collage 7. University
6	Marital status	1. Single 2. Married 3. Widowed 4.Divorced 5. Separated
7	Estimated monthly income (in birr)	……..

B. Associated risk factors of hepatitis B and HIV viruses infection

No	Question	Response Option
1	Have you ever had needle stick or sharp injury ?	1. Yes 2. No
2	Have you ever had surgery?	1. Yes 2. No
3	Have you ever had traditional tooth extraction ?	1. Yes 2. No
4	Have you ever had tattooing?	1. Yes 2. No
5	Have you ever had unsafe injection ?	1. Yes 2. No
6	Have you ever had body piercing ?	1. Yes 2. No
7	Do you have Sharing scissors with others?	1. Yes 2. No
8	Have you ever had unsafe Abortion?	1. Yes 2. No
9	Do you have history of Organ transplantation ?	1. Yes 2. No
10	Do you have Sharing tooth brushes with others ?	1. Yes 2. No
11	Do have history of Circumcision?	1. Yes 2. No
12	Have you ever had history hospital admission?	1. Yes 2. No
13	Have you ever had blood transfusion?	1. Yes 2. No
14	Have you ever-encountered sexually transmitted infection (STIs)?	1. Yes 2. No
15	Have you ever had history of chronic infections ?	1. Yes 2. No
16	Do you have taken Alcohol more than ones per a week?	1. Yes 2. No
17	Do you have inject intra venus drugs?	1. Yes 2. No
18	Do you have household contact with Hepatitis B virus infected person?	1. Yes 2. No
19	Do you have household contact with Human immuno deficiency virus infected person ?	1. Yes 2. No
20	Do you have regular sexual partner?	1. Yes 2. No
21	Do you have experienced sexual contact with multiple sexual partner?	1. Yes 2. No

Participant's signature _____ **Date** _____

12.6. Annex VI: Laboratory request & result form

AFB and Gene-X pert Laboratory request & Report form

Identification Code:	Medical Record No:	Sex:	Age:
Region:	Woreda:	Phone No:	OPD :
Specimen Type:	Date of Sample Collection:		
Lab Sample ID:	Date & time for Result report:		
Sample Quality :			

Results

AFB Microscopy				
Result	ZN		FM	
	spot	spot	spot	spot
Negative				
Scanty				
+1				
+2				
+3				

Gene X pert Rif assay					
	MTB detected	MTB not detected	Rifampicin resistance	Rifampicin sensitive	Indeterminate
MTB					

Comment:
_____
_____

Reported by_____ Signature _____ Date _____
Reviewed By_____ Signature _____ Date _____

Laboratory request form for HBsAg ELISA test

Identification Code :	Medical Record No:	Sex:	Age:
OPD:	Phone number:		

Test Requested	**Result**		**Remark**
HBsAg ELISA	Positive	Negative	

Reported by_____ Signature _____ Date _____
Reviewed By_____ Signature _____Date _____

12.7. Annex VII: Principle & Procedure of tests

12.7.1. Principle & Procedure for HBsAg ELISA

A) HBsAg ELISA test principle

The sample is incubated in microwell strips pre-coated with monoclonal antibodies specific to HBsAg. A secondary antibody conjugated with horseradish peroxidase (HRP) is then added to the sample in the well. During the two incubation steps any HBsAg present in the sample is bound to the well in an antibody-antigen-antibody-enzyme complex. In the absence of HBsAg no conjugate will be bound. After washing to remove sample and unbound Conjugate, a solution containing tetramethylbenzidine (TMB) and hydrogen peroxide is added to the wells.

Wells which contain HBsAg and hence bound Conjugate will develop a purple colour which is converted to orange when the enzyme reaction is terminated with sulphuric acid [Test kit leaflet].

B) Test Procedures for HBsAg ELISA

1. Prepare Substrate Solution and Wash Fluid.
2. Use only the number of wells required for the test.
3. Add 25 µl of Sample Diluent to each well. **25 µl**
4. Add 75 µl of Samples or Controls to the wells. **75 µl**

To each plate add 75 µl of the Negative Control to wells A1 and B1 and 75 µl of Positive Control to well C1. Add the Controls to the designated wells after dispensing the samples.

5. Cover the plate with a lid and incubate for 60 minutes at 37°C ± 1°C. **60 mins**
6. Add 50 µl of Conjugate to each well. **50 µl**
7. Shake the plate using a plate shaker for 10 seconds or manually agitate by gently tapping the sides for 10 seconds. **10 secs**
8. Cover the plate with the lid and incubate for 30 minutes at 37°C ± 1°C. **30 mins**
9. At the end of the incubation time wash the plate 5 times. **5 washes**

After washing is completed invert the plate and tap out any residual Wash Fluid onto absorbent paper.

10. Immediately after washing the plate, add 100µl Substrate Solution to each well.**100 µl**
11. Cover the plate with the lid and incubate for 30 minutes at 37°C ± 1°C while colour develops. **30 mins.** A purple colour should develop in wells containing reactive samples.
12. Add 50 µl Stop Solution to each well. **50 µl**
13. Within 15 minutes read the absorbance of each well at 450 nm using 620 to 690 nm as the reference wavelength if available. **$A_{450/Ref}$**

Interpretation of results

- **Non Reactive Results**

Samples giving an absorbance less than the Cut-off Value are considered non-reactive.

- **Reactive Result**

Samples giving an absorbance equal to or greater than the Cut-off Value are considered initially reactive in the assay. Such samples should be retested in duplicate using the original sample source. Samples that are reactive in at least one of there-tests are presumed to contain HBsAg. Samples that are non-reactive in both wells on retest should be considered non-reactive.

12.7.2. Principle & procedure for HIV test

12.7.2.1. Principle & procedures of HIV 1/2 STAT-PAK™ ASSAY

A) Test Principle

The Chembio HIV 1/2 STAT-PAK™ Assay employs a unique combination of a specific antibody binding protein which is conjugated to colloidal gold dye particles and HIV-1/2 antigens which are bound to the solid phase membrane. The venous or capillary (finger stick) whole blood, serum or plasma is applied to the sample (S) well of test device followed by the addition of Running Buffer. The Buffer facilitates the lateral flow of the specimen and test reagents and promotes the binding of the antibodies to the antigen. The specimen/buffer mixture migrates along the test strip by capillary action, reconstituting the conjugate. If present, the antibodies bind to the colloidal gold conjugated antibody binding protein. In a reactive sample, the dye conjugated-immune complex migrates on the nitrocellulose membrane and is captured by the antigens immobilized in the test (T) area producing a pink/purple line. In the absence of HIV-1 and HIV-2 antibodies, there is no pink/purple line in the test (T) area. The sample continues to migrate along the membrane and produces a pink/purple line in the control (C) area containing immunoglobulin G antigens. This procedural control serves to demonstrate that specimen and reagents have been properly applied and have migrated through the device .

B)Test Procedure

1. Remove the Chembio HIV 1/2 STAT-PAK™ 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. 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.
4. Invert the Running Buffer bottle , hold it vertically & add 3 drops (~ 105 µL) of buffer slowly, drop wise, into the SAMPLE (S) well.
5. Read the test result between 15 and 20 minutes after the addition of the Running Buffer. Reactive Test Results may be observed and read earlier than 15 minutes. To verify a Non reactive test result, wait the entire 15 minutes after starting the test. Do not read results after 20 minutes.

Interpretation of test results

Non reactive:

One pink/purple line in the control (C) area, with no line in the test (T) area indicates HIV-1 and HIV-2 antibodies were not detected in the specimen. The test result is negative.

Reactive:

Two pink/purple lines, one in the test (T) area and one in the control (C) area indicate HIV-1 and/or HIV-2 antibodies have been detected in the specimen. The Test Result is interpreted as Preliminary positive for HIV-1 and/or HIV-2 antibodies.

Invalid: If there is no distinct pink/purple line visible in the control (C) area.

12.7.2.2. Principle & procedures of ABON HIV 1/2/O Tri-Line Human Immunodeficiency Virus Rapid Test Device

A) Test Principle

The HIV 1/2/O Tri-line Human Immunodeficiency Virus Rapid Test Device test strip is pre-coated with HIV-1 and subtype O antigens on T1 test line and HIV-2 antigen on T2 test line. Firstly, specimen and then buffer is added to the specimen well, thus starting the migration of the specimen/buffer. The specimen/buffer passes the conjugate pad which contains a mixture of HIV-1 envelope and capsid antigens and HIV-2 envelope antigen. These detection antigens are conjugated to latex particles. If present, the HIV-1 of HIV-2 antibodies react and bind to the detection antigen-conjugate. The antibody/antigen-conjugate mixture then migrates further and binds to antigens present on the test lines. If the specimen contains antibodies to HIV-1, the specimen will bind to the T1 test line and produce a line, if specimen contains antibodies to HIV-2, the specimen will bind to the T2 test line. As liquid continues to migrate down the test strip, the control line will appear. If the control line is present, in addition to either or both test lines, then the test is reactive for HIV1/2 antibodies. If the specimen does not contain HIV-1 or HIV-2 antibodies, no colored lines will appear for either of the test lines region indicating a non-reactive result. Please note that the appearance of colored lines at T1 and T2 is highly unlikely to be indicative of co-infection with HIV-1 and HIV-2 but rather is a result of cross-reactivity between antigens. A colored line will appear in the control line region if the migration of liquid has been successful and must be present for the test to be valid. Its presence does not confirm sufficient specimen addition.

B) Test Procedure

1. Remove the test device from the foil pouch &use it as soon as possible.
2. Place the test device on a clean & level surface. Label with the specimen ID.

For **serum** or **plasma** specimens: Hold the specimen dropper vertically & transfer 1 drop of serum or plasma (approximately 25 µL) to the specimen well then, add 1 drop of buffer (approximately 40 µL) and start the timer.

For **whole blood** specimens: Hold the specimen dropper vertically & transfer 2 drop of whole blood (approximately 50 µL) to the specimen well then, add 2 drop of buffer (approximately 80 µL) and start the timer

3. Wait for the colored line(s) to appear. Read results at 10 minutes. Don't read results after 20 minutes.

Interpretation of results

Non-reactive for HIV-1 & HIV-2 antibodies: One colored line appears in the control region C and no apparent colored lines in the test line regions T1 and T2.

Reactive for HIV-1 & HIV-2 antibodies: Two or three distinct colored lines appear, one on the control line 'C' and other one or two colored lines in the test line region(s) T1 and or T2.

Reactive for HIV-1 antibodies: Two distinct colored lines appear, one in the control line 'C' and one other colored line in the test region T1.

Reactive for HIV-2 antibodies: Two distinct colored lines appear, one in the control line 'C' and one other colored line in the test region T2.

Invalid: Control line fails to appear in control region, even if colored lines appear in any of the test regions T1 or T2

12.7.2.3. Principle & procedures of SD BIOLINE HIV-1/2

A)Test principle

SD BIOLINE HIV-1/2 3.0 contains a membrane strip, which is pre-coated with recombinant HIV-1 capture antigen (gp41, p24) on test line "1" region and with recombinant HIV-2 capture antigen (gp36) on test line "2" region, respectively. The mixture (recombinant HIV1/2 antigen (gp41, p24 and gp36) - colloid gold conjugate and the specimen moves upward on the membrane chromatographically to the test region (T) and form a visible line as the antigen antibody-antigen gold particle complex forms with high degree of sensitivity and specificity. This test device has the letter of 1, 2 and C as Test Line 1 (HIV-1), Test Line 2 (HIV-2) and Control Line on the surface of the device. All test lines and the control line in the result window should not be visible before applying any sample. The control line is used as a procedural control for the addition of reagents and may still appear if no specimen is added to the test device.

B) Test Procedure for SD BIOLINE HIV-1/2

1. Remove the test device from foil pouch, place it on a flat ,dry surface.
2. Add 20 µL of blood or 10 µL of serum or plasma specimen in to the sample well(s).
3. Add 4 drops (approximately 120 µL) assay diluent vertically in to the sample wells.
4. As the test begins to work , see purple color move across the result window.
5. Time to result is 10-20 minutes. After adding the diluent, read the result after 10 minutes but not more than 20 minutes.

Interpretation of results

Non-reactive for HIV-1 & HIV-2: Presence of only control line (C) within the result window.

Reactive for HIV-1 antibodies: Presence of two lines as control line (C) and test line 1 (T) within the result window .

Reactive for HIV- 2 antibodies: Presence of two lines as control line (C) and test line 2 (T) within the result window.

Reactive for HIV- 1 and 2 antibodies: Presence of three lines as control line (C), test line 1 (T) and test line 2 (T) within the result window.

Invalid: **No presence of control line (C) within the result window**

12.7.3. Principle & Procedures of Ziehl-Neelsen staining method

A) Test principle

The property of acid-fastness is based on the presence of mycolic acids in the cell wall of mycobacterium. Primary stain (fuchsin) binds to cell-wall mycolic acids. Intense decolourization (strong acid or acid/alcohol) does not release the primary stain from the cell wall and the mycobacterium retain the red colour of fuchsin hence acid-fastness. Counterstaining with methylene blue provides a contrasting blue background to see the red mycobacterium.

Reagents

- **1% Carbolfuchsin staining solution**
- 3% Acid- alcohol
- 0.1% Methylene blue counterstaining solution

quality Control

Positive and negative control slides should have stained with the sample to check problems associated with staining solution preparation, staining procedure or reagent used for staining solution preparation.

B) ZN staining procedures

1. Cover the whole surface of the slide with filtered Ziehl's 1% carbol fuchsin solution.
2. Heat the slide until steam rises from the stain &leave it for 5 minutes.
3. Wash the staining solution off with running water & tilt to drain excess rinse water.
4. Cover the whole slide with acid solution (3% Acid Alcohol) & leave it for about 3 minutes. Repeat the procedure until the solution runs clear.
5. Cover the slide with 0.1% methylene blue solution. Leave it for 1 minute. Wash the solution off with running water & tilt the slide to drain excess water. Place the slide on the slide rack upright to dry in the air.
6. Apply one drop of oil immersion, use 10X Objective to focus the first smear carefully rotate to 100X objective & examine at least 100 high power fields (one length) before recording a negative result.

Result interpretation

Observation	Reporting
No AFB found in at least 100 fields	**Negative**
1-9 AFB in100 fields	**Record exact number of bacilli**
10 - 99 AFB per 100 fields	**1+**
1 to 10 AFB per field (check 50 fields)	**2+**
More than 10 AFB per field (check 20 fields)	**3+**

12.7.4. Principle & procedures of auramine O staining method

A) Test Principle

Acid-fast mycobacteria resist decolorization by acid-alcohol after primary staining owing to the high lipid (mycolic acid) content in their cell walls. The identification of mycobacteria with auramine O is due to the affinity of the mycolic acid in the cell walls for the fluorochromes. The dye will bind to the mycobacteria, which appear as bright yellow, luminous rods against a dark background. The potassium permanganate (KMnO4) helps prevent non-specific fluorescence. All acid-fast organisms will be stained by auramine O, including some parasites. The fluorochromes stains are recommended for specimen examination because of their increased sensitivity and speed.

Reagents

- 0.1% Auramine O
- 0.5Acid Alcohol
- 0.5 KMnO4

Quality control

sputum smears of known 1+ or Scanty for positive control and one negative slide stained with routine smears to check that the reagent, staining method and the microscopically examination of smears is satisfactory.

B) Staining procedure of auramine O staining method

1. Cover the whole surface of the slide with 0.1% Auramine O filtered solution & leave it for 20 minutes.
2. Wash the staining solution off with running water ;drain excess water from the slide
3. Cover the whole slide with 0.5% Acid Alcohol; leave it for about 3 minutes.

4. Wash the solution off with running water; tilt the slide to drain off excess rinse water.
5. Cover the slide with 0.5% KMnO4 solution ; leave it for 1 minute.
6. Wash the solution off with running water; drain excess water.
7. Place the slide on the slide rack upright to dry in the air. Apply one drop of oil immersion, use the objective 20-25x for scanning and 40x for confirmation. One length has to be scanned before reporting a negative, corresponding to 300-200 high-power fields and taking 1-2 minutes (20x – 40 x objectives).

Result interpretation

Fluorescence;200-250xmagnification; one length=30 fields=300HPF	Fluorescence;400xmagnification; one length=40 fields=200HPF	Result
Zero AFB/1 length	Zero AFB/1 length	No AFB seen
1-29 AFB/1 length	1-19 AFB/1 length	scanty
30-299 AFB/1 length	20-199 AFB/1 length	1+
10-100 AFB/1 field on average	5-50 AFB/1 field on average	2+
>100 AFB/1 field on average	>50AFB/1 field on average	3+

12.7.5. Principle & procedures of Gene Xpert MTB/RIF assay

A)Test Principle

The Xpert MTB/RIF assay consists of a single use multi chambered plastic cartridge preloaded with the liquid buffers and lyophilized reagent beads necessary for sample processing. The PCR assay detect MTB DNA in sputum samples & rifampicin resistance associated mutations of the rpoB gene. The internal control hemi nested B. globigii assay is multiplexed with the M. tuberculosis assay. M. tuberculosis is detected using five overlapping molecular beacon probes (probes A to E) that are complementary to the entire 81-bp RIF resistance determining core region of the wild type rpoB gene. Mutations in the rpoB gene target inhibit hybridization of one or more of the rpoB specific molecular beacons, reducing or eliminating the signal from the corresponding probes.

Sample preparation:

The sputum samples are treated with sample reagent (SR). The sample reagent contains NaOH and isopropanol. The SR is added at a 2:1 ratio to the sputum sample and incubated for 15 minute at room temperature. Shake the container once during the 15 minute incubation i.e. after 10 minutes. At the end of 15 minutes if the sample is still viscous, shake the sample again and leave it for another 5 min until it is properly liquefied. This mixture can be kept for up to 8 hours at 2-8ºC, in case repeat testing is required.

>2ml of the treated sample is transferred into the cartridge, the cartridge is loaded into the GeneXpert instrument, and an automatic process completes the remaining assay steps.

B) Procedure of Gene Xpert MTB/RIF assay

1. Switch on the GeneX-pert system at the back of the instrument & switch on the computer.
2. Double click 'GeneX-pert' icon on desktop and log onto the GeneX-pert Dx software using your username and password.
3. Click on 'Create test' on the GeneX-pert system toolbar and enter the sample ID barcode in the 'Sample ID Barcode' dialog box, then select 'OK'.
4. The 'Scan barcode' dialog box appears. Scan in the barcode of the MTB/RIF cartridge by placing the X of the barcode scanner in line with the barcode on the cartridge and hold until it beeps. Otherwise select 'Manual Entry' and type in the Cartridge barcode. Once it has been correctly entered, select 'OK'
5. The software automatically fills in the Reagent lot ID, Cartridge SN, and expiration date, as well as Select Assay, Assay version number, Test Type and Sample type.
6. Click on 'Start Test'.
7. In the dialog box that appears type in your password again and presses 'ENTER'.
8. A green light will start flashing above the empty module. Open the instrument module door with the blinking light and load the cartridge.
9. Firmly close the module door till you hear a click, After a few seconds, the green light will stop blinking indicating that the test has started

10. After completion of the run, the green light will switch off and the module lid will open automatically ejecting the cartridge ,remove the cartridge and dispose it in a suitable biohazard waste bin.
11. Click on the 'View Results' icon on the system toolbar, Click on 'View Test' at bottom of the result screen toolbar, Select the client test by clicking on the patient ID field ,then Click 'OK' and the result screen will be displayed.

Result Interpretation

- MTB detected – MTB target DNA is present hence the patient is TB positive
- MTB not detected – MTB target DNA is was not detected
- RIF Resistance detected - MTB present is resistant to Rifampicin
- RIF Resistance not detected – MTB present is sensitive to Rifampicin

12.8. Annex VIII: Thesis Declaration

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

Name of the principal investigator:

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Signature _____Date_____

This thesis has been submitted with my approval as advisor.