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BURDENOF SMEAR NEGETIVE PULMONARY TUBERCULOSIS USING GENEXPERT ANDCULTUREMETHODS AMONG HUMAN IMMUNODEFICIENCY VIRUS INFECTED PATIENTS IN TULUBOLOAND St. LUKEHOSPITALS, ETHIOPIA

By: KinduGetnet

Advisors: KasuDesta (MSc,PhD, fellow, Associate Professor)

RegasaDiriba(MSc, Lecturer)

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This is to certify that the thesis prepared by **KinduGetnet**, entitled:

BURDEN OF SMEAR NEGETIVE PULMONARY TUBERCULOSIS USING GENEXPERT AND CULTURE METHODSAMONG HUMAN IMMUNODEFICIENCY VIRUS INFECTED PATIENTS IN TULUBOLO AND St. LUKE HOSPITALS SOUTH WEST SHEWA, ETHIOPIA and submitted in partial fulfillment of the requirements for Master of Science degree in Clinical Laboratory Sciences (diagnostic and public health microbiology) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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Advisor_____ Signature _____Date _____

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List of Abbreviations

AFB----------Acid fast bacilli

ART----------Anti retro viral therapy

Bp----------Base pair

CD----------Cluster of differentiation

CXR----------Chest x-ray

DR-TB----------Drug resistant tuberculosis

DST----------Drug susceptibility test

FM----------Florescence microscope

FMOH----------Federal ministry of health

HIV----------Human immunodeficiency virus

ID----------Identification

IQR----------Inter quartile range

LED ----------Light emitted diode

LJ----------Lowenstein Jensen

LPA----------Line probe assay

MDR-TB-------Multi drug resistant tuberculosis

MIGT ----------Microbial indicator growth tube

Ml----------Millilitre

MTB DNA-------Mycobacterium tuberculosis deoxyribonucleic acid

MTB/Rif----------Mycobacterium tuberculosis rifampicin

MTBC----------Mycobacterium tuberculosis control

M-TB----------Mycobacterium tuberculosis

NAATs ----------Nucleic acid amplification tests

NAOH----------Sodium hydroxide

NRLM----------National reference laboratory management

NTM----------Non tuberculosis mycobacterium

PCR----------Polymerase chain reaction

PLHIV----------People living with human kimono deficiency virus

PPE----------Personal protective equipment

PTB----------Pulmonary tuberculosis

QC----------Quality control

RIF/DR ----------Rifampicin drug resistance

SPC----------Sample processing control

ST----------Saint

TST----------Tuberculin skin test

WHO----------World health organization

XDR-TB----------Extensive drug resistance tuberculosis

ZN----------Ziehl-Neelsen

Abstract

Background: Smear negative pulmonary tuberculosis(SNPTB) leads to a serious disease to human immunodeficiency virus infected patients. Therefore knowing the correct diagnostic method improves early detection of the disease.The burden of SNPTB is not known in the study area .Therefore, this study aimed to determine the burden of TB, MDR-TB, in Tulu bolo and St. Luke hospitals,south west shewa Ethiopia.

Objective: To determine the burden of SNPTB using genexpert and culture methodsamonghuman immunodeficiency virus infected patients on ART follow up at Tulu bolo Generaland St. Lukas Hospitals in 2012 E.C.

Methods: A total of **197**informed and consented HIV patients with suspected pulmonary tuberculosis who were presented with pulmonary tuberculosis sign and symptoms of at least 2 weeks or more were screened by FM smear microscopy. All **197 HIV** patients' bothsmear negative and smear positive pooled sputum samples were further tested by gene expert in Tulu bolo general Hospital and St. Luke Hospitals. Simultaneously all smear negative patients and smear positive patients were allowed to give samples which were sent to EPHI for MTB culture (LJ and MGIT), and LPA test.All drug resistant isolates were tested by using Genotype MTBDR plus Ver.2 and first- line MGIT DST.Then the result was collected and data analysis was performed by the use of statistical software epiinfo version 7.2 and SPSS version 23. The study was conducted from December 1, 2019 to February 30, 2020

Results: From the total of 197 enrolled HIV patients, 5/197(2.54%)were smear positive,19/192 (9.9%) were Xpert MTB/ RIF and culture confirmed smear negative PTB patients. The positivity rate among male and female was 10/19 (52.6%) and 9/19(48.4%), respectively. From 19 culture positive isolates, 1 (5.26%) was MDR-TB (resistant to both Rifampicin and Isoniazid); this patient was a female patient under retreatment. Among 19 SNPTB cases14/19(73.68%) patients viral load status were greater than 2001.and about 12/19(63.12%) of 19 SNPTB patients CD4cell result were less than 200 cells per millimetre cube

Conclusion: In this study a significant proportion of smear negative pulmonary TB was diagnosed. Further, a smear negative multi drug resistant (MDR) TB was confirmed. Due to the

limitations of smear microscopy which is used as a primary diagnostic tool, these TB strains are missed to be diagnosed, mortality in HIV patients and transmission in the communitycontinues.

Keywords:ART Patient, Smear negative patient, Detection,Culture,Genexpert, FMmicroscopy, burden

1. Introduction

1.1 Background

TB is an infectious disease caused by the bacillus Mycobacterium tuberculosis(1). It typically affects the lungs (pulmonary TB) but can also affect other sites (extra pulmonary TB) (2). The disease is spread when people who are sick with pulmonary TB expel bacteria into the air(3, 4).A relatively small proportion (5–10%) of the estimated 1.7 billion people infected with M. tuberculosis will develop TB disease during their lifetime. However, the probability of developing TB disease is much higher among people infected with HIV it is also higher among people affected by risk factors such as under nutrition, diabetes, smoking and alcohol consumption(1, 2).Worldwide TB is one of the top 10 causes of death and the leading cause from a single infectious agent. Millions of people continue to fall sick with TB each year (5).

In 2017 TB caused an estimated 1.3 million deaths. Globally 9% were people living with HIV (72% in Africa) According to global tuberculosis report of 2018 About 1.7 billion people 23% of the world's population are estimated to have a latent TB infection and are thus at risk of developing active TB disease during their lifetime(1,2).

According to the Global TB Report 2017, 10.6 million people are estimated to have fallen ill with TB in 2016 while an estimated 1.3 million people died of TB. In addition, an estimated 4.1% of these new TB cases and 19% of the previously treated cases are believed to harbour Drug resistant-TB with an estimated 240 000 deaths annually due to Drug resistant-TB (6).

Mycobacterium tuberculosis (MTB) and human immunodeficiency virus (HIV) co-infection is one of the major public health burdens globally (7, 8).According to the World Health Organization (WHO) nearly 15% of individuals diagnosed with MTB were HIV positive and a total of 360,000 deaths were attributed to MTB-HIV co-infection alone. Clinical evidence suggests that MTB is the most common opportunistic infection causing exacerbation of viral load and diminished CD4 count in HIV patients. It also remains the leading cause of death in patients with Acquired Immunodeficiency Syndrome. Reciprocally by decreasing the body's cell mediated immunity HIV increases the risk of MTB progression and reactivation of latent TB infection. HIV co-infection can alter the pathogenesis of MTB and lead to negative sputum

smear results atypical radiographic manifestations and extra pulmonary manifestations which poses difficulty in diagnosing MTB disease(9 ,10).

Mycobacterium tuberculosis (MTB) infection in human immunodeficiency virus (HIV)-infectedpatients is often a challenging diagnosis owing to atypical chest x-ray film patterns, prominent extra pulmonary manifestations, negative tuberculin skin tests, and the presence of concomitant opportunistic infection (11).

HIV-infected people are at markedly increased risk for primary or reactivation tuberculosis and for second episodes of tuberculosis from exogenous reinfection (12).Susceptibility to tuberculosis is related to the pattern of cytokines produced by T lymphocytes. T1 lymphocytes, which produce interferongama, are central to anti mycobacterial immune defences, and fatal mycobacterial disease develops in children who lack the interferon gama receptor. In contrast to T1 lymphocytes, T2 lymphocytes, which produce interleukin-4 and interleukin-10, do not contribute to anti-mycobacterial immunity. The reduced T1 response in HIV-infected patients contributes to their susceptibility to tuberculosis. Exposure of alveolar macrophages and lymphocytes from HIV-infected patients to M. tuberculosis in vitro up-regulates retroviral replication. Pleural fluid from patients with tuberculosis increases HIV replication in activated lymphocytes, and in HIV-infected patients with pulmonary tuberculosis, the concentrations of retroviral RNA in broncoalveolar-lavage fluid are highest in areas of tuberculosisinvolvement. Tuberculosis probably increases HIV replication by inducing macrophages to produce tumour necrosis factor a, interleukin-1, and interleukin-6(13 ,14).

The WHO has classified Ethiopia among the 22 high burden countries with TB. In 2011, it was estimated that in Ethiopia the annual TB incidence was 258/100,000, the prevalence of all forms of TB was 237/100,000 and the TB mortality rate was 18/100,000(15).

Ethiopia has notified 125,836 new TB cases and enrolled 702 drug-resistant TB case in 2016. Although the majority of TB cases has affected the productive age group, 15 100 (12%) cases were reported among children aged under 15 year. HIV co-infection impedes the TB control efforts contributing to around 8% of annually notified TB cases (6).

In 2011 the adult HIV prevalence in Ethiopia was 1.5%(16).The prevalence of HIV in newly diagnosed patients with TB was 17% (15). TB and HIV co-infection presents special diagnostic

and therapeutic challenges and constitutes an immense burden on healthcare systems of countries with high prevalence (15, 16). In patients with HIV, especially patients with low CD4 T cell counts, TB is more difficult to diagnose because they often present with smear negative pulmonary TB and extra pulmonary TB (17). This contributes to low case detection rates and TB diagnosis delay (18).Over the decade, the TB control efforts have further stretched the growing health infrastructures with the efforts to detect, diagnose and managing drug resistant form of TB. Emergence of Tuberculosis that is resistant to essential TB medicines (i.e. Rifampicin with or without Isoniazid) has further impeded the promising gains to control TB epidemic. Though exact estimation of the level of DR-TB is missing, 2.7% of New TB cases and 14% previously treated TB cases notified in the preceding reporting year are used approximate the burden(18).

There is a no data on the burden of smear negative pulmonary tuberculosis, smear negative multidrug resistance tuberculosisamong HIV patients in South west shewa ,Oromia region, Ethiopia. Therefore, the aim of this study was to determine the Burden of tuberculosis, multidrug resistance tuberculosis among HIV patients in the above study area.

1.2 Statement of the Problem

Smear negative pulmonary tuberculosis is defined as being three sputum smear negative but genexpert or culture positive. Although patients with HIV-associated TB mostly have typical clinical pictures, the frequency of atypical manifestations is increased in states of advanced immunosuppression, thus making the diagnosis more difficult(19).

In the immunosuppressed AIDS patient, the clinical features of TB tend to be non-specific, with a predominance of systemic symptoms and other atypical presentation therefore the diagnosis of TB in patients infected with HIV presents certain difficulties depending on the degree of immunosuppression at the time of diagnosis. Smears are negative more often because cavities are less common the organism load in tissues is higher than in individuals who are not infected with HIV .Due to this, Smear negative pulmonary tuberculosis occurs more commonly in HIV patients(20).

Smear-negative pulmonary TB is associated with poor treatment outcomes, including death due to delayed diagnosis or non-diagnosiswhile recommended by the World Health Organization (WHO) for diagnosis of AFB smear-negative TB in HIV-infected patients; sputum culture is still not routinely available at the district-level(21).

Despite the initial clinical suspicion of TB, when a patient's sputum smear results are negative for AFB the diagnosis of TB may be missed. For those patients with a high clinical suspicion, clinicians must face the dilemma of empirically treating or waiting for up to 8 weeks for the final culture results(22).

A problem of patient in delayed diagnosis and treatment, results in possible transmission of TB even though smear negative pulmonary TB is less infectious than smear positive TB patients. In the Netherlands patients with smear-negative culture-positive pulmonary TB are responsible for 13% of TB transmissions(23).

Due to absence of Early smear negative TB diagnosis thechainof transmission of TB disease increases. The studies also reported that approximately 17% of TB disease transmission is caused by AFB smear microscopy– negative TB suspects(24).

Although the burden of SNPTB has been reported in very limited places in Ethiopia, I believe that the data is not quite enough, and in the study area this data is lacking. Having this information in the study area will help us to understand the burden of SNPTB and fill the gaps seen in the two hospitals. It will also contribute for generating more data at national level

1.3. Significance of the study

Considering smear negative mycobacteria tuberculosis pose burden on the health of the community and the country it is necessary to find burden of SNPTBthat will benefit patients, health care workers and policy makers in general.

It will be the researchers hope that the finding this thesis will provide information for the selection of the best TB diagnostic test as country and the best input for Regional health bureau and FMOH to select and distribute the diagnostic test which detect most than the routine test method used in the country. The finding of this research also will use as an input for the health policy of the country which is focus on disease prevention and control, especially communicable disease.

2. Literature Review

2.1 Detection rate of Diagnostic Methods in Smear Negative TB

According to World Health Organization (WHO) report of 2014 on genexpert,Sputum smear microscopy has a particularly low sensitivity for detecting TB among PLHIV. This is because people in later stages of HIV infection and with compromised immune systems often release fewer organisms into their sputum, at concentrations below the threshold for visual detection under a microscope. For PLHIV with a negative smear microscopy result but who are still presumed to have TB, bacterial culture has been the only diagnostic option. However, culture can only be undertaken at central level laboratories, and results are generally available after a number of weeks or months. TB culture is therefore not good enough for PLHIV, who need a speedy TB diagnosis and prompt treatment.GenexpertMTB/RIF should thus be made widely available as the initial diagnostic test in HIV clinics and HIV prevalent settings, as it is a rapid, simple and highly sensitive(25) .

Another report of The World Health Organization onGlobaltuberculosis control in 2014 stated that Smear-negative pulmonary TB (SNPT) represents 30–60% of all pulmonary TB cases, according to region. In Brazil, 24–30% of cases of pulmonary TB in adults are SNPT. The mortality of these patients can reach 25% in populations with high prevalence of HIV infection, which may be largely related to delay in diagnosis .Furthermore, although smear-positive patients are considered to be more infectious, 10–20% of TB transmissions at the population level are attributable to SNPT cases(26).

A systemic review done on Diagnosis of smear-negative pulmonary tuberculosis in people with HIV infection in resource-constrained settings .shows that overall positive rate of a single smear microscopy ranges between 22% and 43.Fluorescence microscopy increases the probability of detecting acid-fast bacilli, especially if the sputum contains few bacteria, and hence improves the sensitivity of microscopy in HIV-positive patients. A systematic reviewof 43 studies that used fluorescence microscopy showed that on average, in comparison with Ziehl-Neelsen microscopy, fluorescence microscopy showed a 10% increase in sensitivity with similar specificity. Culture is the gold standard for the diagnosis of tuberculosis a positive result in solid or liquid medium needs 10–100 viable bacteria per mL of sputum.According to this systemic analysis in South

Africa, 49% of patients on tuberculosis treatment had negative smears on direct microscopy but their sputum cultures were positive(27).

On the systematic review and meta-analysis on Microscopic observation drug susceptibility assay for the diagnosis of TB and MDR-TB in HIV-infected patients: the sensitivity and specificity of 10 studies including 3075 samples were analysed. Overall diagnostic accuracy of MODS for the diagnosis of TB was a sensitivity of 88.3% and specificity 98.2%. For multidrug-resistant (MDR)-TB, sensitivity was 89% and specificity was 100% .For smear-negative pulmonary TB, a sensitivity of 88.2% and specificity of 98.2% were found(28).

A study conducted in Nepal in Patan Hospital clinics in 2013, on smear-negative patients suggests that the use of genexpert increase the yield of confirmed TB cases. Five smear negative patients need to be tested to detect one TB case by applying simple routine criteria to select smear-negative TB suspects. And the implementation of Xpert therefore resulted in a 28.1% (n = 55/196) increase in confirmed TB cases. As every study performed to date and meta-analysis has indicated consistently the specificity is 99%. The ability of Xpert to rapidly confirm TB in smear-negative cases offers the possibility of improving early TB case detection, but due to costs, recommendations for high burden, low resource settings have so far focused on applying Xpert to HIV-infected individuals (where the sensitivity of smear is especially low and the complications of missed diagnosis are more severe) and patients with risk factors for MDR TB (29).

The study conducted in Pakistan on sputum specimens from presumptive TB cases suggests that the positivity rate for MTB was higher in the case of MTB culture by the LJ medium, since it is a more sensitive method. However, Xpert MTB/RIF assay demonstrated high detection sensitivity for MTB complex in sputum specimens in comparison with conventional AFB smear microscopy. In combination with rapid MTB culture, Xpert MTB/RIF assay will definitely improve the detection rate of MTB bacteria. However, GeneXpert assay is advantageous over the culture technique as the results become readily available and in detecting both the MTB and MDR-TB simultaneously. Furthermore, ZN smear microscopy and culture are laborious and require more time to establish clinical diagnosis of TB. When compared to MTB culture by the LJ medium, GeneXpert MTB/RIF is less sensitive but a technique with more rapid and specific results readily available. In addition, it can detect DR-TB, that is, rifampicin resistance

simultaneously with MTB DNA detection and its quantification. MTB culture by the LJ medium is a time-consuming technique that is, up to 8 weeks, and requires additional 4–6 weeks for the diagnosis of DR-TB, that is, rifampicin resistance. Moreover, GeneXpert MTB/RIF assay is not prone to cross-contamination, requires minimal biosafety facilities, and has a high sensitivity in smear-negative pulmonary TB compared to MTB culture by the LJ method which required personal protective equipment (PPE) and special trainings for laboratory workers and biosafety level III laboratory requirement for its setting. This study concluded that GeneXpert assay has shown a much higher sensitivity for the detection of MTB than ZN smear microscopy in pulmonary samples, while the culture technique is considered as the gold standard for MTB diagnosis but takes a longer time to develop MTB growth colonies and is unable to detect RIF/DR simultaneously. Nevertheless, the GeneXpert assay is considered a valuable diagnostic tool for the rapid detection of MTB in AFB smear-positive as well as smear-negative TB suspects with simultaneous detection of MDR-TB. This early detection facilitates in controlling the disease transmission and timely start of TB treatment therapy, and hence the introduction of GeneXpert assay results in a significant reduction of MDR-TB cases (24).

The study done between September 2009 and May 2010 at Athens, Greece, The main purpose of the study was to assess the effectiveness of the Xpert MTB/RIF assay in testing AFB-negative specimens originating from patients with clinical signs highly indicative of active TB. The finding was Xpert MTB/RIF assay correlate well with those reported by others regarding the effectiveness of the assay in accurately detecting the presence of MTBC bacilli in AFB-negative specimens. The study, performed exclusively or mainly on respiratory samples, the reported sensitivities ranged from 72% to 90%. And the specificity was 100%. Since the assay does not detect specific mutations but deviations from the wild-type sequence, it is expected that in certain cases in the presence of mixed infections or silent mutations, discrepancies would occur. Nevertheless, in the most extensive study published so far, the Xpert MTB/RIF assay correctly detected rifampin resistance in 99.1% of patients(30).

Similar study conducted in Vietnam in 2012 stated that although some authors have suggested clinical symptoms can be significant predictors of smear-negative PTB, neither clinical symptoms nor TST were significantly associated with smear-negative PTB in the study. Among available potential predictors, they found only a decrease CD4+ cell level below 200/mm3 and

an abnormal CXR (TB-CXR) as being significant predictors for PTB in HIV-infected patients having sputum AFB-negative smears. Consistent with other studies, but their data failed to identify neither any single symptom nor combination of symptoms which could be a sensitive predictor for smear negative, culture-confirmed PTB.The WHO has recommended using combination of cough,fever,weight loss, and night sweats as the initial screening tool to exclude active TB cases before initiating preventing treatment for persons living with HIV in most resource-constrained settings. However, the study shows that symptom screening may miss up to 54% of AFB smear-negative PTB cases among HIV-infected patients (sensitivity = 46% and negative likelihood ratio = 0.85). Results from this study confirm the need of routinely applying sputum culture for HIV-infected patients. These findings strongly suggest the need for rapid and accurate diagnostic tools for detecting resistant TB cases in HIV patients having AFB-negative sputum smears which is GeneXpert MTB/RIF assay(21).

Another study conducted in india in2015 shows that Gene-xpert testing increases the detection of pulmonary tuberculosis cases and it also shows that at least 3.4 patients need to be tested to detect one genexpert positive pulmonary tuberculosis case. These studies shows that more than thirty percent of smear negative patients were diagnosed positive by this method. this study shows that high intensity of infection present in more than half of gene-xpert positive patients. Prevalence of Rifampicin resistance was found in more than ten percent of smear negative patients which is significantly high. Most of the rifampicin resistance was found in patient with high intensity of infection in gene-xpert and patients who received Ant-tubercular treatment in past(31).

In the study conducted in Pakistan to determine the sensitivity and specificity of GeneXpert assay for the diagnosis of Tuberculosis and rapid detection of Rifampicin resistant in Smear Positive & Smear Negative pulmonary specimen. This assay identified 1264 cases of Tuberculosis from sputum smear positive 100% (1210/1210) and 67% (54/809) in sputum negative specimen. Some studies of GeneXpert MTB/RIF assay have reported sensitivity in cases of Smear & Culture positive were 98 - 100% while in cases of Smear Negative and Culture positive were 57to76.9% while the test specificity remain 99 to 100%. In this study overall sensitivity in Smear Positive and Smear negative cases were 91.15 % and specificity were 100%.

The sensitivity of this study were slightly lower and specificity remain same than those with the other studies (32).

Similar research conducted in Cape Town on evaluation of the Xpert MTB/RIF assay as well the on-going development of new molecular-based diagnostics. Ziehl-Neelsen staining for acid-fast bacilli using light microscopy offered a sensitivity of up to 80% in HIV-uninfected individuals, excellent specificity. However, HIV co-infection has increased the incidence of extra pulmonary, disseminated and smear-negative pulmonary disease. Smears are negative more often because cavities are less common the organism load in tissues is higher than in individuals who are not infected with HIV. The sensitivity of routine smear microscopy has dropped to as low as 20%. Nevertheless, in the majority of resource-limited settings smear microscopy continues to be the frontline TB diagnostic tool(33).

2.2. Prevalence and Diagnosis of smear negative tuberculosis in HIV Patients in Ethiopia

In the national TB prevalence survey in 2010/11 smear-negative cases accounted for 57% of culture positive cases. Diagnosis of smear-negative pulmonary TB remains a challenge. This is because of the lack of rapid and accurate diagnostic modalities that can be applied in resource-limited settings. The GeneXpert MTB/RIF assay detects the presence of MTB complex (MTBC) and its resistance to RIF in a single reaction. In clinical evaluation studies the sensitivity of Xpert in patients with smear-negative pulmonary TB was reported to be moderate 55–86% compared with 99–100% in patients with smear-positive TB. The study conducted at Jimma University Specialized Hospital, in 2014 suggests that application of Xpert on a single smear-negative sputum specimen can substantially increase the yield of confirmed TB cases. This is consistent with those reported by others regarding the effectiveness of Xpert in accurately detecting the presence of MTB in smear-negative specimens. The observed sensitivity of a single Xpert (63.2%) for smear-negative TB is comparable with reported sensitivities ranged from 61% to 71.7% (34).

A study conducted in Addis Ababa St. peters hospital on Prevalence of smear negative pulmonary tuberculosis, between November 2014 and October 2015 suggests that 247/297 (83.2%) patents with suspected pulmonary tuberculosis have had a negative smear results for acid fast bacilli. Abnormal chest x-ray findings were observed in 196 (79.4%) patients. 43/247

(17.4%) patientswhose smears were negative for acid fast bacilli found to be positive for mycobacterial culture (35).

Another study conducted in Addis Ababa on prevalence of smear negative tuberculosis between August 01, 2017 to January 5, 2018 shows that From the total of 418 enrolled patients, 27 (6.5%) were Xpert MTB/ RIF and 26 (6.4%) were culture confirmed smear negative PTB patients. From 26 culture positive isolates 3 (11.54%) were MDR TB; from MDR-TB confirmed isolates 2/23 (8.7%) were among new and 1/3 (33.3%) was among retreatment smear negative presumptive pulmonary TB patients. All Rifampicin resistant smear negative pulmonary TB isolates by Xpert MTB/ RIF assay were found to be MDR TB and 7/26 (26.9%) isolates were INH mono resistant. History of migration found to be a potential factor for developing smear negative pulmonary TB (36).

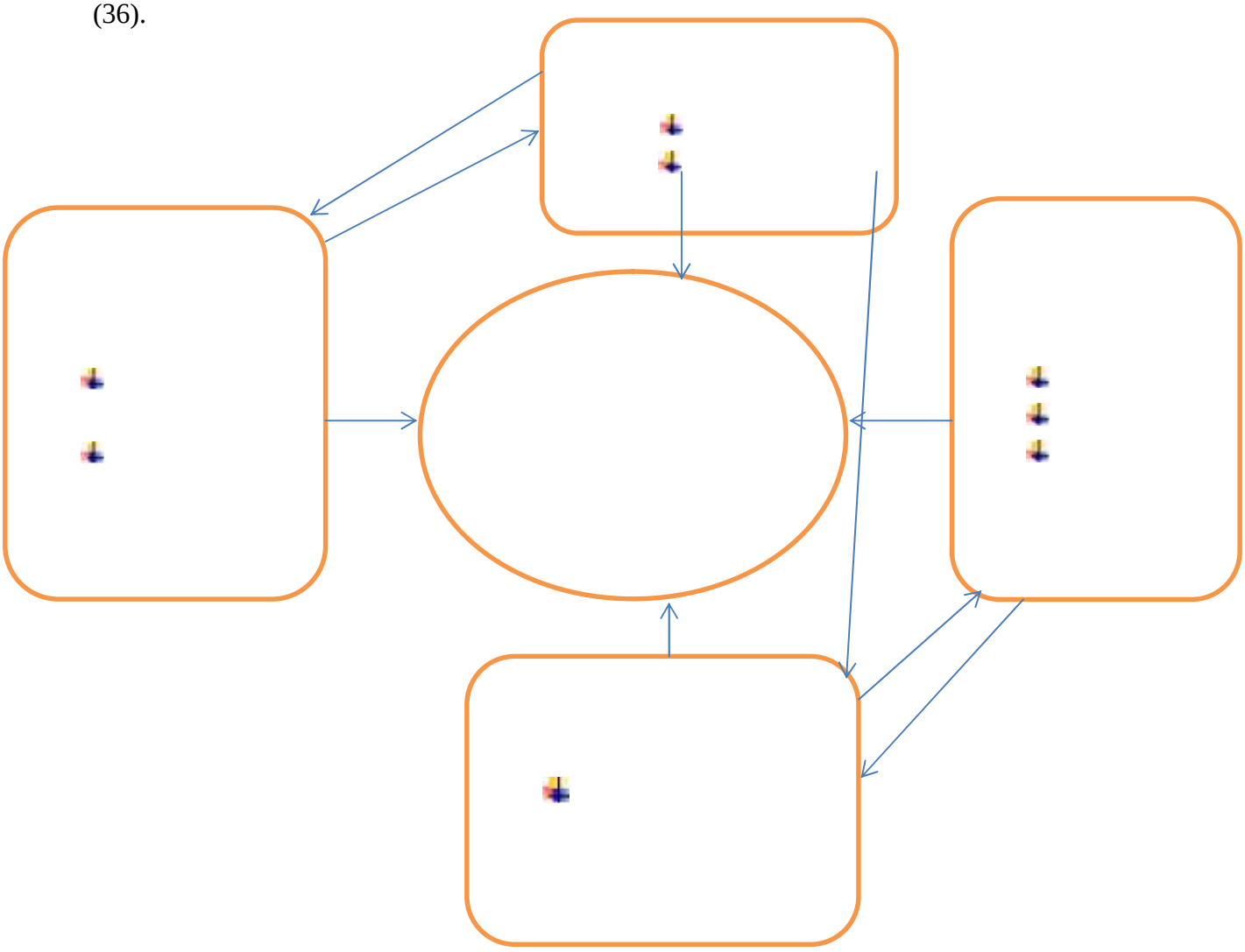

Figure 1 – Conceptual framework

Generally according to the above literatures burden of smear negative PTB, and SNMDR PTB directly influenced by the immunity level of individual .according to the above Conceptual framework developed from different literatures as immunity depletes the risk of SNPTB increases also associated with income of individual's as swells as the capacity of countries to detect the SNPTB .in resource constricted countries the burden of SNPTB is high. In most of the literatures the burden of SNPTB and SNMDRTB were related to low level of CD4 cell level (Figure 1).

3. Objectives

3.1 General Objective

Todetermine the burden of smear negative pulmonary tuberculosis using Genexpert and Culture in human immunodeficiency virus infected (ART) patients in TulubóloGeneral Hospital and St. LukeHospital in 2012 E.C

3.2 Specific objectives

To determine the burden of smear negative pulmonary tuberculosis in the two hospitals

To compare the yield of smear negative PTB using genexpert and culture methods

To describe level of Rif resistance among smear negative PTB cases.

4. Materials and Methods

4.1 Study area

The study was conducted in Tulu Bolo General Hospital and St. Luke Hospital of South west Shewa zone, Oromia regional state, Ethiopia from December 1, 2019 G.C to February 30, 2020 G.C. Tulu Bolo hospital is found in south west shewa zone, Oromia regional state and 80 km from Addis Ababa the capital city of Ethiopia. The catchment population of Tulu Bolo Hospital is around 420,000. St. Lukas Hospital is also located in Woliso town, South West Shewa zone, Oromia regional state and 110 km from Addis Ababa the capital city of Ethiopia. The catchment population of St. Lukas Hospital is around 900,000.

From registration book in ART clinic the level of smear negative PTB in the Tulu Bolo Hospital ART clinic in 2011 e.c was 94 cases found and St.lukassmear negative PTB level in 2011 was 282 cases registered on registration book

The pulmonary tuberculosis suspected patient flow in October, 2019 to Tulu Bolo General Hospital laboratory was on average daily around 25 patients were sent to hospital and weekly around 125 patients were tested for pulmonary tuberculosis also in St. Lukas hospital in month October 2019 around 200 patients were tested in laboratory and daily on average around 40 patients were tested for pulmonary tuberculosis.

The positivity rate of mycobacterium tuberculosis in St. Lukas hospital in the past five years retrospectively from September 2015 up to September 2019 was 1350 total positive rate found out of 7,435 tests done in the past five years. the positivity rate of children less than 10 years was 70 in number out of this 40 were males and 30 were females the positivity rate of age 11—20 were215 in number90 males and 125 females the positivity rate of age between 21 to 30 were 190 males and 210 females were positive for mycobacterium tuberculosis the positivity rate of age between 31to 40 were160 males and 120 females and the positivity rate of age greater than 40 were245 males and 80females ware positive totally 810 males and 540 females ware positive in St Lukas hospital including 7 males and 5 females whose age were not registered in registration book

Also the positivity rate of Tulubolo general hospital were 640 males and 440 females were positive for mycobacterium tuberculosis out of 6,356 total tests donein the past fiveyears

fromSeptember 2015 up to September 2019. But due to absence of age registered in registration book it is difficult to categorize positivity rate of mycobacterium tuberculosis on age variable.

4.2 Study design and period

Cross-sectional study design was utilized and the study was conducted fromDecember 1, 2019 G.C to February 30, 2020 G.C.

4.3 Population

4.3.1 Source population

All ART patients who were on ART follow up in Tulu Bolo General Hospital and St. Luke Hospital ART clinic.

4.3.2. Study Population

All ART patients who were Presumptive TBon ART follow up in Tulu Bolo General Hospital and St. Luke Hospital ART clinic wereincludedbased on inclusion criteria

4.4 Inclusion and exclusion criteria

4.4.1 Inclusion criteria

All ART patients who were on ART follow up in Tulu Bolo General Hospital and St. Luke Hospital ART clinic.

4.4.2 Exclusion criteria

ART patient do not have sign and symptom of TB and ART patients do not exist during data collection

4.5 Study Variables

4.5.1. Dependent variables

Burden of smear negative PTB, Rif resistance level

4.5.2. Independent variables

Age, Sex, length on ART drug, adherence on ART drug, viral load, CD4 count, Previous TB status, Patient monthly income

4.6. Measurement and Data collection

4.6.1. Sample size determination

A single population proportion formula was used to calculate the sample size and sample size of the study was determined taking 17% prevalence of smear negative TB from previous research conducted in Addis Ababa (35) .(17%)0.17 as proportion and taking 5% marginal error.

$$n = \frac{(z\alpha/2)^2 . pq}{d^2}$$

Where:

n= the sample size estimated from a single population proportion

p= proportion which is 17% taken from research conducted in Addis Ababa

d= margin of error (d=5%)

Zα/2=the standard normal value of 1.96

The required sample size was216, but the number of target population was below 10,000 that were 1300, finite population correction formulas was used to estimate the final sample as shown as follows:

N=total ART patient in the two Hospitals which is 1300

$$n = \frac{n}{1 + n/N}$$

Where: n=216

N=1300

$$n = \frac{2}{1+2 \quad /1} = 186$$

This yield sample size 186 and 10% of non-respondent rate wereadded. The final sample size was **203**

4.6.2. Sampling method

The Tulu Bolo General hospital and St. Luke Hospital were selected because they are ART hospitals from all hospital existing in South west Shewa Zone. The number of ART patient which were included in the study from each Hospital were determined in proportion with the total number of ART patient found in each Hospital and convenientsampling techniquewas used. See in the following schematic diagram how the sampling process took place.

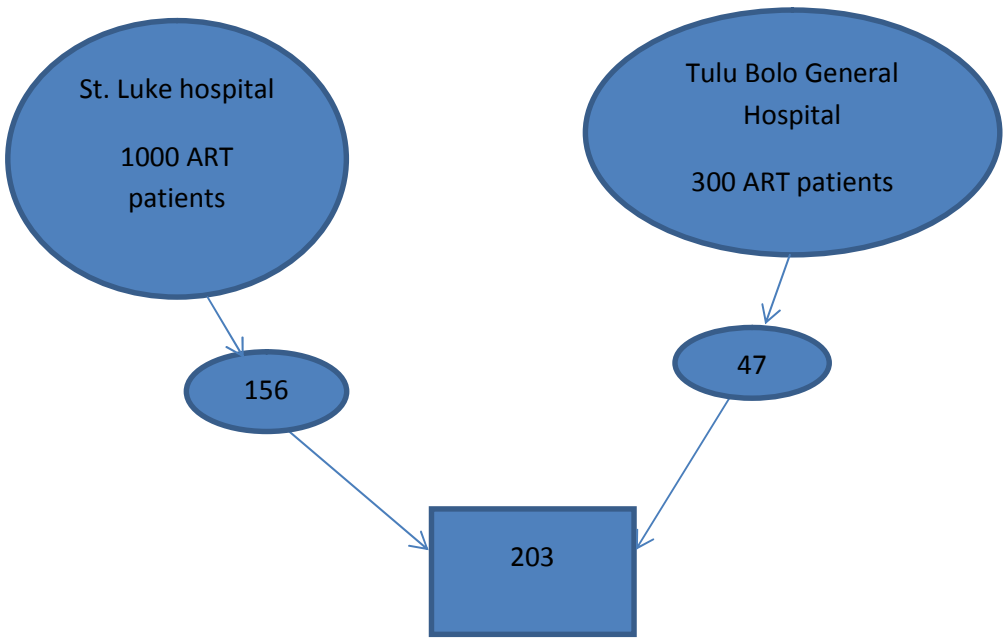

Figure 2:schematic diagram of the sampling process

4.6.3. Data collection procedure

The Clinical data was collected by the laboratory requestwhich were properly filled for each patient without missing each patient identification as the standard afterconsent form signed by individual voluntary study participant and sputum sample was collected by giving the necessary

orientation for the patient about the quality of sputum sample needed by researcher and the resultswere collected by the request form and the socio demographic data was collected by interviewing using questionnaire. Thequestionnaire consists of socio- demographic characteristics of respondents.

4.6.4. Laboratory Methods

4.6.4.1 Specimen collection

Proper collection and transport of sputum specimens was implemented to ensure quality laboratory results. Adherence to procedural details was resulted in collecting adequate and quality sputum specimens for analysis in the bacteriology laboratory and maintained correct identity of the specimen. Specimens were collected in appropriate sterile, disposable containers and Screw caps which fit tightly to avoid leakage was used. Fifty ml polypropylene centrifuge tubes were used. During collection patients were informed that nasal secretions and saliva were not sputum. The desired specimen was produced by a deep cough and was thick, mucoid, white-yellow, and sometimes blood-tinged. It was from the lower airways and lung. Storage of Sputum Specimens were also Checked that the tube is tightly capped, properly labelled, and that the screening and/or subject ID on the tube matches the screening and/or subject ID on the requisition. Specimens were Refrigerated at 2-8°C until ready for transport to the testing laboratory because Refrigeration reduces the growth of contaminants in the specimen. And Transport of Sputum Specimens were delivered to the laboratory the same day as collected for genexpert test and smear microscopy test, but for MTB culture specimens were delivered to EPHI(Ethiopian public health institute) within three days. These specimens were stored in the refrigerator and transported on ice by triple package system (37).See the procedure in the annex 3.5.

4.6.4.2 Conventional light microscopy Ziehl-Neelsen (ZN)

Light microscopy performed directly on sputum specimens is suitable for all laboratory service levels, including peripheral laboratories at primary health care centers or districts hospitals.See the procedure in the annex 3.2

Principles ZN staining;the lipoid capsule of the acid-fast organism takes up carbolfuchsin and resists decolorization with a dilute acid rinse. The lipoid capsule of the mycobacteria is of such high molecular weight that it is waxy at room temperature and successful penetration by the

aqueous based staining solutions (such as Gram's) is prevented .see gram stain procedure in the annex 3.4

4.6.4.3Light-emitting diode (LED) fluorescent microscopy

LED technology allows the use of fluorescent microscopy with a much less expensive light source. LED microscopes or -attachments require less power, are able to run on batteries, the bulbs have a very long half-life and do not release potentially toxic products if broken. See the procedure in the annex 3.1

Principles, of FM staining 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 helps prevent non-specific fluorescence. All acid-fast organisms will be stained by auramine O, including some parasites. Slides stained with auramine O may berestained with Ziehl-Neelsen. This provides a convenient method of confirming and differentiating morphology of positive slides with the traditional stains.

4.6.4.4MTB CULTURE

Mycobacterial culture and identification of M. tuberculosis was done in Ethiopian public health institute **(EPHI)**.Mycobacterial culture and identification of M. tuberculosis provide a definitive diagnosis of TB, significantly increases the number of cases found (often by 30-50%), and can detect cases earlier (often before they become infectious). Culture also provides the necessary isolates for conventional DST. Also Culture is much more complex and expensive than microscopy to perform, requiring facilities for media preparation, specimen processing, and growth of organisms, specific laboratory equipment, skilled laboratory technicians, and appropriate biosafety conditions. Specimens have to be decontaminated prior to being cultured to prevent overgrowth by other micro-organisms. All decontamination methods are to some extent also harmful to mycobacteria, and culture is therefore not 100% sensitive. Good laboratory practices maintain a delicate balance between yield of mycobacteria and contamination by other micro-organisms. Solid and liquid culture methods are suitable for central/national reference laboratories (or regional laboratories in large countries). Usually, one

culture laboratory is adequate to cover 500,000 - 1 million populations. Solid culture methods are less expensive than liquid culture systems, but results are invariably delayed due to the slow growth of mycobacteria. Liquid culture increases the case yield by 10% over solid media, and automated systems reduce the diagnostic delay to days rather than weeks. Liquid systems are, however, more prone to contamination and the manipulation of large volumes of infectious material mandates appropriate and adequate biosafety measures. Positive cultures have to be identified to differentiate M. tuberculosis from NTM. NTM are more common in HIV-infected patients and the prevalence varies from country to country. Treatment of NTM is entirely different from treatment of TB and drug-resistant TB. As a minimum, laboratories performing DST must differentiate M. tuberculosis from other NTM .Confirmation is usually done by a combination of biological characteristics of the culture growth and selected molecular or biochemical tests (which invariable delay the final result).Rapid immunochromatographic assays (so-called strip speciation tests) for species identification on culture isolates provide a definitive identification of M. tuberculosis in 15 minutes and are recommended. Molecular tests, biochemical methods and strip speciation assays are suitable for laboratories where culture and DST are performed(38).

4.6.4.5Genexpert Rif Assay

GeneXpert MTB/RIF assay is nucleic acid amplification (NAA) test which simultaneously detects DNA of Mycobacterium tuberculosis complex (MTBC) and resistance to rifampin (RIF) that is mutation of the rpoB gene in less than 2 hours. In comparison, standard cultures can take 2 to 6 weeks for MTBC to grow and conventional drug resistance tests can add 3 more weeks. (39).

This system integrates and automates sample processing, nucleic acid amplification, and detection of the target sequences.

The primers in the XpertMTB/RIF assay amplify a portion of the rpoB gene containing the 81 base pair “core” region. The probes are able to differentiate between the conserved wild-type sequence and mutations in the core region that are associated with rifampicin see the procedure and materials in the annex part The Xpert MTB/RIF assay includes several internal quality controls that verify specimen processing, success of PCR and cartridge integrity.

Each cartridge includes a sample processing control (SPC), which contains non-infectious spores in the form of a dry spore cake that is included in each cartridge to verify adequate processing of MTB to: verify that lysis of MTB has occurred if the organisms are present,

• We had verified that the specimen processing was adequate,

• detect specimen associated inhibition of the real-time PCR assay. SPC should be positive in a negative sample and SPC can be negative or positive in a positive sample.

The Probe Check Control is a check undertaken before the start of the PCR reaction. The system measures the fluorescence signal from the probes to monitor bead rehydration, reaction-tube filling, and probe integrity and dye stability(40).See the procedure in the annex 3.3

4.6.4.6 Quality control of MTB diagnostic methods

A. A positive and negative control slide were included whenever any acid-fast stain was performed and upon receipt of each new lot of materials, reagents, and media to verify the correct performance of the procedure.

B. The specimen was also checked for adherence to the slide (macroscopically).

C. The microscope was calibrated, and the objectives and oculars used for the calibration procedure were used for all measurements on the microscope. The calibration factors for all objectives were posted on the microscope for easy access (multiplication factors can be pasted on the body of the microscope). Although recalibration every 12 months may not be necessary, this wasvarying from laboratory to laboratory, depending on equipment care and use. Although there is not universal agreement, the microscope should probably be recalibrated once each year. This recommendation should be considered with heavy use or if the microscope has been bumped or moved multiple times. If the microscope does not receive heavy use, then recalibration is not recommended on a yearly basis.

D. Known positive microscope slides and photographs (reference books) were available at the work station.

E. Recordsof all QC results; the laboratory has had an action plan for ``out of control'' results(41).

4.7. Data Quality Assurance

In order to ensure data quality, orientation to the patient was given as much as the patient understand on the quality of sputum sample with due attention to the purpose and the scope of the study. Pre-test was done prior to actual data collection on ART patient in Tulu Bolo Health Center on their understanding about the orientation given to each patient and on the filling of the request form by ART provider, so as to verify the validity of the instruments. Pre tested standardized request form was adopted and investigators were make frequent checks on the request form process to ensure the completeness and consistency of the gathered information.

Quality assurance and quality control of reagents and equipment's were done regularly by testing know positive and negative control slides weakly and for all new batch of reagents .Specimens which had breakage or leakage, incomplete patient information and mislabeling, delayed specimen more than 5 days after collection, inappropriate container, and insufficient volume of sample (less than 3ml) were rejected. Each batch of samples that was processed for culture used start and end controls. All laboratory investigations were done by experienced laboratory technologists in the National Tuberculosis Reference Laboratory, EPHI and in Tulubolo hospital laboratory and St.Luke hospital laboratory. Equipment preventive maintenance, temperature monitoring, monitoring of critical microbiological practices, and instrument function checkups was done. The laboratory performance is also continuously monitored by Proficiency Testing (PT) for all test methods. All laboratory analysis was done based on Ethiopian national TB, HIV and leprosy management guideline and WHO approved methods.

4.8. Data analysis and interpretation

During pre-analytical, analytical and post-analytical phase of laboratory examination for each method of analysis and procedures every patient orientation and data was checked for completeness and appropriateness.

Data entry and analysis methods The data collected from each questionnaire and the laboratory investigation was doubly entered and cleaned by using Epi -info statistical software version 7.2 and exported to IBMSPSS software version 23.0 for analysis. By using central measurement, dispersion and frequency distribution descriptive statistics was done to characterize the study participants and the result of the study was also presented by using tables and figures. The prevalence of SNPT and SNMDR TB was calculated.

4.9. Ethical considerations

Prior to data collection appropriate ethical clearance was obtained from Addis Ababa University College of health science ethical review committee. Formal letter of permission was produced from Regional health bureau and each Hospital. Then each hospital gives written permission the data collection was proceed for the required data collection. Informed consent was taken from each patient after explaining the objective of the study. Confidentiality was assured by using code numbers rather than names in the questionnaire.

4.10. Dissemination of Results

The result obtained from this study will be disseminated through different mechanism. Initially, result of this study will be presented in the final defense at Addis Ababa University and then the result of the study will also be reported to FMOH, Oromia regional health bureau, South west shewa zone Health department and the two hospitals. The finding of the study will be published in different journals. Copies of the final findings will be given to the respective health facilities.

4.11. Operational Definitions:

Smear negative patient: –are tuberculosis patients but their sputum smear is negative due to different factorsbut become culture or Genexpert positive

Detection: is ability of method to detect presence or absence of pathogenic organisms

Adherence on ART drug: taking of anti-retro viral therapy drugs according to advised or counseled by health professionals

5.0. Results

5.1. Socio demographic characteristics of study participants

A total of 197 pulmonary tuberculosis suspect HIV patients were included in the study. Five individuals excluded due to their AFB smear-positive result. One hundred nighty two (192) AFB smearnegative pulmonary tuberculosis suspects were included in the study but for comparison purpose total of 197 HIV patients' epidemiological and bacteriological data were analysed. The mean and median age of the study participant was 37.2± 11.5and 36 respectively. All patients were from ART clinic. Majority, 104(52.8 %) of the patients were females. Most of the participants, 64(32.5%) were in the age group of 31-40 years. Among all participants, 121(61.4%) were below grade 12. The participants had different occupation; the most frequent was house wife, 52(26.4) .One hundred seven 107(54.3%) participants were married whereas 49 (24.9) were single (Table 1).

Table 1 Socio demographic characteristics age, sex and educational status of study participants from, December 01, 2019- February 30, 2020

Variable		Frequency	Percent
Sex	Male	93	47.2
	Female	104	52.8
	Total	197	100.0
Age group of the study participants			
<10		3	1.5
11-20		6	3.0
21-30		54	27.4
31-40		64	32.5
41-50		45	22.8
>51		25	12.7
Total		197	100.0
Level of education of study participants			

None or non-formal education	43	21.8
<12 grade	121	61.4
12 complete	8	4.1
Diploma	18	9.1
Degree and above	7	3.6
Total	197	100.0

Among all participants, 121(61.4%) were below grade 12(Table1). The participants had different occupation; the most frequent was house wife, 52(26.4 %).One hundred seven 107(54.3%) participants were married whereas 49 (24.9%) were single (Table 2).

Table 2 Socio demographic characteristics marital status, work status and religion of study participants from, December 01, 2019- February 30, 2020

Variable	**Frequency**	**Percent**
Marital status of study participants		
Married	107	54.3
Single	49	24.9
Divorce	22	11.2
Separated	7	3.6
Widowed	12	6.1
Total	197	100.0
Work status of study participants		
Farmer	19	9.6
Government employee	12	6.1
Driver	14	7.1
NGO employee	4	2.0
Merchant	34	17.3

Dailylabourer	18	9.1
Sex worker	11	5.6
House wife	52	26.4
Other	24	12.2
Student	9	4.6
Total	197	100.0
Religion of participants		
Orthodox	138	70.1
Muslim	33	16.8
Protestant	26	13.2
Total	197	100.0

5.2. Clinical presentation, CD4 follow up status and recent viral load test result status of study participants

Clinical presentation, Signs and symptoms CD4 and viral load status of patients were assessed in the study. From all the participants, 141 (71.6%) had cough as a chief compliant, and 19 (9.6%) of them has had loss of weight as chief complaint.About 75/197(38.1%) of patients HIV viral load test follow up status were shows less than 1000copies followed by67/197 (34.0%) of patients viral load status is undetected and 28(14.2%) ofpatients recent viral load result was greater than 2000 copies, 27 (13.7%) of patientsviral load status was between 1000 and 2000copies.Sixty five, 65/197(33.0%)patients CD4level wasbetween 201and 350per microliters, whereas 52/197 (26.4%) of patients CD4 level between 351-450per microliters, 45 (22.8%) and 35(17.8%), hashad greater than 450 and less than 200per microliters CD4 result respectively.(Figs. 3and,Table3)

Table 3 Chief Complaints of study participants, CD4 and viral load follow up status of study participants from, December 01, 2019- February 30, 2020

Variable	Frequency	Percent

Chief complaints of study participants

Cough	141	71.6
Night sweat	17	8.6
Loss of weight	19	9.6
Chest pain	12	6.1
Fever	6	3.0
Other	2	1.0
Total	197	100.0

Recent Viral load result of study participant from ART clinic registration book

<1000	75	38.1
1001-2000	27	13.7
>2001	28	14.2
Undetected	67	34.0
Total	197	100.0

Recent CD4 result of study participant from ART clinic registration book CD4follow up status of participants

<200	35	17. 8
201-350	65	33.0
351-450	52	26.4
>450	45	22.8
Total	197	100.0

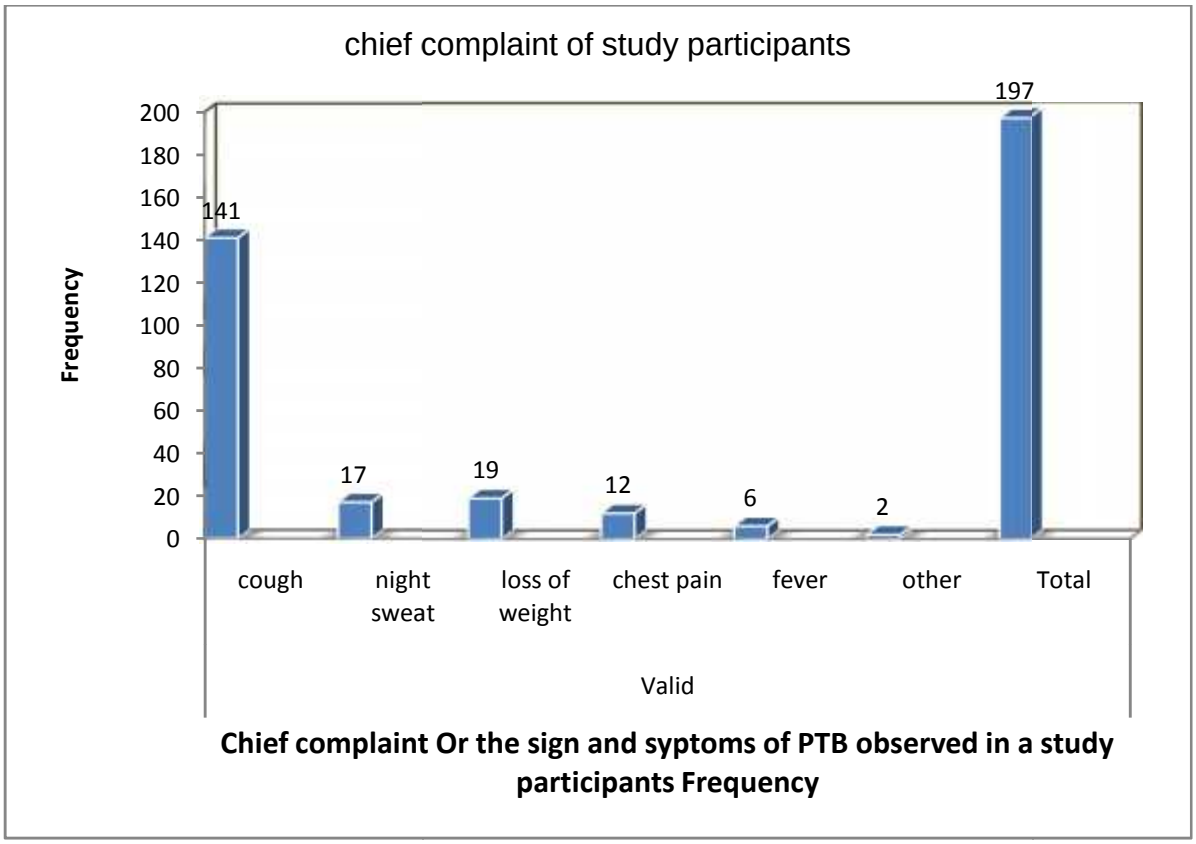

Figure 3: chief complaints of study participants

5.3. Burden of smear negative pulmonary tuberculosis (SNPTB)

SNPTB burden among presumed smear negative PTB patients was 9.9% by Xpert MTB/RIF assay.About 19 (9.9%) were smear negative and culture confirmed / by either of the culture techniques (LJ and MGIT), SNPTB patients (Table4). More TB was detected in male than female patients. The positivity rate among male and female was 10/19 (52.6%) and 9/19(48.4%), respectively. There was significant difference in TB prevalence among male and female .High rate of TB detection was observed in the age range 31-40 9/19(48.4%). All positive SNPTB were detected from the age groups between 21 and 50 years. Majority, 17/19 (89.47%) of the total SNPTB were found to be positive in those who were below grade 12, Among 19 SNPTB cases14/19(73.68%) patients viral load status were greater than 2001 copies and Among 19 SNPTB cases 12/19(63.12%) patients CD4 result were less than 200 per microliters.(Table 4)

Table 4Burden of Smear negative PTB, andburden of MDR TB of study participants from, December 01, 2019- February 30, 2020

Variable	Frequency	Percent
Smear microscopy result of study participants		
	Frequency	Percent
Negative	192	97.5
Positive	5	2.5
Total	197	100.0

genexpert result of study participants		
	Frequency	Percent
Negative	173	90.1
MTB detected RR not detected	18	9.4
MTB detected RR DETECTED		10.5
Total	192	100.0

Culture RESULT of study participants		
Frequency	Percent	
Negative	173	90.1
Positive	19	9.9
Total	192	100.0

5.4. Burden of Multidrug resistant tuberculosis

A total of 19 smear negative, genexpert and culture confirmed TB was detected. All clinical isolates were tested by using Genotype MTBDR plus Ver.2, first- line MGIT DST and Genotype MTBDRsl Ver.2(Second line Line Probe Assay). Among these isolates, 1 (5.26%) was MDR-TB (resistant to both isoniazid and rifampicin). This rifampicin resistant (RR) TB by GeneXpert was found to be MDR-TB by Genotype MTBDR plus Ver. 2(Second line Line Probe Assay) and MGIT DST methods. The age of patient was 34 years old and sex of the patient was female and has had history of previous treatment and completed the treatment given at that time and comes with chief complaint ofcough viral load result of the patient was less than 2000 and the CD4 cell count was less than 200 cells per millimetre cube (Table5).

Table 5:Antituberculosisdrugs resistance of MDRTB patient

s.no	Drugs	Culture result Second line Line Probe Assay	Genexpert result
1	Rifampicin	Resistant®	Resistant®
2	Isoniazid	Resistant®	
3	Kanamycin	Sensitive(S)	
4	Amikacin	Sensitive(S)	
5	Capreomycin	Sensitive(S)	
6	Viomycine	Sensitive(S)	

5.5. Comparison of detection rate ofsmear microscopy, GeneXpert and MTB culture methods in diagnosis of PTB patients

Both GeneXpert and MTB culture methods detect equal number of SNPTB (Table 6).

Table6. Comparison between ZN/AFB smear microscopy, GeneXpert MTB/RIF assay, andMTB culture of all 197 study participants

Result/method	ZN/AFB smears microscopy	GeneXpert MTB/RIF assay	MTB culture
Positive	5 (2.54%)	24 (12.18%)	24(12.18%)
Negative	192 (97.46%)	173(87.82%)	173(87.82%)
Total	197 (100%)	197 (100%)	197 (100%)

Smear microscopy detects 5/197 (2.54%) of suspected PTB cases andgenexpert Rif detects24 /197(12.18%)of suspected PTB cases also MTB culture detects 24(12.18%) of suspected study participants in this study genexpert and MTB culture shows the same resulthowever smear microscopy missed 19 of PTB patients which later detected by genexpert and confirmed by MTB culture. According to our finding the detection rate of smear microscopy is 5/24 (20.83 %) whereas the detection rate of genexpert Rif assay and MTB culture is 24/ 24(100 %)(Table6).

6.0. Discussion

According to the Global TB Report 2017, 10.6 million people are estimated to have fallen ill with TB in 2016 while an estimated 1.3 million people died of TB. In addition, an estimated 4.1% of these new TB cases and 19% of the previously treated cases are believed to harbour Drug resistant-TB with an estimated 240 000 deaths annually Ethiopia is among the 30 High TB, HIV and MDR-TB Burden Countries, with annual estimated TB incidence of 177/100,000 populations and death rate of 25 per 100,000 populations for 2016(6).

According to WHO more than 9 million people who become ill with TB each year from this more than 3 million are not diagnosed treated or officially registered by national TB programmes. The emergence of MDR TB makes the situation worse. Collectively these “missed” million TB patients are aglobal public health problem (42). Similarly diagnosis of MTB in developing countries largely depends on smear microscopy which has poor sensitivity (43).resulting in increased smear negative pulmonary tuberculosis (SNPTB) that remains undiagnosed or delay in diagnosis and treatment (44). SNPTB and SNMDR TB prevalence in South west shewa zone was not well known. Therefore determining the magnitude and distribution of SNPTB and smear negative MDR TB in different settings can contribute to the national efforts for fighting TB.

Our study finding showed that the prevalence of smear negative pulmonary tuberculosis among smear negative presumptive pulmonary tuberculosis patients was 19/192 (9.9%) which was Xpert MTB/ RIF confirmed and 19/192 (9.9%)by the collaborative diagnostic performance of BACTEC MGIT 960 and LJ culture. This is in line with the 9.6 %prevalence of study conducted in Tanzania Dares Elam in 2015 (45).How ever therewas no difference between GeneXpert and culture results in our study. From the total, majority 104(52.8 %) of the study participants were female, but more SNPTB10/19 (52.6%) were detected from male which is in line with the fact that TB is more common in men than women (1). Our study revealed higher smear negative PTB prevalence than a report from Vietnam which was 5.6% (smear negative culture positive PTB) (46) and study conducted in Addis Ababa Ethiopia in 2019 which shows 6.5% SNPTB prevalence (36). And the other study conducted in Addis Ababa, Ethiopia between May 25,

2017, and September 30, 2017whichshows 14/206 (6.8%) prevalence of SNPTB (47)also shows lower prevalence than our study finding. But Our study shows lower prevalence of SNPTB when compared to the 20.0 %prevalence of the study conducted in Nepal in Patan Hospital clinics in 2013 (29) and the study conducted in Addis Ababa Ethiopiaat St. Peter TB specialized hospital Addis Ababa, Ethiopia between November 15, 2004 and October 30, 2005.Which shows 17.4% SNPTB,(35),A study conducted in Adama, Ethiopia in 2013, also showed a higher SNPTB prevalence that was 12.1% (48)than our study results however the study conducted in Addis Ababa in St. Peter TB specialized hospital, Ethiopia in 2019 and the study conducted in Adama Ethiopia in 2013 shows similarity with our study finding in that men were more affected by SNPTB than females.

The differences in the prevalence of SNPTB may be due to different reasons like geographical variation, change in sample collection strategy, difference in time that the study conducted, change in TB diagnostic and treatment algorithm and diagnostic method differences. Another justification for these differences might be, our study tried to cover sample size of our study in short period of time that is within three month. Our study shows wide range of difference withthe study conducted in Ethiopia at St. Peter TB specialized hospital Addis Ababa, Ethiopia between November 15, 2004 and October 30, 2005.Which shows 17.4% prevalence of SNPTB this difference shows mainly change in TB diagnostic and treatment algorithm and diagnostic method differences the country implemented.

Although the study included all age groups, which are on ART follow up all (100%) of the SNPTB were detected in the age group 21–50years' participants which are the economically productive age group of the population. This finding is in line with WHO estimation that states TB affects mostly adults in the economically productive age groups around two-thirds of are estimated to occur among people aged 15–59years (1).

According to ourstudy findings from the 19 isolates of smear negative pulmonary TB patients 01 (5.26%) patient had MDR TB. This SNMDR TB isolates was treated once before, comes with relapse case. Our study demonstrated low MDR TB prevalence as compared to a finding by a study in Belarus which was 47% SNMDR-TB: (49). But, our study showed high prevalence of SNMDR TB in comparison with a study conducted in Australia which was 2 to 3% prevalence were resistant to at least isoniazid and rifampicin (50).Even if our study showed lower

prevalence ofSNMDR-TB when compared to the Study conducted in Addis Ababa Ethiopia in 2019 our study shows higher smear negative PTB prevalence than this report which shows 6.5% SNPTB (smear negative Genexpert Rif test positive & culture positive PTB)(36).

A study conducted in Cape Townshows detection rate ofXpert MTB/RIF assay 80% and detection rate of AFB smear microscopy 20% (27)which is similar to our study smear microscopy detection rate which is 20.83% however the detection rate ofXpert MTB/RIF assay is not similar to our study finding which is 100% detection rate in our study .our smear negative PTB detection rate 100 % but our study prevalence 9.9% had similarity with the study conducted inTanzania Dares Elam in 2015 which is 9.6 prevalence and detection rate of 76.77%(40).also the study conducted in Pakistan shows 76.9 detection rate of genexpert and MTBculture methods from SNPTB cases which had not similarity with our study finding(32).

Our study shows among 19 SNPTB cases14/19(73.68%) patients had viral load levelmore than 2001copieswhich means 73.68% of study participants had high HIV viral load. Among 19 SNPTB cases 12/19(63.12%) patients had CD4 count less than 200 cells /mm3 this shows significant similarity with World Health Organization (WHO) report of 2014 on genexpert, Sputum smear microscopy has a particularly low sensitivity for detecting TB among PLHIV. This is because people in later stages of HIV infection and with compromised immune systems often release fewer organisms into their sputum, at concentrations below the threshold for visual detection under a microscope. (19) .The study conducted in Vietnam in 2012 shows that only a decrease CD4+ cell level below 200 cells /mm^3 related to SNPTB which is similar to our finding where 12/19(63.12%) patients CD4 cell count below 200cells /mm^3(21).

Our study finding on clinical presentation of study participants 141(71.6%) had cough as a chief compliant, and 19 (9.6%) of them had loss of weight as chief complaint. About 17(8.6%) had night sweat and 12(6.1%) chest pain 6 (3.0%) has had fever and 2 (1%) had other symptoms like loss of appetite as chief complaint .This is in line with WHO recommendationwhich said using combination of cough, fever, weight loss, and night sweats as the initial screening tool to exclude active TB cases before initiating preventing treatment for persons living with HIV in most resource-constrained settings. However, the study conducted in Vietnam in 2012 shows that symptom screening may miss up to 54% of AFB smear-negative PTB cases among HIV-infected

patients (sensitivity = 46% and negative likelihood ratio = 0.85). Results from this study confirm the need of routinely applying sputum culture for HIV-infected patients (21).

7.0. Strength and Limitation

7.1. Strength

The Line probe assay test was done for all study samples can be seen as strength of the study

7.2. Limitation

We are not able to see any association due to incomplete data on the registration book.

8. Conclusion and Recommendations

8.1. Conclusion

The study revealed that the burden of genexpert and culture-confirmed SNPTBwas 9.9% The overall finding of this study showed that high burden of PTB among the males than female's and there is high burden of SNPTB in the young productive age group

8.2. Recommendations

Since SNPTB cannot have specific sign and symptoms and there is high prevalence of SNPTB in HIV patients all ART patients should be screened for Mycobacterial diseases using genexpert.

Since Genexpert machine is simple and accurate it should be available at all health facilities to control both PTB and SNPTB

Further large scale study is warranted in wider places

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Annex I Information sheet and consent form in English Version

Title of the Research Project:Burden ofsmear negativepulmonary tuberculosis using grenexpert and culture methods amonghuman immunodeficiency virus infected patients in Tulu bolo and St .Luke hospitals, south west shewa ,oromia ,Ethiopia

Principal Investigator: KinduGetnet (BSc, MSc candidate)

Name of the Organization: Department of Medical Laboratory Sciences, College of Health Sciences, Addis Ababa University

Introduction

You are invited to participate as a study subject in a research conducted by MSc candidate, from Addis Ababa University. Your participation is voluntarily. The research teams will include one principal investigator, two advisors; from Addis Ababa University from Microbiology department. Please take as much time as you need to read or listen in the information sheet.

Purpose of the Research Project

We are asking you to take part in this study because we will try to assess the burden of smear negative pulmonary tuberculosis in HIV patients.

Purpose of the research:

I am conducting this study in order to assess the burden of smear negative pulmonary tuberculosis in HIV patients. Similar studies were conducted in certain parts of the country however previously there has not been any study done to establish the prevalence ofsmear negative pulmonary tuberculosis in HIV patients in Tulu bolo General Hospital and St. LukeHospitals. Although there have been a number of smear negative pulmonary tuberculosis were reported empirically inHIV patients' the real prevalence of smear negative pulmonary tuberculosis has not been quantified.

You have been chosen for this study. Please read this information or have read to you so that you can understand what we are going to ask you, Feel free to ask question so that you understand fully. So the finding of this will initiate the physicians to screen and request all important tests used to diagnose in order to identify the co infection so that the appropriate antibiotic is given for the patients in time for better outcome.

Procedures and the expected participation

If you agree to participate in this study, you need to understand the purpose of the study and give your consent. We will also conduct a face-to-face interview for additional questions about you and not only this but also specimen collected from you will be used for the research purpose, and the results of your sample will be exposed to some concerned professional staffs as it is needed. The required clinical sample will be collected by residents of microbiology department. Then, you are requested to give your consent to the sample collector. After consent, a sample will be collected.

Procedures: After agreeing that you can take part, one or more of our research staff will ask you some questions which will take up to 15 minutes. Your weight, height and vital signs will be measured. You will be asked to provide fresh sputum on a particular container we provide. We will also collect two sputum specimens within one hour difference or gastric lavage (for <5 years) from you using disposable screw capped leak proof container. Then we will conduct microbiological laboratory examination.

Potential risks and Discomforts

There will be minimal discomfort in giving sputum samples. However, there might be some minimal risk and discomfort in case of gastric aspiration procedure. Nevertheless, we will try to minimize the discomfort as much as possible, since will be collected by experienced physicians.

Confidentiality

We respect your privacy and confidentiality. Any information that identifies you will not be shared with anyone else outside the study team. The information we will collect from you as part of the study will be kept in a locked file cabinet, or be protected by a password on the computer only accessible to personnel involved in the study. There is no sensitive issue that you will be asked related with your social desirability but any information that is obtained in connection with this study and that can be identified with you will remain confidential.

Potential benefits to subjects and/or to the society

If you agree to take part in this study you will not receive any payment except that when you are found the pulmonary tuberculosis you will be treated free according to the guide line. In addition, the result of the study will be beneficial for the prevention and control of smear negative pulmonary tuberculosis. Hence, you are indirectly benefiting other patients and the society in this respect.

Participation and Withdrawal from the Study

The participation is voluntary and you are free to decide if you want to participate in this study. If you agree you can still withdraw at any time and place without consequences of any kind. You may also reject to give any sample. You can ask any questions regarding to this study and you have a right to get a laboratory diagnosis result free.

Contact information

If you have any questions about this study you can contact the following principal investigators and advisors for further information.

Name: KinduGetnet Phone: +251912885773 E-mail: kindugetnet@yahoo.com

Annex I. Informed consent form in English version

Card no…..……

I had been informed that the objective of this study is to determine the burden of smear negative pulmonary tuberculosis using Genexpert and Culture in human immunodeficiency virus infected (ART) patients in Tulubolo General Hospital and St. Lukas Hospital. The results of this study have an importance to treat me and other patients, and to be used as an input for the future development of strategies or guidelines for diagnosing of smear negative pulmonary tuberculosis in Ethiopia. I had been also informed about the confidentiality of this study. The principal investigator requested me to participate in the study that would require my willingness to provide the required data that include sputum sample, and filling questionnaire. Therefore, with full understanding of the importance of the study, I agreed voluntarily to provide the requested samples and my benefit will be only from the free laboratory investigation result/s.

I_____ hereby give my consent for providing the requested information and specimens as the doctors find best for me.

Signature: _____ Date_____

Annex I Information sheet and consent form in Afaan Oromo Version

Unkawaliigaltee

YuunivarsitiiFinfinnee (Addis Ababaa) ttikolleejjiisaayinsiifayyaadipppaartimeentiisaayinsiilaaboraatooriittiwaraqaaqorannoodigiriilam maffaairrattiakkahirmaattaniifafferamtaniittu.Maalooyaadakanattiaaneejiru gad fageenyaandubbisuudhaanwaanisiniihingalledursaagaafadhaa.

Seensa

Mata-dureeqorannichaa ”Burden of smear negative pulmonary tuberculosis using grenexpert and culture methods among human immunodeficiency virus infected patients in Tulu bolo and St .Luke hospitals ,south west shewa ,oromia ,Ethiopia”.

Hirmaannaankeessanqorannoo kana keessattiguutuuguutuuttitola-ooltummaairrattikanhunda’eedha.

Hirmaachuudhiisuuyknhirmaaachuufwaliigaltaniiaddaankutuuyoobarbaaddantajaajillihospitaalakanattikennamukamuisinirraahincitu.Hirmaachuufeyyemamaayootaatanunkaakanarrattimallattessuuisinirraaeegama.

Qorannoo kana keessattihirmaachuufmaaltuisinirraaeegama?

Qorannoo kana keessattihirmaachuufyoomurteessitansumudiisinirraafuudhamuqorannoofakkaooluwaliifgaltu.

Ragaansumudakeessaniinamoota(qorattoota) qorannoo kana keessattiga’eeqabanyoobeekanakkahinmorminenimirkanneessitu. Inumaayyuuragaalee kun odeeffannoowwankanneenakkamaqaa, teessookeessanii fi bilbilakeessanhindabalatu. Inumaayyuutajaajila kana qofaafkanoolulakkoofsaaddaatukennama. Dabalataanisgaaffileetokkotokkokanneenfayyummaakeessaniinwalqabatannigaafatamtu.

Qorannookanarrattihirmaachuunrakkooninniqabumaali?

Sumuda kana yerookennitanrakkoocimaanisinhinmudatu.Ta’usogeesotamuuxannooqabanii fi eegumsacimaagodhantusumuda kana fuudhawaanta’eefdhukkubbiindhaga’amuhinjiru.

Ragaaleenfayyaadhoksaanisaaeegameeakkamittituruudanda’a?

RagaanSumudairraaargamuu fi odeeffannoonkeessanqorannoo kana qofaafoola. kanas kanbeekannamootamuraasaqorannoo kana keessattihirmaataqofatubeeka. Dabalataniskoomputerakeessattiolka'ameelakkoofsadhoksaannicufama.

Qorannoo kana keessattihirmaachuunbu'aanargamiisumaali?

Qorannoon kun waraqaaqorannookandigiriilammaffaafwaanta'eefqarshiinbu'aanargattanjiraachuubaatusragaaqorannookanaatiingaruuofiisfayyadamootaataniihawaasasfayyadamaanitaasiftu. .

Qorannichattihirmaachuufmirgoonnikeessanmaalfa'aa?

Qorannookanarrattihirmaachuunguutuuguutuuttifedhiikeessanirrattihundaa'eewaanta'eefyeroo fi bakkafedhiikeessaniittiaddaankutuukeessaniiftajaajillihospitaalichaankennamukamuuisinirraacituhinjiru.Dabalataaniswaanisiniihingallerrattiodeeffannoodabalataaargachuufmirgaguutuuqabdu.Ba'iiqorannoostolaanargattu. Haata'umaleeodeeffannoonkennitanrakkoo kana furuufwaan nu gargaaruudeebiisirriiakkanuufkennitankabajaangaafanna.

Gaaffiiyoonqabaadheyknrakkoonyoonamudatemaalangochuudanda'a?

Qorannookanaanwalqabateerakkootasaayooisinmudateykngaaffiiyooqabaattanteessoo kana gadiifayyadamaa.

Maqaa: KinduuGeetinnatLakkbilb: 09-12-88-57-73

Email: kindugetnet@yahoo,com

Annex I Informationsheet and consent Form in Amharic Version

የተሳታፊዎችፈቃድና መተማመኛቅፅ

በአዲስአበባዩኒቨርሲቲጤናሳይንስኮሌጅየሕክምናላቦራቶሪሳይንስት/ክፍልበማስተርስዲግሪተማሪየመመረቂያጥናትላይእዲሳተፉተጋብዘዋል፡፡ እባክዎበዚህጥናትለመሳተፍከመስማማትዎበፊትከዚህቀጥሎየሚገኘውንምንባብበጥሞናያንብቡናግልጽያልሆነ ልዎትንማንኛውንምሳብዩጠይቁ፡፡

መግቢያ

የጥናቱርዕስ ”Burden of smear negative pulmonary tuberculosis using grenexpert and culture methods among human immunodeficiency virus infected patients in Tulu bolo and St .Luke hospitals ,south west shewa ,oromia ,Ethiopia”.

የእርስዎበዚህጥናትላይየሚኖርዎትተሳትፎሙሉበሙሉበበጎፈቃደኝነ ትላይየተመሰረተነ ው፡፡ በዚህጥናትውስጥላለመሳተፍወይምለመሳተፍከወሰኑበኋላለማቋረጥየሚወስኑበሆንምእንኳንከዋበዚህሆስፒታልየሚሰጡዎትንማንኛውምአገ ልግሎትአይቋረጥም፡፡ በጥናቱለመሳተፍየማይስማሙከሆነ የስምምነ ትቅጹላይበጹሁፍወይምበጣትፊርማስቀመጥይጠበቅዎታል፡፡

የጥናቱተሳታፊለመሆንየሚጠበቅበዎትምንድንነ ው?

በዚህጥናትለመሳተፍየሚስማሙከሆነ ናሙናዎለጥናቱእንዲውልመስማማትይጠበቅብዎታል፡፡ ከተወሰደውናሙናላይየሚገኙመረጃዎችከዚህሆስፒታልውጪለሚገኙናለስራውአግባብነ ትላላቸውሰዎችበነ ገርየሚቃወሙትንመስማማትይጠበቅብዎታል፡፡ ይሁንእንጂይህአይነ ቱመረጃየርስዎንማንነ ትየሚገልጡመረጃዎችንማለትምስም፣ አድራሻናየስልክቁጥርየመሳሰሉትንመረጃዎቸንአይጨምርም፡፡ ይልቁንምለዚህአገ ልግሎትብቻየሚውልእርስዎንለማወቅየማያስችልመለያቁጥርጥቅምላይእንዲውልይደረጋል፡፡ በተጨማሪምስለርስዎአጠቃላይየጤናሁኔታለማቁርበአንዳንድተጨማሪጥያቄዎችመልስመስጠትይኖርብዎትአል፡፡

በዚህጥናትመሳተፍየሚያስከትላቸውችግሮችምንድንናቸው?

ናሙናበሚሰበሰብበትወቅትምንምአይነ ትየከፋችግርአያጋጥምዎትም፡፡ ሆኖምግንናሙናውንለመሰብሰብልምድያለውባለሞያስለሚወስድበአስፈላጊውጥንቃቄእርምጃስለሚወሰድየህመምስሜትአይኖርም፡፡

የህክምና መረጃዎ በሚስጥር ተጠብቆ መቆየት የሚችለው እንዴት ነው?

ስለ እራስዎ የሰጡት ማንኛውም መረጃና ከተወሰደው ናሙና ላይ የተገኘው የላቦራቶሪ ውጤት የሚያዙት ለጥናቱ አላማ ብቻ ነው፡፡ ይህንን ማህደር ሊያገኙ የሚችሉት የተወሰኑ የጥናቱ ተባባሪዎች ብቻ ናቸው፡፡ ከዚያም ባሻገር ስለ እርስዎ ያለው ማንኛውም መረጃ የተለየ የይለፍ ቃል ባለው ኮምፒውተር የመረጃ ማህደር ውስጥ እንዲቀመጥ ይደረጋል፡፡

በዚህ ጥናት መሳተፍ የሚያስገኛቸው ጥቅሞች ምን ምን ናቸው?

ይህ ጥናት የማስተርስ ዲግሪ መመረቂያ እንደመሆኑ መጠን በዚህ ጥናት በመሳተፍዎ በገንዘብ የሚያገኙት ጥቅም አይኖርም ጥናቱ በሚገኘው ውጤት ግን ተጠቃሚ ይሆናሉ፡፡

በዚህ ጥናት ተሳታፊ የመሆንዎ ጉዳቶች ምን ምን ናቸው?

በዚህ ጥናት መሳተፍ ሙሉ በሙሉ በእርስዎ ፈቃደኝነት የተመሰረተ በመሆኑ በማንኛውም ሰዓት ና ቦታ የማቋረጥ መብትዎ የተጠበቀ በመሆኑ ምንም ላይ እራስዎን ከጥናቱ በማግለልዎ ምክንያት የሚቀርብልዎት ምንም አይነት የሆስፒታል አገልግሎት አይኖርም፡፡ ከዚህ በተጨማሪ ጥናቱን በተመለከተ ማንኛውም አይነት ጥያቄ የመጠየቅ ና ገለጻ የማግኘት መብት አለዎት፡፡ የላቦራቶሪ ምርመራ ውጤቱን በነጻ ማግኘት ይችላሉ፡፡ ነገር ግን እርስዎ በሚሰጡን መረጃ የችግሩን ስፋት ለመከላከል እና ለመቆጣጠር ጠቃሚ ስለሆነ ለሚቀርብልዎት ጥያቄ ቀጥተኛ መልስ ይሰጡን ዘንድ በታላቅ አክብሮት እንጠይቃለን፡፡

ጥያቄ ካለኝ ወይም ችግር ቢያጋጥመኝ ማንን ማናገር ይገባል?

ይህንን ጥናት በተመለከተ ወይም ከዚህ ጥናት ጋር በተዛመደ መልኩ ስለሚያጋጥምዎ ነገር ተጨማሪ አደጋዎች ወይም ጥያቄ ካለዎት በሚከተለው አድራሻ ይጠቀሙ፡

ሞባይል፡ +251-9-12-88-57-73

ኢሜል፡ kindugetnet@yahoo.com

Annex I. Informed consent form in Amharic version

የተሳታፊዎችስምምነት ትሚጋገጫ

የሚከፕርቁፕር ----------

የተሳታፊዉስም----------

እኔስሜልላይየተጠቀሰዉተሳታፊ “To determine the burden of smear negative pulmonary tuberculosis using Genexpert and Culture in human immunodeficiency virus infected (ART) patients inTulubolo General Hospital and St. Lukas Hospital” ጥናትላይበቂገለጻተደርጎልኛል፡ ፡ ለጥናቱም sputumናሙናእንደሚጂስፊልግተግ ልዶልኛል፡ ፡ የጥናቱንምአላማ ዎችምተረድቻለሁ፡ ፡

በቃለመጠይቁላይየገለጽኳቸዉመረጃዎችብሙስጢርየተጠበቁእንደሚሆኑተነ ግሮኛል፡ ፡ በጥናቱላይያለመሳተፍ ናሜንኛዉንምመረጃያለመስጠትእንዲሀንሜንኛዉሚዜከጥናቱራሴንየማገለልመብቴየ ተጠበቀእንደሆነ ተገ ልዶልኛ ል፡ ፡

ስለዚህለዚህጥናትመረጃናየ ስምምነ ትቃሌንየ ሰጠሁትብአጠቃላይህሄ ታወንበመረዳትናበፍጹምፍቃደኝነ ትነ ው፡ በተ ጨማሪምኮያቄለመጠየ ቅተፈቅዶልኛለሜመቅየ ፈለኩትንያህልማቢራያአግኝቻለሁ፡ ፡ የዚህጥናትተሳታፊበመሆኔየማ ገኘወጥቅምሞ ሀሉንምሜርመዉጤበነ ጻማገኘትእንደሆነ ተረድቻለሁ፡ ፡

በአጠቃላይእኔከላይበመሞሜቅፅየተጠቀሱትንሀሉብሚ ባናበተረጋጋመፈስአንብቤዋለሁኝ፡ ፡ ስለዚህበዚህጥ ናትለመሳተፍፈቃደኛመሆኔንበፊርሜሔአረጋግጣለሁ፡ ፡

ፊርማ---------- ቀን ----/---/----------

(የ ስምምነ ትቅጽንማበብለሚቸሉተሳታፊዎች)

የአጣሪነ ርስስም---------- ፊርማ----------

ቀን----------

Annex II: Questionnaire

ADDIS ABABA UNIVERSITY COLLEGE OF HEALT SCIENCES

DEPARTMENT OF MEDICAL LABORATORY SCIENCES

Instruction I

It is aimed to screen that ART patients haveSymptoms of pulmonary tuberculosis, smear negative pulmonary tuberculosis, and to know HIVstatus of ART patients. This questionnaire contains series of brief questions that take 10 minutes to complete. Please, answer every question. Instructions for how to respond to the different questions are provided at the top of each page and answer each question according to you interviewed or asked. Note that there is no right or wrong answer, just what you perceive and what you think. All the information that you provide in this session will be confidentiality. Your response will be kept by the researcher and I will aggregate responses from all interviews so that no one individual will be identifiable.

Please, after you have completed answering all questions, check once that all questions are answered and finally submitted it to the data collector

Instruction II

1. Name of health facility_____
2. Please make 'X' mark in a given circle for close ended question
3. Please give your suggestion or short answer for the question items that requires your opinion

Section one: Socio- demographic Characteristics of Respondents

1. Sex_____ Male ☐ Female ☐

2. Age_____

< 10 ☐ 11-20 ☐-30 ☐

31-40 ☐ 41-50 ☐

>51 ☐

3. Level of education

Less than grade 12 ☐ grade 12 complete ☐ diploma ☐

1st degree ☐ MA/MSc ☐ MD ☐

4. Marital status

Married ☐ divorce ☐

Single ☐ widow ☐

5. Job category

Farmer ☐ private sector employee ☐

Government employee ☐

Driver ☐

NGO employee ☐

Merchant ☐

Other ☐

6. Monthly in come

0-600 ☐ 1651-3200 ☐ 5251-7800 ☐

601-1650 ☐ 3201-52500 ☐ 7801-10900 ☐

>10900 ☐

Section Two: Put circle on the rating that best reflects your feeling.

Ser. No	**Question**	**Response**	**addition information if any**
1	**PTB and SNPTB related awareness**		
1.1	What do you know about Pulmonary tuberculosis?	1,viral infection 2,bacterial infection 3,I don't know	

1.2	How you think PTB transmitted?	1,air borne 2,blood contact 3 , I don't know	
1.3	What you know about active and latent TB?	1,latent TB converted to active TB 2,Active TB converted to latent TB 3,I don't know	
1.4	What you know about SNPTB?	1,It is active TB 2,It is TB which is not detected by smear microscopy 3,I don't know	
1.5	What you think the Risk factor to SNPT?	1,co infection with HIV 2,Imunity depletion 3,all 4, I don't know	
2.	**PTB SIGN AND SYMPTOMS**		
2.1	Do you have the sign and symptoms of PTB?	1,Cough 2,night sweet 3,loss of appetite 4,weight loss 5,chest pain 6,fever	
2.2	Have you ever tested for PTB	1Yes 2No	
2.3	IF yes in question no2.2 what was your result	1,positive 2,Negative 3 , I don't know	
2.4	If positive in question 2.3 do you get the treatment	1Yes 2No	
2.5	If yes in question 2,4 for how long you take the treatment ?	1,For <2month 2,for 2-4 month 3,completed	
2.6	Is there PTB, MDR TB or extra PTB infected person in your family co-worker or relative?	1, yes 2 ,no	
2.7	Is there a history of previous TB treatment in family members, co-workers, friends with TB?	1, yes 2 ,no	
2.8	If yes in question no 2.7When and for how long Are there family members, co-workers, friends with TB or TB?	1,<1 year 2.1-2year 3,2-3year 4,>4 years	
2.9	What do you know about MDR TB?	1,it is the same to active TB	

		2,it is TB that is resistance to atleast2 drugs 3, I don't know	
3	**HIV status**		
3.1	How long have you been on ART drug	1<1 year 21-4 years 3.5- 8years 4,> 8 year	
3.2	Do you participate regular in CD4 follow up?	1,yes 2,n0	
3.3	Do you know your last CD4 count result	1 <200 2, 200-350 3,350-450 4,>450 5,I don't know	
3.4	What you know about HIV and TB co infection	1,strengthen immunity 2,reduce immunity 3,I don't know	
3.5	Dou you have tested for viral load?	1,yes 2,no	
3.6	Do you know your viral load result so how much?	1,<1000 2,1000-2000 3,>2000	

Annex III: Materials and procedures

1, Materialsand procedures of FM staining

A. Reagents, Supplies and equipment's

1. Auramine O

2. 0.5% Acid-alcohol

3. Counterstain (potassium permanganate)

a. Glass slides (25 by 75 mm), frosted ends desirable

b. sterile 50-ml conical polypropylene screw-cap tubes (aerosol free and graduated)

c, applicator stick

d, Bunsen burner

e, face mask

Binocular microscope with 10X, 40X, and 100X objectives

B. PROCEDURE

1. Cover the slide with auramine O stain for 20 minute

2. flood the slide with water.

3. cover the slide with 0.5% acid-alcohol for 3 minute

4.flood the slide with water.

5. cover the slide with potassium permanganate for 1 minute.

6. Air dry; do not blot.

7. Examine the smear with a fluorescent microscope 20x and 40 x objectives Mycobacteria are approximately 1 to 10 µm long and typically appear as slender rods. However, they may also appear curved or bent, coccobacillary, or even filamentous. Some may be beaded or banded.

2, materials and procedures of Z staining

A. Reagents,Supplies and equipments

1, Ziehl-NeelsenCarbol-Fuchsin Solution.

2, 1% Acid Alcohol:

3, Methylene Blue Working Solution:

4. Glass slides (25 by 75 mm), frosted ends desirable

5. Sterile 50-ml conical polypropylene screw-cap tubes c, applicator stick

6, Bunsen burner

7, face mask

8,Binocular microscope with 10X, 40X, and 100X objectives

B , PROCEDURE:

1.Make a smear on slide using applicator stick.

2 .Allow smear to dry in air

3. Fix with flame 2-3times

4. Cover the slide with Carbol-fuchsin solution

5. Heat the slide gently by flame then wait for 5 minutes.

6. Wash in running tap water.

7. Cover the slide by 1% Acid alcohol and wait for 3minutes

8. Wash in running tap water.

9. Cover with methylene blue for 30 seconds.

10. Wash slowly.

11. clean, and observe by 100x objective for presence of red rods

3, Materialsand procedures of Genexpert

A, supplies and equipment

GeneXpert System Equipped with GX2.1 software

Computer

Printer

Barcode reader

GeneXpert Cartridge: Single-use disposable XpertMTB/RIF cartridges

Sterile (individually packed) disposable transfer pipettes–

B ,Procedure for sputum sample (for Xpert MTB/RIF only)

1. Collect sputum in a sterile falcon tube (graduated from 4ml to 15ml).

2. Add approximately 2:1 ratio of sterile NaOH buffer

3. Mix the sample and buffer by then vigorously shake the tube 10 to 20 times

4. Incubate for 10 minutes at room temperature, and then shake the specimen vigorously again for another 10–20 times

8. Incubate the specimen at room temperature for an additional 5 minutes.

9. Using a fresh transfer pipette, transfer 2 ml of the processed sample to the Xpert MTB/RIF cartridge.

10. Load the cartridge into the GeneXpert instrument following the manufacturer's instructions.

C, reporting (result)

MTB DETECTED, Rif resistance NOT DETECTED

MTB DETECTED, Rif resistance DETECTED

MTB DETECTED, Rif resistance INDETERMINATE

MTB NOT DETECTED

4, materials and procedures of gram staining

A, materials and supplies

Reagents; gentian violate or crystal violate

Grams iodine

Acetone alcohol

Safranin

Equipments

Applicator stick

Slides

Binocular microscope

Immersion oil

Procedures

1,Make a smear on slide by sterile applicator stick

2,Fix by heat or alcohol

3,Cover withgentian violate or crystal violate for 1 minute

4 ,flood the slide by water

5,cover the slide with grams iodine for 1 minute

6, flood the slide by water

7, decolorize by acetone alcohol for 30 seconds

8, flood the slide by water

9, cover the slide with safranin for 1 minute

10, Wash the slide gently and allow it to dry by air observecharacteristics of the stain by 100x objective

5, materials and Procedure for specimen collection

Materials

• Sterile, disposable, single-use, screw-capped, conical centrifuge tubes (50 ml)

• Sponsor-provided Sputum Collection Kit

• Permanent marker

• Study Labels

• Disposable gloves

• Refrigerator with detached thermometer

• Cooler

• Cold packs

Sputum Collection

1. Collect specimens in appropriate sterile, disposable containers. Screw caps must fi t tightly to avoid leakage. 50 ml polypropylene centrifuge tubes are preferred.

2. Discuss the following collection procedures with the patient:

• Emphasize the nature of the desired specimen. − Inform the patient that nasal secretions and saliva are not sputum. − Explain that the desired specimen is produced by a deep cough and is thick, mucoid, white-yellow, and sometimes blood-tinged. It is from the lower airways and lung.

• Instruct the patient not to touch the inside of the collection container or lid with their fi ngers or other objects.

3. Positively identify the patient. Prepare two study labels (three for patient-collected samples) with the screening and/or subject ID number, date and time of collection, sputum specimen number (#1 or #2, or N/A if Visit 2 or Visit 3) and visit number for which the specimen is being collected. Place one study label on tube/container in which the sputum will be collected.

4. If able, instruct the patient to stand.

5. Give the patient a glass of water (bottled or boiled) to rinse the mouth free of food particles. Instruct the patient to rinse twice.

6. Instruct the patient to produce sputum, after rinsing mouth as described above. Use a demonstrator glass and tube/container to show the patient the procedure if this will help.

• Take a deep breath.

• Hold breath for a moment.

• Cough deeply and vigorously at the same time the breath is coming out. • Release sputum into the labeled tube/container by holding it to the lower lip and gently releasing the specimen.

• Close the tube/container tightly with the screw-on lid without touching the inside of the lid. Avoid spills or soiling the outside of the container.

7. If the patient cannot cough spontaneously, instruct the patient to take several deep breaths and hold the breath momentarily. Repeating this several times may induce coughing.

8. If a patient is unable to spontaneously expectorate a sputum sample, an attempt will be made to induce sputum production by aerosol inhalation. Contact the study physician to conduct this

9. After the specimen is collected, complete the following:

Annex IV: TB Laboratory Request and Report Form

TB Laboratory Request and Report Form (for AFB microscopy,GeneXpert and Culture)

1. PATIENT IDENTIFICATION:

Patient Full Name: _____Age(Yrs):_____Sex (M/F): _____MRN_____ Unit TB No:_____

Co-infection: (Y/N/Unknown)_____

Referring Unit/ Health Facility:_____Woreda_____Zone:_____

Address: Region: _____Zone/Sub-city: _____Woreda:_____Kebele:_____ Tele.: _____

Name of Contact person:_____Address:-Zone:_____Woreda_____Kebele_____Tele:___

2. TB DISEASE TYPE & TREATMENT HISTORY:

Site: ☐ Pulmonary ☐ Extra pulmonary (specify):_____**Previous TB drug use**: ☐ New ☐ First line ☐ second line

3. **REQUEST FOR TESTING AT TB LABORATORY:**

Reason: ☐ Diagnosis: If diagnosis, ☐ presumptive TB ☐ Presumptive Drug Resistance TB

☐ If presumptive Drug resistance TB, Patient registration Group (☐ New ☐ Relapse ☐ treatment after loss to follow-up ☐ after failure of first treatment ☐ after failure of re treatment ☐ MDR TB contact ☐ other

☐ Follow up: If Follow up, at _____months during treatment

Specimen Type: ☐ Sputum ☐ Other (Specify): _____

Requested tests: ☐ Microscopy ☐Xpert MTB/RIF test☐ Culture ☐ Line probe assay ☐ Phenotypic Drug Susceptibility Testing (DST)

Person requesting examination: Name_____ Signature: _____Date_____

Date specimen collected: ____/____/____ (Ethiopian Calendar) Collected By:-Name_____Sign_____

4. LABORATORY RESULT:

Lab. Ser. Number: _____ Date specimen received: ____/_____/____ (Ethiopian Calendar)

Specimen Appearance: Mucus/saliva/muco-purulent/bloody/other_____

4.1. Microscopic Examination Result:

Date of specimen collected	**Specimen Number**	**AFB Result**				
		Negative	**Positive**			
			Scanty	**1+**	**2+**	**3+**

☐Ziehl-Neelsen (ZN) ☐ Fluorescence
☐ Direct Smear ☐ Concentrated Smear

4.2. Xpert MTB/RIF test result (*to be completed in the laboratory)*

M. tuberculosis: ☐Detected ☐ Not detected ☐ Invalid / No result / Error

Rifampicin resistance: ☐ Detected ☐ Not detected ☐ indeterminate result

4.3. TB Culture result:

Date of specimen collected	Media used (liquid or solid culture)	Lab. serial number (s)	Result (Tick One)						
			Negative (0 colonies)	1-9 (<10 colonies)	+ (10-100 colonies)	++ (>100 colonies)	+++ (Innumerable/confluent growth)	NTM [1]	Contaminated

4.4. Drug susceptibility test (DST) and Line Probe Assay (LPA) results

Method [a]	Laboratory Serial number(s)	Results [b] (mark for each drug)									
		INH	RIF	Emb	Sm	Amk	Km	Cm	FQ	Other( )	Other( )

Comment: _____

_____

[a] Method, Specify: solid media DST; liquid media DST; direct LPA; indirect LPA

[b] Results codes: R = Resistant; S = Susceptible; C = Contaminated ; ND = Not done; NTM; Non Tuberculosis Mycobacterium

Date reported: ____/____/____ (E.C), Name/ Signature: _____Reviewed by: _____

Declaration

I, the undersigned agree to accept responsibility for the scientific ethical and technical conduct of the research project and for provision of required progress reports as per terms and conditions of the research publications office.

M.Sc. candidate: **KinduGetnet (BSc, MSc candidate)**

Signature: _____

Date of submission: _____

This thesis has been submitted with our approval as advisors.

Advisor: **KasuDesta (MSc, PhD)**

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.

Advisor: **RegasaDiriba(MSc, PhD candidate)**

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.