**ADDIS ABABAUNIVERSITY**

**COLLEGE OF HEALTH SCIENCE**

**SCHOOL OF ALLIED HEALTH SCIENCE**

**DEPARTEMENT OF MEDICAL LABORATORY SCIENCE**

# The burden of Pulmonary Tuberculosis and co-morbid disease and its associated risk factors among patients with malignant conditions visiting oncology department of Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia


**By:**

**Yonas Sebsibe (Bsc)**

**Advisor:**

**Kassu Desta (MSc, PhD fellow)**

**Abebaw Kebede (Msc, PhD fellow)**


**A thesis Submitted to Department of Medical Laboratory Sciences, School of Allied Health Science, College of Health Science, Addis Ababa University, for Partial Fulfillment of Masters Degree in clinical Laboratory Sciences. (Diagnostic and public health microbiology)**

**June, 2018**

**Addis Ababa, Ethiopia**

## Addis Ababa University

## School of Graduate Studies

This thesis prepared by **yonas sebsibe** which is entitled with "**The burden of TB/MDR TB and its associated risk factors among patients with all types of malignant conditions visiting oncology department of Tikur Anbessa Specialized Hospital"** is my original work and submitted for the partial fulfillment of the requirements for the degree of Master of Clinical Laboratory Sciences (Public health and diagnostic microbiology Specialty) complies with the regulations of the University and meets the accepted standards with respect to originality and quality. All sources of materials used for the thesis have been duly acknowledged.

**Approved by the Examining Board**

| | | |
|---|---|---|
| _____ | _____ | _____ |
| **Chairman, Dep. of Graduate Committee** | **Signature** | **Date** |
| Kassu Desta (MSc. PhD fellow) | _____ | _____ |
| Abebaw kebede (MSc. PhD fellow) | _____ | _____ |
| **Advisors** | **Signature** | **Date** |
| _____ | _____ | _____ |
| **External examiner** | **Signature** | **Date** |
| _____ | _____ | _____ |
| **Internal Examiner** | **Signature** | **Date** |

## Acknowledgement

I would like to thank Kassu Desta (Msc, PhD fellow), for thoughtful reviews of the manuscript and Abebaw Kebede(Msc,PhD fellow) for conscientious assistance in title selection.

I am also indebted to the Addis Ababa University College of Health Sciences, School Allied Health Science, Department of Medical Laboratory Science and its staff for financing my research under taking.

I extend my thanks to the Tikur Anbesa hospital oncology clinics, chest unit and laboratory staff of the hospitals for their good cooperation. We gratefully acknowledge the study participants for their willingness to participate in our study.

# Contents

## List of tables

## List of figures

## List of abbreviations

| | |
|---|---|
| EPHI | Ethiopia public health institute |
| LJ | Löwenstein Jensen |
| LPA | Line prob assay |
| MDR | Multi drug resistance |
| MGIT | Micobacterium growth indicator tube |
| NALC | N-acetyl L-cysteine |
| NaOH | Sodium Hydroxide |
| NTM | Non tuberculosis mycobacteria |
| TASH | Tikur anbessa specialized Hospital |
| TB | Tuberculosis |
| UV | Ultra violate |
| WHO | World health organization |
| ZN | Ziehl Neelsen |

## Operational definitions

**MDR-TB**: TB caused by Mycobacterium tuberculosis (M.Tb) strains resistant to at least isoniazid and rifampicin.

**PTB:** Pulmonary tuberculosis.

**TB co-morbidity**: Presence of culture confirmed TB, presenting with a cancer disease.

**Abstract**

**Background:** Tuberculosis (TB) and cancer are two major public health problems associated with significant morbidity and mortality. TB is one of the major causes of death amongst infectious diseases and it is estimated that one-third of the human population is infected with *Mycobacterium tuberculosis* .The burden of cancer is increasing globally. Co morbid conditions influence the survival of cancer patients.

**Objectives:** The objective of this study is to evaluate the burden of pulmonary TB/Rf resistance Tb,co morbid disease and associated risk factors among patients with all types of malignancies visiting the oncology unit of Tikur Anbessa Hospital

**Methods:** A Cross sectional study was performed in Tikur Anbessa Specialized Hospital from May 2017 to April 2018. Convenience sampling methods was used. Sputum sample was collected from all patients with malignancy that had cough. The sputum was treated with NaOH and isopropanol at a ratio of 2:1then added in to cartridge for Xpert test. Smear was prepared for conventional microscopy.

**Results:** A total of 206 patients with cancer were enrolled. Of these patients, 82 were male. Median age was 49 years, and range was from 18 to 80 years. 76 (36.9%) patients diagnosed with lung cancers; 20 (9.7%) had breast cancer; 18(8.7%) had hematologic malignancy; 17 (8.2%) patients had thyroid cancer; 15(7.3%) had Cervical ca; 13 (6.3%) esophageal cancer 11 (5.3%), colon cancer 9 (4.4%) Adenocarcinoma 8(3.9%) patients with Nasoparyngial cancer 7 (3.4%) had gastric cancer; 7(3.4%) and 12(5.8%) pancreatic carcinoma. During the study period, 6 patients developed tuberculosis .The prevalence rate of tuberculosis in the general population was 192 per 100,000 p, and the prevalence rate tuberculosis in the population with diagnosed malignancies was 2912 per 100,000 p.

**Conclusion and recommendation:** Patient with anti cancer treatment was more likely to develop tuberculosis than a patient without treatment. Patients on chemotherapy or on steroid for longer period of time (6-11months) are in more risk than who had for 24 months and more or not taken at all. Recognition, timely prevention and control of tuberculosis and associated factors may reduce morbidity and mortality in malignant patients.

**Keyword**: tuberculosis, cancer.

# 1. Introduction

## 1.1. Background

Tuberculosis (TB) is an old and well-known infectious disease; however, it remains a serious public health problem around the world [1]. *M. tuberculosis* is the causative agent of TB. It is an acid fast bacillus, weakly gram-positive and belongs to the genus Mycobacterium that includes more than 50 species. *M. tuberculosis* is a member of the *M. tuberculosis complex*. *M. tuberculosis,* M. *bovis, M. africanum,M. canetii, and M. caprae*, being *M. tuberculosis* the responsible for the vast majority of TB cases in humans. Infection by *M. tuberculosis* starts with inhalation of droplets of 1-5 μm in diameter droplets that contain tubercle bacilli from a person with pulmonary TB [2].

The World Health Organization estimates that one third of the population of the world has *Mycobacterium tuberculosis* infection. Eight million new cases of active tuberculosis are diagnosed annually, and 2 million people die of tuberculosis each year [3].Ethiopia ranked second after Nigeria in Africa and rated 7$^{th}$ among the 22 high burden countries (WHO, 2016) the incidence of TB is estimated at 192 new cases per 100,000 populations[4].

Tuberculosis (TB) and cancer are two major public health problems. The burden of cancer is increasing in economically developing countries as a result of aging, growth of the world population in addition to the increase in the adoption of cancer-associated lifestyle choices including smoking, physical inactivity and consumption of westernized calorie-dense food [5]. WHO has reported that 48.4% Ethiopian died annually due to cancer [4].

Cancer patients are at increased risk of tuberculosis infections due to their immunocompromised state, because of the underlying malignancy and its treatment. Since 1970, the American Thoracic Society and Centers for Disease Control and Prevention have recognized that cancer increases the risk of tuberculosis [6]

The incidence of tuberculosis has been reported to increase in patients with malignancies [7]. In cancer patients, TB occurs 9-22 times more frequently than in the general population [8]. Deterioration of immunity due to malignancy or immunosuppressive therapeutic interventions such as chemotherapy, corticosteroids and radiation therapy are responsible for immune defects in such patients and likely to play an important role in TB infection or reactivation [10, 11].

Although the association between tuberculosis and cancer (co morbidity) is an important clinical problem that needs consideration in order to increase the survival time of cancer patients and number of cancer survivors, least has been done in the area of especially tuberculosis prevalent countries. Moreover, there is no documented data concerning tuberculosis and its related determinants among cancer patients in Ethiopia. Therefore, the present study investigated the magnitude of TB among cancer patients and the cancer type-specific risk factors associated with TB infection in cancer patients visiting Tikur Anbesa hospital oncology centre, Addis Ababa, Ethiopia.

## 1.2. Statement of the problem

Ethiopia is one of the high burden countries for TB. However, the extent and the magnitude of the problem are not studied. TB and cancer are very common diseases and also the risk of tuberculosis is higher in people with immunodefiecient, the association between tuberculosis and cancer (co morbidity) is an important clinical problem that needs consideration in order to increase the survival time of cancer patients and number of cancer survivors [12].

Although several studies have reported on the Patients with malignant disease are immunocompromised, Patients with comorbid diseases may ignore symptoms or delay reporting. Delayed diagnosis is detrimental to patient outcomes, and untreated infectious pulmonary disease leads to have poorer survival, poorer quality of life, higher health care costs and further disease transmission possibility to others. As the identification of risk groups can help us in the early detection of individuals in need of TB treatment, it will help us to clarify the incidence and risk factors for TB among cancer patients [10]. More over there were no documented data concerning tuberculosis and its related risk factor in cancer patients in Ethiopia. We were try to assess the burden of Tb/Rif resistance Tb and identify association risk that refers to the correlation between TB and cancer in order to derive clinically useful information and to provide appropriate treatment to cancer patients in need of anti-Tb.

## 1.3. Significance of the study

TB is mainly occurred as results of immunosupresion, poor treatment adherence, comorbid disease and poor infection control practices. Ethiopia is one of the high burden countries for TB. However, the extent and the magnitude of the problem are not studied. So it is important to study the burden of TB /Rif resistance Tb and association risk factor in patients with malignancy. Such studied was not conducted before in the country. Thus,

- It will give new in sight for the Hospital and oncology centers that are working in patients with malignancy.
- Policy maker, programmer, manager and the public in general in ordered to set new technique to intervene of TB in malignant patients.
- Moreover, the study will be step stone for further research on association of cancer and TB/MDRTb infection.

# 2. Literature review

## 2.1. Burden of Tuberculosis in Cancer patients

The prevalence of TB is higher in immunocompromised patients, particularly those with lung cancer, head and neck cancer, and lymphophroliferative disorders [10].

Kim HR, *et al* has done research on 1809 eligible cancer patients in Korea. They found out that Active TB developed in 11 patients (0.6%) with cancer. The incidence of active TB per 1000 person-years was 3.07 in patients with cancer. Their study showed that patients with solid-organ malignancy have a 4.69 times higher risk of developing active TB than patients without malignancy [1].

Gi Hyeon *et al* have done a study in Korea that a total of 855,382 cancer patients and 1589,876 persons were observed. A total of 5745 patients developed tuberculosis; the mean incidence rate was 361.3 per 100,000, and the SIR was 2.22 (95% confidence interval [CI], 2.17–2.27).they demonstrated that the risk of tuberculosis in patients with cancer is higher than general population and the risk differs according to the type of cancer and remains elevated even 24 months after cancer diagnosis in a country of intermediate burden of tuberculosis [8].

Aoki et al. and Tamura *et al*. reported that in Tokyo National Hospital, the incidence of active TB among lung cancer patients was 1%–2%. Furthermore, Aoki *et al*. observed that active pulmonary TB was significantly higher in lung cancer patients than in those without ones (5.6% vs. 0.52%) [24].

## 2.2. Risk of Tb and association risk factor in Cancer patients

Chronic renal failure and anticancer chemotherapy were identified as independent risk factors for the development of active TB among patients with cancers [1].

Yoo CG *et al* have been conduct a study on Twenty-four patients and 48 control subjects were enrolled. They found that malignant disease and anticancer chemotherapy are risk factors for the development of tuberculosis [3].

From 1980 to 2004, Mini Kamboj et al has been following a total of 290 patients received a diagnosis of microbiologically-confirmed tuberculosis. Of these, 103 patients developed tuberculosis at the same time as or after receiving a cancer diagnosis. They confirmed that

following types of cancer and treatment were the risk of tuberculosis: "prolonged corticosteroid treatment, gastrectomy, leukemia, silicosis, Hodgkin's disease, pneumoconiosis, severe or poorly controlled diabetes, pregnancy, and children with measles or whooping-cough" Malignant disease and anticancer chemotherapy are risk factors for the development of tuberculosis or poor prognosis in tuberculosis patients [6].

Akinosoglou K.S *et al* have found that the increased utilization of anti-neoplastic agents in the treatment of hematological malignancies is associated with an increase tuberculosis infections and frequent complications in patients with hematological malignancies [7].
Chien-Yuan Chen *et al* in Taiwan found that the increased utilization of anti-neoplastic agents in the treatment of hematological malignancies is associated with an increase tuberculosis infection [14].

On their first population-based nationwide study Fang, Yi-Ping *et al* clearly demonstrate that significantly increased TB risk in patients with gastric cancer. Furthermore, old age, male sex, diabetes mellitus, and COPD were identified as independent risk factors for developing TB among patients diagnosed with gastric cancer [16].

*Lin JN et al* study showed that TB infection is related to the risk factors diabetes mellitus and hypertension. [27]
*workneh et.al* reviewed different studies in different countries and found out that the prevalence of TB among DM patient ranged from 0.38% in Taiwan to 14% in Pakistan and the Prevalence ranged from 0.38% in Taiwan to 14% in Pakistan .and the prevalence ranged from1.3 in Tanzania To 6.2% in Ethiopia .There was study in North America (Mexico) that showed a prevalence rate of 4.9%. Prevalence study from Europe that showed prevalence rate of 1.82% [28]

*Nissapatorn et al* found out that pulmonary tuberculosis was considered the most common form in diabetic patients and it showed a much higher proportion than non-diabetic ones. Relative risk of developing PTB the risk is 26 times higher in diabetes than the general population [31].

*Bates et al* reviewed several cohort studies that showed the relative odds of developing active TB ranges from two to eight in diabetic patients versus non diabetic controls [32].

Ibrahim EM et al had been on study for over six years period on CA patients, Of 2,143 biopsy proven cancer patients and 4 (0.19%) patients developed active tuberculosis (TB) during anticancer therapy or shortly after its completion. The cancer diagnoses of those patients were non-Hodgkin's lymphoma, breast cancer, chronic myelogenous leukemia [33].

*Nair R et al* pointed out certain malignancies and anticancer chemotherapy schedules are considered as risk factor for the development of tuberculosis [34].

Marais B et al conduct a Study that consistently show a twofold to three fold higher risk of developing tuberculosis in patients with diabetes mellitus compared with people without diabetes. In a study from the Indian state of Tamil Nadu, nearly 50% of patients with tuberculosis had either diabetes mellitus (25·3%) or pre diabetes (24·5%).n a Chinese survey of 8886 registered tuberculosis cases, 1090 (12·4%) patients had diabetes mellitus [35].

### 2.3. Cancer type and risk of Tuberculosis

C-Y. Wu *et al* in Taiwan have conduct a study on suspected group and found that the incidence of TB per 100 000 person-years was 339 in the cancer patients. TB incidence among cancer patients in total, 205 persons developed active TB (1.2%).Patients with aero digestive tract, lung and hematological cancers are especially vulnerable to TB. The results showed a significantly higher TB incidence rate among oral, esophageal and lung cancers as well as non-Hodgkin's lymphoma, together with an increasing trend for nasopharyngeal cancer and leukemia [11].

Chen et al. and Mishra *et al.* reported that patients with AML rather than other types of hematologic malignancies had a higher rate of tuberculosis infections due to Mycobacterium tuberculosis. It can precede or occur simultaneously or during treatment of hematological malignancies. The prevalence in those patients ranges between 2.1 and 2.6% and when present it is usually disseminated [15].

Kaplan *et al.* reviewed 201 cases of TB in United State, Carcinoma coexisting in various organs and found that certain neoplasms like Hodgkin's disease, lymphosarcoma, reticulum cell carcinoma, and lung cancer had a high prevalence of TB [17].

*Omoti et al* pointed out 3.4% the incidence of TB patients in haematological cancer patients in an endemic area were undergoing anticancer chemotherapy. [24]

El sharawi et al, pointed out coexistence of TB with cancer was more common in the solid tumour malignancy group, followed by the haematological malignancy group. The frequencies

of TB-positive cases in bronchogenic, haematological and solid tumour malignancy groups were 21%, 25% and 30%, respectively. [25]

A case series in multiple hospitals in Japan in 1990 involving 445 cases had been observed by *Tubura et al* showed that various types of solid-organ malignancies placed patients at risk for active TB and that the introduction of chemotherapeutic agents increased the risk of TB progression and also the authors concluded that the analyzed results were the major underlying diseases of compromised hosts with tuberculosis were diabetes mellitus and various types of cancer. In terms of risk factors they mentioned that major risk factors for advancement of tuberculosis were nutrition and immunosuppressions due to long term use of corticosteroids and anticancer agents [26].

*Dobler et al* conducted meta-analysis that showed a statistically significantly increased risk of TB in cancer patients, compared to the general population. Most solid cancers were associated with an approximately two-fold increase in the risk of developing TB, compared to the general population [29].

*Suzuki et al* conducted observational study included 904 consecutive patients diagnosed with histologically confirmed lung cancer and nine lung cancer patients (1.00%) developed TB during the observation period. In all cases, TB occurred within 2 years of the diagnosis of lung cancer. The cumulative incidence of TB at 6 months, 1 year, and 2 years was 0.65%, 1.15%, and 1.38%, respectively [30].

*Nair R et al* pointed out that the prevalence of tuberculosis in patients with hematological malignancies has been reported to be between 0.72% and 2.6%. [34]

# 3. Objectives

## 3.1 General Objectives

To determine the burden of pulmonary Tuberculosis associated risk factors and Rif resistance among patients with all types of malignancies visiting the oncology unit of Tikur Anbessa Hospital Addis Ababa, Ethiopia

## 3.2 Specific Objectives

- To determine the magnitude of TB case among patients with all types of malignancies
- To determine the magnitude of Tb/ Rif resistance among the different types of malignancies
- To determine the risk factors associated with TB.

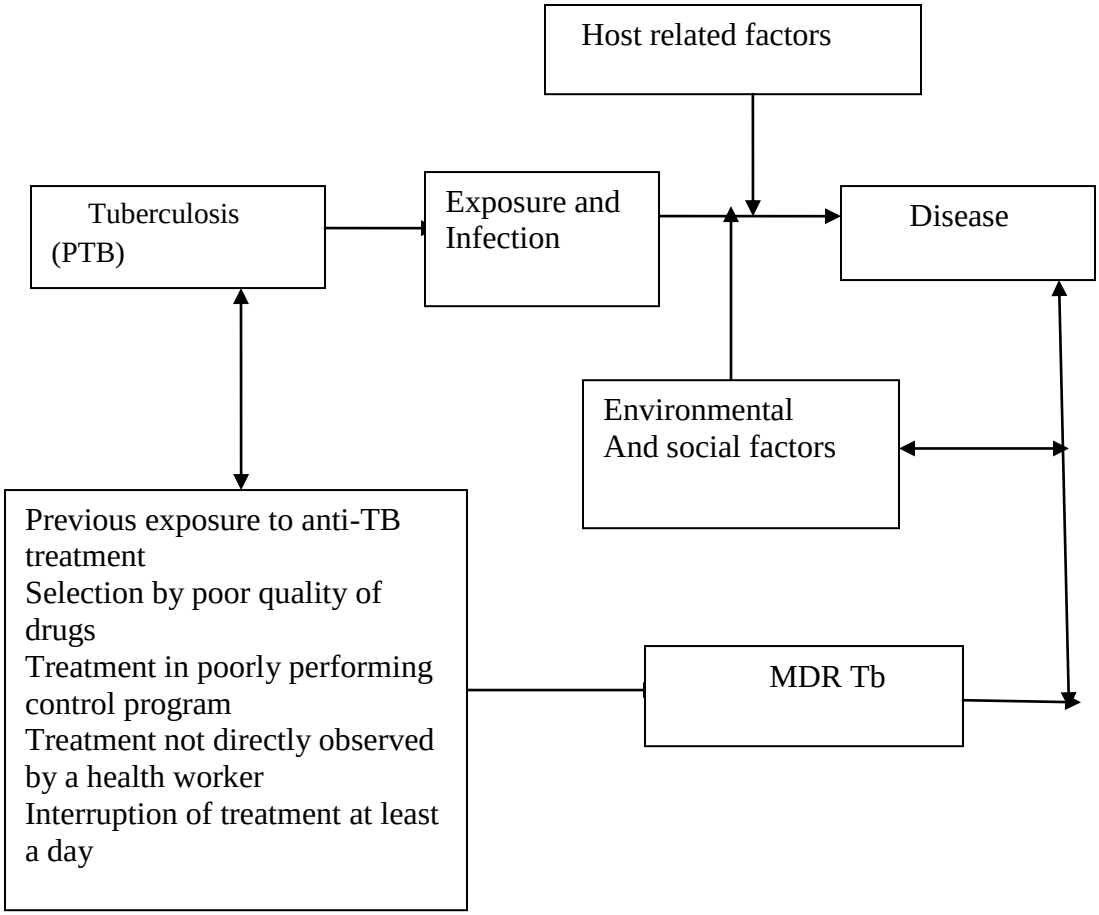


Fig1. Risk factors associated to the occurrence of MTB in Ethiopia

## 4. Hypothesis

Null hypothesis of the research: No association between *M. Tuberculosis* infection and cancer patients

# 5. Materials and methods

## 5.1. Study area

The study was conducted in Tikur Anbesa Specialized Hospital Radiotherapy Oncology Center found in Addis Ababa city in kirkos sub city. Addis Ababa 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.

Tikur Anbessa Specialized Hospital (TASH) is the largest referral hospital in the country .The hospital start service in 1972. In 1998, the TASH was transferred to the School by the Federal Ministry of Health, and it has since become a University teaching hospital. The Tikur Anbessa Specialized Hospital is now the main teaching hospital for both clinical and preclinical training of most disciplines. It is also an institution where specialized clinical services that are not available in other public or private institutions are rendered to the whole nation.

The hospital has 600 beds, of which 18 are allocated for cancer treatment. Of the 201 physicians at the hospital, only two are hematologists, four are medical oncologists, four are radiotherapists, two are surgical oncologists, and one is a pediatric oncologist. Three palliative pain specialists also work at the hospital. Only 26 of the Tikur Anbesa hospital627 nurses are dedicated oncology nurses. The hospital has one CT scanner and one MRI scanner. In 2010, the hospital saw more than 260 000 patients in total, including more than 2000 adults and more than 200 children with cancer. The Federal Ministry of Health estimates that there could be more than 150,000 cancer cases in Ethiopia each year.

## 5.2. Study design and period

A hospital-based cross sectional study was conducted from May 2017 to April 2018

## 5.3. Source of population

The source populations were all Hospital Clients or patients who visit Tikur Anbesa Hospital Radiotherapy Oncology Center during the study period

## 5.4. Study population

The study populations were adult cancer patients' age ≥ 18 with sign symptom of TB infection during the study period.

## 5.5. Inclusion and exclusion criteria

### 5.5.1. Inclusion criteria

All type of cancer patients manifesting clinical symptom of TB include fever, chronic cough for more than 2 weeks, fever, night sweats and willing to participate in the study was included.

### 5.5.2. Exclusion criteria

- Previously confirmed pulmonary tuberculosis (PTB) cases
- Known pulmonary tuberculosis patients who were on anti-TB treatment
- Pulmonary TB co-infection with HIV was excluded from the study.

## 5.6. Study variables

### 5.6.1. Dependant variable

Prevalence of Mtb/Rif resistance tuberculosis disease

### 5.6.2. Independent variables

- Age
- sex
- previous history of TB infection
- specific cancer type
- History of chronic illness
- Smoking history
- Alcohol abuse
- Steroid

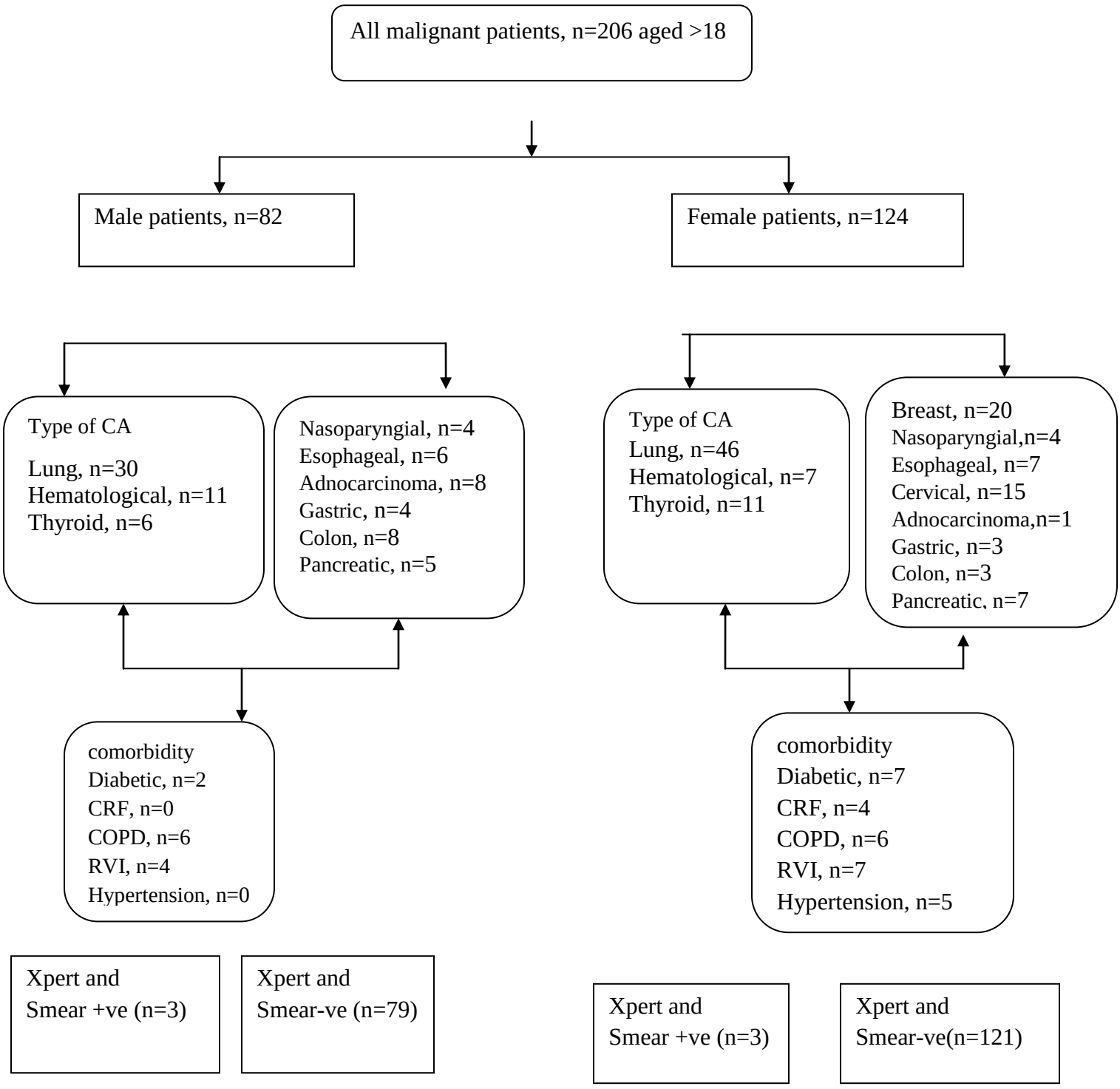


Fig 2.Summery of Diagnosis of sputum on Xpert and direct microscopy for detection of M. tuberculosis from patients with malignancy

## 5.7. Measurements and data collection

### 5.7.1. Sample size determination

All malignant patients who visited the clinic and met selection criteria from January 1, 2017 up to December 2018 had taken sampling size so I didn't need calculated.

### 5.7.2. Sampling method

A convenient sampling technique was applied to recruit study participants who fulfilled the inclusion criteria were utilized in this study.

### 5.7.3. Data collection procedure

Structured and proper data collection form was used to collect/record demographic information of the patients, contact number, type of disease, and physical examination or the preclinical findings. Data from all cancer patients with sign and symptom of TB at the time of the study and volunteer to participate in the study period was utilized for this study. Sample collection, transportation, processing and interpretation of result were as sop of the hospital policy.

## 5.8. Laboratory methods

### 5.8.1. Specimen collection

Sputum samples were collected from the oncology centers as per the collection and transportation policy of the laboratory and transport to St.peter hospital laboratory. Xpert assay and AFB smear was performed from one fresh 2-5mL amount of the sputum sample.

### 5.8.2. Direct microscopy

Direct Smear microscopy was performed to investigate presence of acid fast bacilli with the specimen using conventional ZN staining method. Slides showing red coloured acid fast bacilli were taken as positive and negative slides were those without any acid fast bacilli [36].

### 5.8.3. GeneXpert MTB/RIF assay

GeneXpert testing performed according to the manufacturer's instructions. Sample reagent added to untreated sputum was treated with sample reagent (SR) containing NaOH and isopropanol at a

ratio of 2:1, manually agitated and kept for 10 min at room temperature, then shaken again and kept for 5 min; 2 ml of the inactivated material transferred to the test cartridge and inserted into the test platform. [22]

## 5.9. Data quality assurance

### 5.9.1. Pre analytical

Data collection was conducted after the participant informed the purpose of the study and when gives consent. Demographic data was collected .Standard operational procedures of the host laboratory had been ensured the reliability and validity of test result. Specimens were collected in clean, sterile containers with a tight-fitted lid or cap. Sputum sample and data collection were done by trained personnel .The specimens were expectorated sputum and not saliva, with a volume of about 2-10 ml each. Specimens were transported to the laboratory as quickly as possible. Specimens were transported in a container, such as an ice box, in which temperature was maintained as low as possible. Samples were Receive according to the Sample Reception procedure. The specimens were refrigerated and processed as soon as possible. For samples which cannot be possibly analyzed the same day had been Stored in the 2-8°C overnight and placed in sample racks in the same order as registered in the worksheet and request forms.

### 5.9.2. Analytical

All laboratory tests were performed by well-trained laboratory personnel. Standard operational procedures of the host laboratory had been ensured the reliability and validity of test result. All Samples brought to the testing bench were analyzed immediately. To avoid subjective interpretation of test results the laboratory personnel processing the sputum samples for TB culture and Xpert MTB/RIF were blinded to the results of the other test.

### 5.9.3. Post analytical

Standard operational procedures of the host laboratory were used to ensure the reliability and validity of test result. Reports were recorded as soon as results are ready. Sample rack were Stored at -20°C

## 5.10. Data entry analysis and analysis

All laboratory and clinical data were recorded on logbook during the study period. Each completed data were properly coded and key was prepared for each code. Data were recorded in

standard spreadsheets separately for quality purpose. Collected data were entered and cleaned into a computer using SPSS version 24 statistical package. Frequency distribution and percentage calculation were made to describe socio-demographic characteristics and to determine the magnitude of the relative burden of Tb in cancer patients. Crude and adjusted odds ratio were done to determine whether any association existed with a 95% confidence interval. Binary logistic regression analysis was made to see the relative effect of independent variable (socio-demographic variable) on the dependent variable (burden of Tb in cancer patients). P-value less than 0.05 considered statistically significant.

## 5.11. Ethical considerations

This study was carried out after getting ethical clearance and formal letter of cooperation obtained from 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 to conduct the study. The letter was taken to Tikur Anbesa Specialized Hospital. Permission was obtained from TASH ethical review board. The purpose of the study was explained to radiology department head and all research ethical issues to keep the privacy, confidentiality, beneficence and justice to make the study under the legal frame work of the institutional board review strictly adhered to. An informed written consent was obtained from all participants. All the necessary precautions were taken to keep all documents in a safe and secure place. Names and other identification of the study subjects were omitted during data collection.

# 6. Results

## 6.1. Tuberculosis prevalence in patients with cancer

A total of 206 patients with sign symptom of TB particularly with cough were enrolled between May-feb 2018. Median age was 49 years, and range was from 18 to 80 years. The majority were female124 (60.8), married 164(79.6) and were illiterate 75(36.4). Seventy one participants were housewives (**Table 1**).Including 76 (36.9%) patients diagnosed with lung cancers; 20 (9.7%) patients with breast cancer; 18(8.7%) patients with hematologic malignancy; 17 (8.2%) patients had thyroid cancer; 15(7.3%) patients with Cervical ca; 13 (6.3%) patients with esophageal cancer 11 (5.3%), colon cancer 9 (4.4%) Adenocarcinoma 8(3.9%) patients with Nasoparyngial cancer 7 (3.4%) had gastric cancer; 7(3.4%) and 12(5.8%) pancreatic carcinoma.

During the study period, 6 patients developed tuberculosis. 2(33.3%) patients were with hematological malignancy. 4(66.7%) patients had solid-organ tumors (T**able 3**). Among symptomatic patients, the most common symptoms were fever (86 patients) and cough (206 patients). 31 (15%) had received steroids. Out of 6 patients who had tuberculosis infection, one Patient (1 of 6) was on steroids. No patients had ever received anti-tuberculosis drugs. No MDRTb had been observed.2.9% (6/206) were positive for TB on genexpert, 1.9% (4/206) on smear microscopy (Table 5).

The most prevalent comorbidity was COPD 12(29.2%), followed by DM 9(21.9), HT5 (12.1%) and CRF 4(9.7%).Among the 41confirmed comorbidity case 9 (22%) were with diabetes, 12 (29%) with COPD; 11 (27%), Hypertension; 4(9.7%), CRF. 2(6.9%). Diabetic patients were more likely to develop TB disease than patients with other comorbidity( T**able 2**) .

**Table1.** *Socio demographic data of patients with malignancies, Addis Ababa Ethiopia 2018*

| Variables | Frequency | Percent |
|---|---|---|
| **Age** | | |
| **18-44** | 88 | 42.7 |
| **44-54** | 65 | 31.6 |
| **55-64** | 33 | 16.5 |
| **65-74** | 17 | 7.8 |
| **>74** | 3 | 1.5 |
| **Sex** | | |
| **Male** | 82 | 39.2 |
| **Female** | 124 | 60.8 |
| **Marital status** | | |
| **Single** | 27 | 13.1 |
| **Married** | 164 | 79.6 |
| **Divorced** | 6 | 2.9 |
| **Widowed** | 9 | 4.4 |
| **Educational status** | | |
| **Illiterate** | 75 | 36.4 |
| **Primary** | 68 | 33.0 |
| **Secondary** | 41 | 19.9 |
| **Tertiary** | 22 | 10.7 |
| **Occupation** | | |
| **Government** | 39 | 18.9 |
| **Private** | 45 | 21.8 |
| **Daily laborers** | 23 | 11.2 |
| **House wife** | 71 | 34.5 |
| **Student** | 7 | 3.4 |
| **Farmer** | 18 | 8.7 |
| **Retire** | 3 | 1.5 |

Of the 206 participants, 18% drank alcohol, 11.7% had smoking history, and 15% of participant had taken steroid. 11.2% participant had previous TB history. Out of those patients who are on treatment 42.7% taken for 1-5months, 24.8% had taken for 6-11months, 3.4% had taken for 12-24months.28.6% of patients were not on treatment (**Table 2**)

**Table 2 C***linical characteristics patients with malignancies and clinical presentation, Addis Ababa Ethiopia 2018*

| Variables | Frequency | Percent |
|---|---|---|
| **Alcohol drinking** | | |
| **Yes** | 37 | 18 |
| **No** | 169 | 82 |
| **Smoking** | | |
| **Yes** | 24 | 11.7 |
| **No** | 182 | 88.3 |
| **Steroid** | | |
| **Yes** | 31 | 15 |

| | | |
|---|---|---|
| **No** | 175 | 85 |
| **Tb history** | | |
| **Yes** | 23 | 11.2 |
| **No** | 183 | 88.8 |
| **Treatment duration** | | |
| **No treatment** | 59 | 28.6 |
| **1-5months** | 88 | 42.7 |
| **6-11months** | 51 | 24.8 |
| **12-24months** | 7 | 3.4 |
| **>24months** | 1 | 0.5 |
| **CA stage** | | |
| **Insitu** | 41 | 19.9 |
| **I** | 25 | 12.1 |
| **II** | 53 | 25.7 |
| **III** | 46 | 22.3 |
| **IV** | 41 | 19.9 |
| **Clinical presentation** | | |
| **Cough** | | |
| **Yes** | 157 | 76.2 |
| **No** | 49 | 23.8 |
| **Fever** | | |
| **Yes** | 91 | 44.2 |
| **No** | 115 | 55.8 |
| **Night sweat** | | |
| **Yes** | 58 | 28.2 |
| **No** | 148 | 71.8 |
| **Diagnostic time** | | |
| **<1year** | 78 | 37.9 |
| **1-3** | 104 | 50.5 |
| **4-6** | 22 | 10.7 |
| **7-9** | 2 | 1.0 |
| **>10years** | 0 | 0 |
| **Comorbidity** | | |
| **Diabetes** | 9 | 4.4 |
| **CRF** | 4 | 1.9 |
| **COPD** | 12 | 5.8 |
| **Hypertension** | 5 | 2.4 |

## 6.2. Prevalence of TB on specific cancer types

Among the 206 enrolled patients, 17 (8.3%) had thyroid cancer and 17 (8.3%) had gastric cancer; 12 (5.8%), pancreatic carcinoma; 11 (5.3%), colon cancer; 20 (9.7%), breast cancer; 18(8.3%), hematologic malignancy; 13 (6.3%), esophageal cancer; 8(3.9%) Nasopharyngeal cancer; 15(7.3%) Cervical ca; 9 (4.4%), Adnocarcinoma and 76 (36.9%), had lung cancers. The overall incidence of TB disease in patients with malignancies during the study period was 3.9% lung cancer, 5.5%hematological,

5.9% breast cancer and 8.3% in pancreatic cancer patients. Pancreatic cancer was more likely to develop TB disease than patients with other malignancies (**Table3**).

**Table 3** *the prevalence rate of tuberculosis to the cancer type and co morbid disease, Addis Ababa Ethiopia 2018*

| Variables | No.of patients with cancer | No. of patients with cancer who developed tuberculosis | Prevalence of of tuberculosis |
|---|---|---|---|
| **Lung cancer** | 76(36.9) | 3 | (3.9) |
| **Hemtological** | 18(8.7) | 1 | (5.5) |
| **Thyroid** | 17(8.3) | 1 | (5.9) |
| **Breast cancer** | 20(9.7) | 0 | (0.0) |
| **Nasoparyngial cancer** | 8(3.9) | 0 | (0.0) |
| **Esophageal** | 13(6.3) | 0 | (0.0) |
| **Cervical ca** | 15(7.3) | 0 | 0(0.0) |
| **Adnocarcinoma** | 9(4.4) | 0 | (0.0) |
| **Gastric cancer** | 7(3.4) | 0 | (0.0) |
| **Colon cancer** | 11(5.3) | 0 | (0.0) |
| **pancreatic cancer** | 12(5.8) | 1 | (8.3.) |
| **Diabetes mellitus** | 9(4.4) | 2 | (6.9) |
| **CRF** | 4(1.9) | 0 | (0.0) |
| **COPD** | 12(5.8) | 0 | (0.0) |
| **Hypertension** | 5(2.4) | 0 | (0.0) |

### 6.3. Associate risk factor and Prevalence of TB on patients with malignancy

The prevalence rates of tuberculosis on patient with malignancy differed depending on treatment . Prevalence rate was 59 (1.7%) for those with no treatment, 88(1.1%) had taken for1-5months, 51(5.9%) for 6-11months, 7(14%) for 12-23months and 1(0.0%) patients had taken for 24 months and more (Table4).Out of 206 patients with malignancies that categorized in stages 19.9% was Carcinoma insitu, 12.1% stage I, 25.7% was stage II, stage III was 22.3% and stage IV was 19.9% (table4).

**Table4.** Prevalence of Tb and multivariable regression model measured adjusted ratio and 95% confidence interval (CI) of type of cancer by demographic status, co-morbidity, stage of lung cancer and anti CA treatment.

| Variables | Cancer patients (n=206) n (%) | Tb positive | COR | P | 95%CI | AOR | P | 95%CI |
|---|---|---|---|---|---|---|---|---|
| **Age** | | | .966 | .282 | .908-1.028 | | | |
| **<44** | 88(42.7) | 1(16.7) | | .999 | | | | |
| **45-54** | 65(31.6) | 3(50) | | .999 | | | | |
| **55-64** | 34(16.5) | 0(0) | | 1.00 | | | | |
| **65-74** | 16(7.8) | 2(33.3) | | .999 | | | | |
| **>74** | 3(1.5) | 0(0) | | | | | | |
| **Sex** | | | | | | | | |
| **Male** | 82(39.8) | 3 | .653 | .607 | .129-3.316 | | | |
| **Female** | 124(60.2) | 3 | | | | | | |
| **Comorbidity** | | | | | | | | |
| **Diabetes mellitus** | 9(21.9) | 2 | .065 | .006 | .009-.452 | .106 | .049 | .011-.991 |
| **Chronic renal failure** | 4(9.7) | 0 | | .999 | | | | |
| **Hypertension** | 5(12.1) | 0 | | .999 | | | | |
| **COPD** | 12(29.2) | 0 | .185 | .160 | .018-1.945 | | | |
| **RVI** | 11(26.8) | 1 | | .999 | | | | |
| **Marital status** | | | | | | | | |
| **Single** | 27(13.1) | 1 | 3.2 | .423 | .182-58.0 | | | |
| **Married** | 164(76.6) | 4 | 5.0 | .171 | .5-50.0 | | | |
| **Divorced** | 6(2.9) | 0 | | .999 | | | | |
| **Widowed** | 9(4.4) | 1 | | | | | | |
| **Educational level** | | | | | | | | |
| **Illiterate** | 75(36.4) | 1 | 7.4 | .109 | .638-85.8 | | | |
| **Primary** | 68(33.0) | 2 | 3.3 | .247 | .437-24.9 | | | |
| **Secondary** | 41(19.9) | 1 | 4.0 | .269 | .342-46.8 | | | |
| **Tertiary** | 22(10.7) | 2 | | | | | | |
| **Smoking** | | | .118 | .247 | .043-1.429 | | | |
| **Yes** | 24(11.7) | 2 | | | | | | |
| **No** | 182(88.3) | 4 | | | | | | |
| **Alcohol drinking** | | | .333 | .424 | .075-2.408 | | | |
| **Yes** | 37(18) | 2 | | | | | | |
| **No** | 169(82) | 4 | | | | | | |
| **Steroid** | | | .882 | .910 | .100-7.820 | | | |
| **Yes** | 31(15) | 1 | | | | | | |
| **No** | 175(85) | 5 | | | | | | |
| **CA stage** | | | | | | | | |
| **Insitu** | 41(19.9) | 0 | .967 | | | | | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **I** | 25(12.1) | 0 | .998 | | | | | |
| **II** | 53(25.7) | 3 | .998 | | | | | |
| **III** | 46(22.3) | 2 | .456 | .417 | .042-4.160 | | | |
| **IV** | 41(19.9) | 1 | .631 | .550 | .048-6.30 | | | |
| **TB history** | | | .111 | .010 | .021-.588 | .436 | .402 | .062-3.042 |
| **Yes** | 23(11.2) | 3 | | | | | | |
| **No** | 183(88.8) | 3 | | | | | | |
| **Night sweat** | | | .072 | .018 | .008-.631 | .114 | .065 | .011-1.146 |
| **Yes** | 58(28.2) | 5 | | | | | | |
| **No** | 148(71.8) | 1 | | | | | | |
| **Treatment duration** | | | .443 | .074 | .181-1.083 | | | |
| **No treatment** | 59(28.6) | 1(1.7%) | | | | | | |
| **1-5months** | 88(42.7) | 1(1.13%) | | | | | | |
| **6-11months** | 51(24.8) | 3(5.9%) | | | | | | |
| **12-23months** | 7(3.4) | 1(14.2%) | | | | | | |
| **>24months** | 1(0.5) | 0 | | | | | | |
| **Diagnosis time** | | | .830 | .754 | .260-2.650 | | | |
| **<1year** | 78(37.9) | 1 | | | | | | |
| **1-3years** | 104(50.5) | 5 | | | | | | |
| **4-6** | 22(10.7) | 0 | | | | | | |
| **7-9** | 2(1.0) | 0 | | | | | | |
| **>10years** | 0(0) | 0 | | | | | | |
| **CA type** | | | 1.079 | .472 | .878-1.326 | | | |
| **Lung** | 76(36.9) | 3 | | | | | | |
| **Gastric** | 7 (3.4) | 0 | | | | | | |
| **Colon** | 11 (5.3) | 0 | | | | | | |
| **Breast** | 20(9.7) | 0 | | | | | | |
| **Hemato** | 18(8.7) | 1 | | | | | | |
| **Thyroid** | 17(8.3) | 1 | | | | | | |
| **Panceatic** | 12(5.8) | 1 | | | | | | |
| **Nasopha** | 8 (3.9) | 0 | | | | | | |
| **Esopha** | 13(6.3) | 0 | | | | | | |
| **Cervical** | 15(7.3) | 0 | | | | | | |

All of the 206 who reported to have symptoms suggestive of TB gave morning sputum for xpert, and 6 (2.9%) of them were positive for Mycobacterium tuberculosis. From the 6 xpert positives, 24 (11.7%) had smoking history, 37 (18%) were alcoholic, 31(15%) had been taking steroid. A

total of 206 individuals were enrolled in the study. Of these, 41(19.9%) were insitu, 25(12.1%) were stage I, 53(25.7%) were stage II, 46 (22.3%) were stage III and 41(19.9%) were stage IV. Of those patients 59(28.6%) without treatment, 88(42.7%) had been taking for about1-5mon, 51(24.8%) for 6-11mon, 7(3.4%) for12-23mon and 1(0.5%) for more than 24months (Table4). Of the 6 M. tuberculosis xpert positive, 2 (33.3%) were found to be negative for AFB to. Primary MDR-TB was not detected.

## 7. Discussion

This is the first study, to my knowledge, to investigate the burden of TB and its relationship with co morbid disease, cancer type and others associated risk factors. 48.4% per 100,000 population mortality occurred in Ethiopia due to cancer annually. Ethiopia is a high-burden country for TB, with an estimated incidence of 192 of 100,000 populations per year in 2015 [4].In the current study, we estimated the prevalence of Tuberculosis among malignant patients to be approximately 2912 per 100,000. It is increased by fifteen fold of general population. Our finding was relatively higher than with a study in united state by *Libshitz et al.* reported that incidence of TB disease in cancer patients a rate nine times greater than the general population [9].This difference might be due to higher Tb prevalence in Ethiopia. There is also similar study by *KIM et al* showed that patients with solid-organ malignancy 4.69 times higher risk of developing active TB than patients without malignancy [1].

We demonstrated that the risk differs according to the type of cancer in a country high burden of tuberculosis. In our study, 1(8.3%) pancreatic cancer is the most prevalent malignancy, followed by 1(5.9%) thyroid cancer, 1(5.5%) hematological and 3(4.3%) lung malignancy .On the contrary, *Kaplan et al.* reported Hodgkin's lymphoma followed by sarcoma, leukemia and lung cancer[17]. it might be due to the small sample size.

*Chen et al* pointed out that the incidence of TB disease for patients with hematological malignancies two-fold above the general population. (14) This finding was in accordance with our result 2.5 fold above the general population. This finding is in agreement with those of previous studies [20, 29].And also in agreement with *Al-Anazi et al.* reported the prevalence of TB in patients with hematological malignancies ranges between 2.1 and 2.6% [20]However; *Kamboj et al* pointed out those patients with hematologic neoplasm have tuberculosis rates approximately 40 times greater than the current rate among the US population [6]. It's because of high immigrants and HIV epidemic at the time.

In present study Frequencies of tuberculosis infection in hematological and solid tumor malignancy groups were 17% and 83%, respectively. This finding is in agreement with *El-Sharif et al* that pointed out frequencies of TB-positive cases in bronchogenic, haematological and solid tumour malignancy groups were 21%, 25% and 30%, respectively. Therefore, coexistence of TB

with cancer was more common in the solid tumour malignancy group, followed by the haematological malignancy group [25].

Our finding prevalence of tuberculosis among cancer type were pancreatic, thyroid hematological, lung cancer prevalence was 8.3%, 5.9%, 5.5%, 4.3%, respectively. This result comparable with *Hyeon et al* that showed among the solid organ cancers, the pancreatic cancer had the highest prevalence [8].

Patients with solid organ tumors do not have the same risk of infection as patients with underlying hematological malignancies. This is largely because the standard chemotherapeutic regimens used to treat these malignancies do not usually result in either long-term or profound neutropenia. Aggressive chemotherapeutic regimens used to treat these malignancies may result in periods of neutropenia for 7–10 days or more [29].

In our study diabetes was found as risk factor for developing Tb on malignant patients. 6.9% individual of DM patients had TB. This correlated with reviewed by W*orkneh et.al* different studies in different countries and found out that the prevalence of TB among DM 6.2% in Ethiopia [28]. Similarly, *Bates et al* reviewed several cohort studies have shown that the relative odds of developing active TB ranges from two to eight in diabetic patients versus non diabetic controls [32]. another study by *Nissapatorn et al* pointed out that Relative risk of developing PTB in diabetes patients 26 times higher risk than the general population [31]. It might be due to DM patients have poor nutrition absorption, and are at risk of higher glucose level, which may also lead to weaken the immunity.33.3% of patients with tuberculosis had DM in our study. it is a little bit of lower than a study from the Indian state of *Tamil Nadu,* nearly 50% of patients with tuberculosis had either diabetes mellitus (25·3%) or prediabetes (24·5%).n a Chinese survey of 8886 registered tuberculosis cases, 1090 (12·4%) patients had diabetes mellitus [35].it might be due to different life style,geographical or race difference could be the reason behind.

In present study, our result 5.5% of hematological malignancy patients had taken chemotherapy for 6-11months. The finding was relatively higher than a study in Nigeria by *Omoti et al* that pointed out 3.4% the incidence of TB patients in haematological cancer patients in an endemic area were undergoing anticancer chemotherapy [24]. On different study, *Deog Kyeom Ki*m *et al* believed that anticancer chemotherapy was risk factors for the development of tuberculosis [3]. Another

study by *Chien-Yuan Chen et al* in Taiwan found that the increased utilization of anti-neoplastic agents in the treatment of hematological malignancies is associated with an increase of tuberculosis infections [14].

Chemotherapeutic agents predispose to infection in a variety of ways many of these agents damage the body's anatomical barriers. Most notably, they can cause ulceration of the gastrointestinal tract, allowing for erosion and invasion by endogenous microorganisms.

Compared to the general population, cancer patients with adaptive cellular immune dysfunction are clearly at increased risk of active TB [15].It is also probable that the treatments of cancer, such as chemotherapy and chemo, impair immunity, leading to increased infection or reactivation of tuberculosis. We therefore suggest that taking cancer treatment for longer period of time should be considered as a risk factor of tuberculosis.

Prevalence Tb is higher in those patients with steroid.3.2% (1of 31) than without 2.8 %( 5of175).among patients those developed Tb 16.7 %( 1of6) had steroid. *Libshitz et al* reported that among patient develop TB during treatment, 41 %( 11of 27) of patients received steroids [9].

In our result that 50% Tb observed within 1-3years of diagnosis of cancer. This result consistent with *Suzuki et al*. that reported TB occurred within 2 years of the diagnosis of lung cancer. The cumulative incidence of TB at 6 months, 1 year, and 2 years was 0.65%, 1.15%, and 1.38%, respectively [30].

Comrbidity increased with advancing cancer stage, from an average for those diagnosed with cancer at stage I no Tb observed, at stage II the incidence of Tb was 5.6% at stage III 4.3%, stage IV was 2.4%.this result was comparable with *Christopoulos et al* showed that most lung cancer patients diagnosed with active TB are advanced-stage (III–IV) cancer patients [19].

The emergence and global spread of MDR tuberculosis is a serious concern. Fortunately, in the present report, all clinical isolates of M. tuberculosis from cancer patients were susceptible to first-line anti microbial agents.

In summary, our study shows that the incidence of tuberculosis increases in patients with malignancies. Among the specific types of cancer, patients with pancreatic, thyroid cancer, hematologic malignancy and lung carcinoma had increased the incidence of tuberculosis. Tuberculosis should be considered an important comorbidity in patients with malignancies.

# 8. Strength and limitation of the study

## 8.1. Strength of the study

- It has provided the high light on burden of the tuberculosis infections in different types of malignancy which will be useful for monitoring and improving controlling programs in the oncology clinics.

## 8.2. Limitation of the study

- Since, we used Small sample size for the study, the question on reliability of describe specific types of malignancy and associated of risk factors could arise.
- Smear microscopy and Xpert were used as a reference standard, though both of these methods are not sufficient to detect all TB cases. Thus, further prospective studies are required to get concrete result using a more sensitive liquid and LJ culture as a reference standard.

- We studied only patients with cough and those able to produce sputum. Therefore, our results do not represent the full spectrum of clinical presentation of malignant patients towards tuberculosis.

# 9. Conclusion and recommendations

## Conclusion

The present finding of tuberculosis among malignant patients is 2.9% .regarding associated risk factor particularly in diabetes the risk was higher recorded 6.8%. Patient with anti cancer treatment was more likely to develop tuberculosis than a patient without treatment. Diabetes and anticancer chemotherapy were identified as independent risk factors for the development of active TB among patients with cancers Patient with anti cancer treatment was more likely to develop tuberculosis than a patient without treatment. Patients on chemotherapy or on steroid for longer period of time (6-11months) are in more risk than who had for 24 months and more or not taken at all.

## Recommendation

Tuberculosis is common among patients with malignancies in countries with a high incidence of tuberculosis. If TB is masked by malignancy, the therapy instituted for the cancer may cause immunosuppression and thereby result in dissemination of TB with fatal results. On other hand, the presence of TB may lead to drastic over-staging of a cancer. The presence of unsuspected active pulmonary tuberculosis indicated a large number of undiagnosed cases in the community. It has to be tackled seriously, so that we can reduce prevalence of TB /MDRTb resistance Tb. The following recommendations are given:

- Malignant patients with diabetes mellitus and undergoing cancer treatment should be suspect and screen for tuberculosis.
- Patients on chemotherapy or steroid for longer period of time should be screened early for tuberculosis.
- More Sensitive and specific methods like on MGIT and LJ culture for detecting TB/MDRtb are recommended.

Future studies are required to investigate the overall burden of tuberculosis among patients with comorbid disease and association of anticancer treatments.

## 10.References


1. Kim HR, Hwang SS, Ro YK, Jeon CH, Ha DY, Park SJ. *et al*. Solid-organ malignancy as a risk factor for tuberculosis. *Respirology 2008; 13:413–419.*
2. Anibarro L, Pena A.Tuberculosis in Patients with Haematological Malignancies. *Mediterr.J Hematol Infect Dis 2014, 6(1)*
3. Kim DK, LeeWS, Yoo CG, Kim YW, Han SK, Shim YS, Yim JJ .Clinical Characteristics and Treatment Responses of Tuberculosis in Patients With Malignancy Receiving Anticancer Chemotherapy. *Chest 2005;128(4):2218-2222*
4. WHO global report .2016
5. Shieh SH, Probst JC, Sung FC, Tsai WC. Decreased survival among lung cancer patients with co-morbid tuberculosis and diabetes.*BMC Cancer2012;12:174*
6. Kamboj M, Sepkowitz KA. The risk of tuberculosis in patients with cancer. *Clin Infect Dis 2006; 42:1592–1595.*
7. Akinosoglou K.S., Karkoulias K.,Marangos M.Infectious complications in patients with lung cancer. *Eur Rev Med Pharmacol Sci. 2013 ;17(1):8-18*
8. Seo GH, Hyeon G , Hwang B, Kim MJ, Jae M ,Seo S et al. Cancer-specific incidence rates of tuberculosis: A 5-year nationwide population-based study in a country with an intermediate tuberculosis burden. *Medicine 2016; 95(38).*
9. Libshitz HI, Pannu HK, Elting LS, Cooksley CD .Tuberculosis in cancer patients: an update. *J Thorac Imaging* 1997; *12: 41-46.*
10. Stover DE, Kaner RJ .Pulmonary complications in cancer patients.*CA: A Cancer Journal for Clinicians 2008**; 46(5**):303-320*
11. Wu CY, Hu HY, Pu CY, Huang N, Shen HC, Li CP, Chou YJ. Aerodigestive tract, lung and haematological cancers are risk factors for tuberculosis: an 8-year population-based study. *Int J Tuberc Lung Dis* 2011; 15:125–130.
12. Woubishet Y, Teklu A, Girma B.Cancer in ethiopia,*The Lancet Oncology, 2013; 14*
13. Sarfati D., Koczwara B.,Jackson C.The Impact of Comorbidity on Cancer and Its Treatment. *CA CANCER J CLIN ,2016;66:337–350*
14. Chen CY, Sheng WH, Cheng A, Tsay W, Huang SY, Tang JL et al. Clinical characteristics and outcomes of Mycobacterium tuberculosis disease in adult patients with hematological malignancies.*BMC Infect Dis 2011;11:324.*

15. Mishra P. Kumar R, Mahapatra M., Sharma S, Dixit A, Chaterjee T.et al., "Tuberculosis in acute leukemia: a clinico-hematological profile," *Hematology, 2006; 11(5-6): 335–340.*
16. Fang WL, Hung YP, Liu CJ, Lan YT, Huang KH, Chen MH, et al .Incidence of and Risk Factors for Tuberculosis (TB) in Gastric Cancer Patients in an Area Endemic for TB: A Nationwide Population-based Matched Cohort Study. *Medicine 2015; 94(47).*
17. Kaplan MH, Armstrong D, Rosen P. Tuberculosis complicating neoplastic disease: a review of 201 cases. *Cancer 1974; 33:850–851.*
18. GeneXpert MTB/RIF assay manual: Operator Manual Software Version-4. Cepheid: 2011.
19. Christopoulos A, Muhammad W. Saif, Evangelos G. Sarris, Kostas N. Syrigos. Epidemiology of active tuberculosis in lung cancer patients: a systematic review. *Clin Respir J,2014; 8: 375–381*
20. Al-Anazi K.A, Al-Jasser A.M, Alan D. Infections caused by mycobacterium tuberculosis in patients with hematological disorders and in recipients of hematopoietic stem cell transplant, a twelve year retrospective study. *Annals of Clinical Microbiology and Antimicrobials2007; 6:16*
21. Al-Anazi K.A, Al-JasserA.M.Tuberculosis in the Era of Globalization.*www.esciencecentral.org/ebooks*
22. Dutkowska A, Antczak A. Comorbidities in lung cancer. *Pneumonol Alergol Pol 2016; 84: 186–192*
23. Fujita, T, Endo M, , Gu Y, Sato T, Ohmagari N. Mycobacterium tuberculosis infection in cancer patient At tertiary care cancer center in Japan. *J Infect Chemotherapy 2013; 1-4*
24. Omoti C, Olu-Eddo A,Nwannadi A .Co-existence of TB and adult haematological cancers in Benin City, Nigeria. *Tropical doctor 2009; 39: 205 – 207*
25. El-Sharif A., Afifi S., El-Dahshan R, N. Rafehand N. and Eissa S. Characterization of Mycobacterium tuberculosis isolated from cancer patients with suspected tuberculosis infection in Egypt: identification, prevalence, risk factors and resistance pattern. *Clinical Microbiology and Infection 2012; 18: 11*
26. Tubura E. Pulmonary tuberculosis in the compromised host—report of the 30th B series of controlled trials of chemotherapy-Cooperative Study Unit of Chemotherapy of Tuberculosis of the National Sanatoria in Japan. *Kekkaku. 1991 ;66(2):95-99*

27. Lin JN, Lai CH, Chen YH . Risk factors for extra-pulmonary tuberculosis compared to pulmonary tuberculosis. *Int J Tuberc LungDis2009; 13: 620–625*
28. Workneh MH,Bjune GA,Yime SA. Prevalence and associated factors of tuberculosis and diabetes mellitus comorbidity: A Systematic review**.** *PLoSONE2017; 12(4).*
29. Dobler C, Cheung K, Nguyen J, and Martin A. Risk of tuberculosis in patients with solid cancers and haematological malignancies: a systematic review and meta-analysis. *Eur Respir J. 2017; 50:2.*
30. Suzuki Y , Imokawa S, Sato J, Uto T, Suda T, Cumulative incidence of tuberculosis in lung cancer patients in Japan: A 6-year observational study . *The Japanese Respiratory Society2016; 54: 179–183*
31. Nissapatorn V, Kuppusamy I, Jamaiah I, Fong MY, Rohela M and Anuar K .Tuberculosis in diabetic patients: a clinical perspective. *Southeast Asian J Trop Med Public Health.2005; 36:(4) 213-20.*
32. Bates M, Marais B, and Zumla A. Tuberculosis Comorbidity with Communicable and Noncommunicable Diseases. *Cold Spring Harb Perspect Med 2015;5*
33. Ibrahim EM, Uwaydah A, al-Mulhim FA, Ibrahim_AM, el-Hassan AY **.**Tuberculosis in patients with malignant disease**.** *Indian J Cancer. 1989 Jun; 26(2):53-7.*
34. Nair R., Prabhash K., Sengar M, Bakshi A, Gujral S., Gupta S, Parikh P. The effect of short-term intensive chemotherapy on reactivation of tuberculosis. *Annals of Oncology2007;18: 1243–1245*
35. Marais B, Lönnroth K, Lawn S, Migliori G, Mwaba P, Glaziou P,et al. Tuberculosis 2013: 3Tuberculosis comorbidity with communicable and non-communicable diseases: integrating health services and control efforts. *Lancet Infect Dis2013; 13: 436–48*
36. *Forbes BA, Sahm DF, Weissfeld AS. Bailey and Scott's Diagnostic Microbiology2007; 12(45): 478-509.*

# 11.Annexes

## Annex I: Participant information sheet

### Participant Information Sheet

Dear participant:

We would like to ask you to take part in this study which aimed to assess the burden of TB/MDR Tb in patients with cancer which helps to prevent treatment failure and medication resistant types of TB. This study protocol and consent form will be approved by 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 and Tikur Anbessa Specialized Hospital's ethical review board to make sure that your rights and autonomy are protected.

If you agree to participate in this study, these are what will be happen:

- You tell interviewer that you agree to be part of the project.
- You will be answer questions about your socio-demographic status
- You may be provided education program
- No identifying information about you, will be collected for this project, except some socio-demographic ,TB and cancer disease and its treatment related information, such as your age, gender, education level, economic level,
- This study result will be reported through publication with your full approval. During, publications of its result, no any identifying information about you or any other participant will appear in the publishing report.
- Data collected for this study will not be used for other purpose, without your consent.
- Regarding risk or discomfort: there is no any risk or discomfort in this study procedure.
- Regarding benefits of the study, you as individual may get comprehensive knowledge about TB disease, its treatment and the consequences of treatment non-adherence. As general benefit, your participation in this study will greatly benefit for the improvement of TB control program at national level based on this study result report.

Project Manager: Yonas Sebsibe Phone Number:-251911915729

Work Address: St.Peter Hospital, Addis Ababa,

**Annex II: Participant information sheet (Amharic version)**

**የተከበራችሁ የጥናቱ ተሳታፊዎች**

**ይህ ጥናት የሳንባ ነቀርሳ በሽታ□በካንሰር ህመማን ላይ የሚሳድረውን ጫና ለመለየትና በተጨ□ ቲ□ ህመማን የመከላከያ መድሀኒት እንዲያገኙ ለመርዳትና የህመማኑን በህይወት የመቆያ ጊዜ ከአ ለማትረ□ እንደሚረዳ ስለታመነበት በዚህ ጥናት እንድትሳታኘ እንጠይቃለን፡፡**

- **በጥናቱ ላይ ለመሳታአ □ቃ□ኛ መሆኖን ለጠያቂው ያሳውቁት**
- **ስለ ዕድሜና ጾታዎን መግለጽ ይኖርቦታል□**
- **ስለ ሳንባ ነቀርሳ በሽታና ስለ ናሙና አሰጣጥ ገለጻ ይደረግሎታል**
- **ሳንባ ነቀርሳና ካንሰርን የተገናኘ ካልሆነ በስተቀር የግል ሚስጥሮን መግለጽ አይኖርቦትም**
- **ከአእርስ- እውቅና ውጪ እርስ- ን የሚመለከት ውጤት ይፋ አይደረግም**
- **የምንወስደውን መረጃ ከአእርስ- እውቅና ውጪ ለሌላ ጥናት አናውልም**
- **በጥናቱ ላይ መሳታፍ ምንም አይነት የጤና ጉዳት አያስከትልም**
- **ሳንባ ነቀርሳን በተመለከተ የሚኖሮት ግንዛቤ ከፍ ያለ ይሆናል፡፡ ጥናቱ ላይ በመሳተ□ ሳንባ ነቀርሳ በሽታን ለመቆጣጠር በሚደረገው ሀገራዊ አንቅስቃሴ ላይ እቱ□ □□ረፉሉ፡፡□**

**የጥናቱ ባለቤት፡-ዮናስ ሰብስቤ**

**ስል□ ቁ□ር፡-*251911915729***

**የስራ አድራሻ፡-ቅ□ስ ጆ□ሮስ ሆስ□ተል**

## Annex III: Informed consent form

### Informed Consent Sheet

Dear participant, 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 of 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 TB disease after he/she explain for me the procedure of collection, | ☐ |
| 5. The interviewer explain for me as there is no any risk or discomfort, and extra treatment | ☐ |
| 6. I understand that as information collected from me are confidential, and they will be reported with my approval and the information will be reported are only the result without my personal information. | ☐ |
| 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 and Tikur Anbessa Specialized Hospital's ethical review board | ☐ |
| This form and 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: ----------/--------/2017

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

I project manager yonas sebsibe, 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: Yonas Sebsibe

Phone Number:-2519---------- work address: St.peter hospital, Ethiopia, Addis Ababa,

Signature: ----------, Date: -------/------/2017

**Annex VI: Informed consent form (Amharic version)**

**በጥናቱ ለመሳተፍ ፍቃደኛ ከሆናችሁ ፎርሙን በሚገባ ከነበባችሁ/ከተነበበላችሁ በ□ላ በ□ርሙ ላ□ በሚቶ ክፍት ሳጥኖች ላይ ምልክት በማድረግና በፎርሙ መጨረሻ ላይ በሚቶ ክፍት ቦታ ላ□ በመ□ረም እንድተረጋግጡልን እንጠይቃለን። ያልተረዳችሁት/ያልገባችሁ ነገር ከለ እባኮት ከመጠየቅ ወደ □ላ አይበሉ።**

| | |
|---|---|
| 1. **የጥናቱ ዋና አላማና አተገባበሩ ከተነበበልኝ /ከነበብኩኝ በ□ላ በጥናቱ ብሳተፍ ከኔ ምን □እንደሚጠበቅ ተረድቻለሁ** | □ |
| 2. **በጥናቱ የምሳተፈው በፍቃዴ ነው።የምሰጠው መረጃ በጥብቅ እንደሚቀመጥ ተረድቻለሁ።** | □ |
| 3. **በጥናቱ መቀጠል ከልፈለኩ በማንኛ□ም ቲ□ ማቋረ□ እንደምችል ተረድቻለሁ።** | □ |
| 4. **የሳንባ ነቀርሳ ምንነትና ለጥናቱ የሚሆን ናሙና እንዴት አንደምሰጥ ገለጻ ተደርጎልኛል።** | □ |
| 5. **በጥናቱ በመሳተፌ የሚደረስብኝ ጉዳት እንደሌለ ጠያቂ□ አስረድቶኛል።** | □ |
| 6..**ያለኔ ፍቃድ የጥናቱ ውጤት የግል መረጃዎቼን ይፋ እንደማያደርግ ተረድቻለሁ።** | □ |
| 7..**ምንም አየነት የገንዘብ ወጪ እንደሌለብኝ ከጠያቂየ ተረድቻለሁ** | □ |
| 8. **በአሰራሩ ላይ ችግር ባይበት/ባገኝበት ለጥቁር አንበሳ ሆስፒል ኢቲከል ኮሚ ማሳወቅ እንደምችል ተረድቻለሁ።□** | □ |

**ከላይ የተዘረዘሩት መረጃዎች ተረድቼ በጥናቱ ለመሳተፍ የተስማማሁ መሆኑን በፊርማ□ አረጋግጣለሁ።**

**ኝርማ**: ----------, **ቀን**: ----------/--------/2017

**ስል□ ቁ□ር/ ከለ/**: ----------

**አኔ ዮናስ ሰብስቤ የጥናቱ ተሳታፊዎች ደህንነት፤መብትና ጥቅም የተጠበቀ መሆኑን በፊርማየ አረጋግጣለሁ። ኝርማ**…….

**የጥናቱ ባለቤት: ዮናስ ሰብስቤ**

**ስል□ ቁ□ር**:-2519---------- **የስራ አድራሻ: ቅዱስ ጴጥሮስ ሆስፒተል አዲስ አበባ ኢትየጵያ**

**ኝርማ**: ----------, **ቀን**: -------/------/2017

## Annex IV: Data collection format

Dear participant

I am going to ask you few questions about your willingness to participate this study. After the following statement is read to you or yourself read it, please give your response as either I agree or I disagree to participate in the study. You may confirm your agreement or disagreement by either giving your signed or verbal consent in the respective space give below.

I the undersigned participate, given that I read all relevant information concerning the purpose of this particular study participants to be included, the procedure of selection the study participate, the study procedure, benefits, the withdraw effect on the service provided, consent and confidentiality or read and explain to me, I decided to agree/ or disagree to participate in the respective study mentioned above. (Please put your signature in the respective space provided below or your verbal consent.

Data collector Name: ----------

Signature: ----------

Date: ----------

CONSENT FORM

DATE...../...../.....

This is a survey planned to assess the burden of TB/MDR TB and its associated risk factors among patients with all types of malignant conditions visiting oncology department of Tikur Anbessa Specialized Hospital.

Are you willing to respond the following questions?

Yes..... No.....

If you are willing confirm with signature.....

**Data collection format**
Unique patient ID number ……

| No | **Variable** | **Descriptive categories** |
|---|---|---|
| | Part 1. Socio Demographic characteristic | |
| | Age incomplete years | |
| | Sex | 1.Male ☐2.Female ☐ |
| | Marital status | 1.Single ☐2.Married ☐3.Divorced/widow ☐ |
| | Level of education completed | 1.No education ☐2.Primary education ☐<br>3.Secondary education ☐4.Tertiary education ☐ |
| | Occupation | 1.Government employee ☐2.private employee ☐<br>3.Daily laborer ☐4.House wife ☐5.Merchant ☐<br>6.Factory worker ☐ Other specify ☐ |
| | Part II; Stage of cancers | 1.Carcinoma in situ☐2.Stage 1 condition ☐<br>3.Stage II condition ☐4.Stage III condition ☐<br>5.Stage IV condition ☐ |
| | Co morbidity | 1.Diabetes mellitus ☐2.Chronic renal failure ☐<br>3.Autoimmune diseases ☐4.COPD☐ |
| | Type of cancer | 1.Lung cancer☐2.Gastric cancer ☐3.Colon cancer☐<br>4.Breast cancer☐5.Liver cancer☐<br>6.Hematologic malignancy ☐7.Thyroid cancer ☐<br>8.Pancreas cancer ☐ Other cancer☐ |

Questionnaire related to clinical outcome of the study variable groups.

1. Durations on CA treatment...../...../.....

2. Have you been contacted with TB patients?

1. Yes □ 2. No ☐

3. Did you have previous history of TB?

1. Yes ☐ 2.No ☐

4. Did you MDR-TB patient contact history in the past?

1. Yes☐ 2. No ☐

5. Have you had Smoking history?

1. Yes ☐ 2. No ☐

6. Have you been Alcohol abuse?

1. No ☐ 2.Yes ☐

7. Have you been Drug abuse?

1. No ☐ 2. Yes ☐

## Annex V laboratory procedure

**PURPOSE**

This procedure provides instructions for sample collection, transport and handling.

**ABBREVIATIONS**

BAL---------- Bronchoalveolar Lavage

CPC---------- Cetylpyridinium chloride

MOTT---------- Mycobacterium other then tuberculosis

NTP ---------- National Tuberculosis Program.

**MATERIALS**

| Supplies | |
|---|---|
| **1. sterile absorbent cotton swab** | 3.wide mouthed sterile sputum cup(Fulcune tube of 50 ml) |
| **2.sterile glass container** | |

| Reagents |
|---|
| **1. 0.9% saline solution** |
| **2. Potassium oxalate** |
| **3. Heparin** |
| **4. Cetylpyridinium chloride** |

| Equipment |
|---|
| **1. Refrigerator** |
| **2. Triple package** |

**SAMPLE**

| Sample type | Amount | Transport and Storage | Stability |
|---|---|---|---|
| **Sputum** | 3-5ml* | As soon as possible. If not, kept refrigerated and could be sent at least 3 times a week | Up to 3days on refrigerator Or CPC should be added It has to be kept at room temperature (> 20°C) for up to 7 days. |
| **CSF** | at least 3 ml | As soon as possible. If not, kept refrigerated and could be sent at least 3 times a week | |
| **Bronchial secretion** | 2 - 5 ml | As soon as possible | |
| **Pleural effusions** | 20 - 50 m | As soon as possible | |

* Ideally, a sputum specimen should have a volume of 3- 5ml, although smaller quantities are acceptable if the quality is satisfactory.

**LIMITATIONS:** The following specimens should not be processed

- Broken containers
- Dried swab
- Interval too long, > 3 days from collection

**SPECIMEN IS LIABLE FOR REJECTION FOR CULTUR**

1. Specimen is unlabeled or mislabeled.
2. Specimen without request form.
3. Specimen name and request form does not match.
4. Container is full up to the lid, because of pooling of specimens.
5. Specimen breakage or leakage.
6. Specimen not collected in an appropriate container.
   Note: The specimens should not be rejected without proper reasons.

## SPECIAL SAFETY PRECAUTIONS

Aerosols containing tubercle bacilli may be formed when the patient produces a sputum specimen. Patients should, therefore, produce specimens either outside in the open air or away from other people and not in confined spaces such as toilets, laboratories, waiting room or reception room. The sample should also transport using triple package

## STORAGE SAMPLE

### Pre Testing storage and handling of samples

- Receive samples according to the Sample Reception procedure
- As soon as samples have been checked according to sample acceptance/ rejection criterion, registered in the worksheet and process the samples and send them to the testing bench for analysis

### Pre test Storage

- All Samples brought to the testing bench should be analyzed **IMMEDIATELY**.
- For samples which cannot be possibly analyzed the same day
    - Store in the 2-8°C overnight
    - Samples should be placed in sample racks in the same order as registered in the worksheet and request forms

### Post test sample storage and retention

- After analysis, put samples in appropriate position on the sample rack.
- Label sample racks
- Arrange the samples in their ascending laboratory numbers.
- Store sample rack at -20°C
- Store samples for 2 weeks for follow up and for the primary slide result negative new case and store sample until LPA result released for new case with positive slide result.
- Make sure that all sample need to autoclave at $121^0$ c for 15 min before removing from the Lab.
- The cleaner should burn the autoclaved waste in the incinerator.

.

**PROCEDURE**

**1. Sputum**

| Step | Action |
|---|---|
| 1. | It is best to obtain sputum early in the morning before the patient has eaten or taken medication |
| 2. | Ask the patient whether she/he is informed how to produce sputum |
| 1. | If necessary instruct the patient. |
| 2. | collect sputum in mouth and spit carefully in to a wide mouth, unbreakable, leak proofed container |
| 3. | Close the lid tightly. |
| 4. | Compare label with patient's data on the request form |
| 5. | Maintain the desirable temperature |

**2. Laryngeal swab**

| Step | Action |
|---|---|
| 1 | Use a sterile absorbent cotton swab for collection. |
| 2 | Collect early morning before food and drinks are taken |
| 3 | This may be useful in children and patients who cannot produce sputum or may swallow it. |
| 4 | Transport specimen with a little 0.9% saline solution in a container in order to keep the swap wet |

**3. Gastric lavage**

| Step | Action |
|---|---|
| 1 | The collection should be made early in the morning with an empty stomach |
| 2 | The specimen must be transported immediately into the lab |
| 3 | Neutralized by adding 1 to 2 ml of disodium-hydrogen-phosphate solution to the gastric aspirate depending on the amount. |

**4. Aseptically collected fluid**

| Step | Action |
|---|---|
| 1 | All liquid specimens should be collected in a sterile glass container or sterile saline solution. |

**PRINCIPLE**

Good conditions of specimens are the responsibility of the setting where the specimen has been collected, either the peripheral laboratory where patients were given sputum containers or the clinics where sampling/biopsy have been performed. Conditions have to be considered from collection to the arrival in the laboratory where culture will be carried out

### Collection

Specimens should be collected in clean, preferably sterile containers with a tight-fitted lid or cap. At least two morning specimens collected on separate days should be processed for each new case. For patients with respiratory symptoms, the specimens should be expectorated sputum and not saliva, with a volume of about 2-10 ml each.

### Transportation

Specimens should be transported to the laboratory as quickly as possible. Delays in transportation, especially in hot weather, result in an increase in contaminating bacteria that result in higher contamination rate of the medium. Specimens should be transported in a container, such as an ice box, in which temperature is maintained as low as possible. This is especially important in countries with high ambient temperatures.

### Storage

Upon receipt, the specimens should be refrigerated and processed as soon as possible

# SOP on Sputum Processing using NALC-NaOH

## PURPOSE

This Procedure Provides Instructions on Sputum Processing For Culture Using 4%Nalc-NaOH.

## ABBREVIATIONS

BSC ----------Biological Safety Cabinet

ID---------- identification

LJ---------- Löwenstein Jensen

MW---------- Molecular weight

NALC ----------N-acetyl L-cysteine

NaOH ---------- Sodium Hydroxide

RCF ----------Relative Centrifuge Force

## MATERIALS

| Supplies | |
|---|---|
| **1.Powdered Glove,N95 mask and surgical gown** | 6. Paper towel and Cotton |
| **2.waste containers for solid and liquid wastes** | 7. Marker, Pen and pencil |
| **3. Tips 1000ml and Tips 1-200ml** | 8. Frosted slide and Slide rack |
| **4.Absorbable pad and plastic Pasteur pipettes** | 9. Crynovial tube and Falcon tube 50ml |
| **5.Biohazard bag and Safety bag** | 10. Buckets, stainless steel or polypropylene |
| **Reagents** | |

| | |
|---|---|
| 1. 4% Sodium hydroxide (NaOH) solution | 2. NALC |
| 3. Phosphate buffer 0.067M, pH 6.8 | 4. NALC-NaOH solution |
| 5. 2.94%Trisodium citrate-$3H_2O$ | |

**Reagents preparation:**

NALC-NaOH solutions, freshly prepared for daily use only add 0.25 g NALC to the mixture of sodium hydroxide-trisodium citrate just before use.

| Equipment |
|---|
| 1.Vortex mixer |
| 2.Refrigerated centrifuge with safety shield |
| 3.BSC certified |
| 4.Pipettes for 1.0 ml |
| 5.Timer |

## SAFETY PRECAUTIONS

- No material may leave the laboratory unless it has been decontaminated or autoclaved.
- Procedures that can cause the generation of aerosols must be minimized and carried out in a class II bio-safety cabinet
- 
- Minimize the aerosol production by opening the caps of specimen containers slowly, avoiding vigorous shaking of the specimen and avoiding the expulsion of the last drop from pipette

## QUALITY CONTROL

| Control | Level | Frequency |
|---|---|---|
| PBS | Start | For each test run |
| Distilled water | End | For each test run |

**Note:** Start and End shouldn't show any growth.

**PROCEDURE**

| step | Action |
|---|---|
| 1 | Adjust the sputum (at least 5 ml, not more than 10 ml) |
| 2 | Add equal volumes of NALC-NaOH solution |
| 3 | Tighten cap of container and vortex slowly for 10 sec |
| 4 | Shake intermittently to aid homogenization and decontamination |
| 5 | Invert each bottle to ensure that NaOH solution contacts all the sides and inner portion of caps |
| 6 | Keep at 20°C – 25°C for 15 min for decontamination |
| 7 | Fill the tube with phosphate buffer up to 45 ml mark on the tube |
| 8 | Centrifuge at 3,000 g for 15 minutes |
| 9 | Carefully pour off the supernatant into a discard can containing 5% phenol or 5% concentrated Bleach |
| 10 | Smear the concentrated sputum on a slide with the ID number for microscopic examination |
| 11 | Re suspend each tube with 1ml PBS and vortex for homogenization |
| 12 | Prepare the BSC for inoculation of the decontaminated sputum on LJ and MGIT tubes(*refer to SOP on Inoculation and Incubation specimen on LJ and MGIT* ) |

**PRINCIPLE**

Specimens for tubercle bacilli isolation do usually contain associated flora which has to be eliminated before inoculation of the specimen onto culture media.

Specimens for tubercle bacilli isolation do usually contain associated flora which has to be eliminated before inoculation of the specimen onto culture media. Isolation of *Mycobacterium species* from non-sterile clinical specimens requires a digestion, decontamination and concentration treatment prior to inoculation of media for growth detection. Digestion and liquefaction of mucous and organic debris allows eradication of rapidly growing contaminants which will overgrow slower growing mycobacteria especially *Mycobacterium tuberculosis complex*. The N-acetyl L-cysteine–sodium hydroxide (NALC/NaOH) method is the mildest and most widely used decontamination procedure for recovery of *Mycobacterium species* from sputum and other clinical specimens. The starting concentration of NaOH is 4% with a final concentration of 1% in the specimen. The mucolytic agent NALC enhances rapid liquefaction and digestion of sputum. Tri-sodium citrate binds heavy metal ions that might be present in the specimen that might inactivate NALC. The NaOH exposure time is rigidly controlled to prevent over-kill of tubercle bacilli. The efficacy of this procedure is strongly influenced by buffer neutralization of NaOH, centrifugation efficiency and heat buildup during the concentration process

## Declaration

I, the under signed, declared that this thesis is my original work, and has not been presented for a degree in any other university and that all source of material used for this thesis and all people and institution that gave support for this have been duly acknowledge.

Name: Yonas Sebsibe Signature_____

Place: Addis Ababa
Date of submission: _____

This Thesis work has been submitted with my approval as University Advisor.

Advisor's Name Signature

Kassu Desta (PhD fellow) _____

Abebaw Kebede(PhD fellow) _____