Addis Ababa University College of Health Sciences, School of Medicine, Department of pathology

Assessment of possible tuberculous lymphadenopathy by Xpert MTB/RIF assay compared to non-molecular methods at St. Paul's hospital millennium medical college, Addis Ababa, Ethiopia.

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This is to certify that the thesis prepared by **Mengistu Fantahun** entitled: "**Assessment of possible tuberculous lymphadenopathy by Xpert MTB/RIF assay compared to non-molecular methods at St. Paul's hospital millennium medical college, Addis Ababa, Ethiopia.**" submitted in fulfillment of the requirements for the degree of Master of Science in histotechnology complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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Table of contents page

Acknowledgements........ II
List of tables........ II
List of figures II
List of Annexes II
List of Abbreviation/acronyms........ II
Operational definition........ II
Summary........ II
1. Introduction........ 2
1.3 Significance of the study........ 2
2. Literature review........ 2
3.1 General objective........ 2
3.2 Specific objectives:........ 2
4. Materials and Methods........ 2
4.1 Study area........ 2
4.2 Study Design and Period........ 2
4.3 Source of Population........ 2
4.4 Study Population........ 2
4.5 Sample size Calculation and Sampling Technique........ 2
4.5.1 Sample collection and transportation........ 2
4.5.2 Sample Size Calculation........ 2
4.6.1 Administration of questionnaire........ 2
4.6.2 Quality control........ 2
4.7. Specimen collection, transportation and processing and laboratory investigation........ 2
4.8 Eligibility........ 2
4.8.1 Inclusion Criteria........ 2
4.8.2 Exclusion criteria........ 2
4.9 Study Variables........ 2
4.9.1 Dependent variable........ 2
4.9.2 Independent variable........ 2
4.10 Authorship and Intellectual Property Right........ 2
4.11 Statistical Analysis........ 2
4.12 Dissemination of Results........ 2
4.13 Ethical considerations........ 2
5. Results........ 2
6. Discussion........ 2
7. Conclusion and recommendation........ 2
8. Strength and limitation of the study........ 2
9. Reference........ 2
Annex I: Physical examination form........ 2
Annex II : Laboratory examination form........ 2
Annex III: Patient Information, Consent Form and assent........ 2
Annex IV-Questionnaire........ 2

የዉል ስምምነት.. 2
Annex IIV Standard operating procedures.. 2

List of table **page**

Table1. Socio-demographics characteristics of presumptive TBLN patients from SPHMMC, Addis Ababa, Ethiopia, December 2015- May 2016 (N=152)………………………………...25

Table 2. Association of TBLN with socio-demographic characteristics of presumptive TBLN patients from SPHMMMC, Addis Ababa, Ethiopia, December2015- April 2016 (N=152)…27

Table 3. Association of TB lymphadenitis with clinical characteristicsof presumptive TBLN patients from SPHMMMC, Addis Ababa, Ethiopia, December 2015- May 2016 (N=152**)** ….29

Table 4. Comparison of diagnostic yield of different laboratory methods for diagnosis of TBLN with aspirate types at SPHMMC, Addis Ababa, Ethiopia, December 2015- May 2016 (N=152) ……………………………………………………………………………………......32

Table 5. TBLN positivity by different diagnostic methods (N=152)…………………………33

Table 6. Comparison and kappa values of diagnostic yield of different laboratory methods used for diagnosis of TBLN with each other's at SPHMMC, Addis Ababa, Ethiopia.(N=152)….34

Table 7. Sensitivity, specificity, positive and negative predictive values of the laboratory methods against composite reference standard: direct and/ or concentrated LJ culture for the diagnosis of presumptive TBLN patients SPMMC, Addis Ababa Ethiopia……………………………………………………………………………………..35

List of figures **page**

Figure 1.Distribution of lymph node sites from presumptive TBLN patients at SPHMMC, Addis Ababa, Ethiopia……………………………………………………………………………………28

Figure 2. Distribution of FNA types obtained from presumptive TBLN patients at SPHMMC, Addis Ababa, Ethiopia, December 2015- May 2016 (N=152)………………………………..31

List of Annexes **Page**

Annex I Physical examination form--52

Annex II Laboratory examination form--53

Annex III Patient Information, Consent and assent Form--55

Annex IV-Questionnaire---60

Annex V የዉል ስምምነት

---64

Annex VI Standard operating procedures --69

List of Abbreviation/acronyms

AA	Addis Ababa
AFB	Acid-fast bacilli
BLH	Black Lion Hospital
DNA	Deoxyribonucleic acid
DOTS	Directly observed treatment, Short-course
EPTB	Extra pulmonary tuberculosis
FMOH	Federal Ministry of Health
FNAC	Fine needle aspirate cytology
HIV	Human Immunodeficiency Virus
LJ	Löwenstein–Jensen
MTC	Mycobacterium tuberculosis complex
NTM	Non-tuberculous mycobacteria
PCR	Polymerase Chain Reaction
RIF	Rifampicin
SPC	Sample processing control
SPMMC	Saint Paul's Millennium Medical College
TB	Tuberculosis
TBL	Tuberculosis lymphadenitis
WHO	World Health Organization
ZN	Ziehl Neelsen

Operational definition

- Presumptive Tuberculosis lymphadenitis case: Any person who presents with signs and symptoms of Tuberculosis lymphadenitis.
- Composite culture: the M.TB colony grew on direct and/or concentrated LJ culture
- Bacteriological positive: MTB positive in any of diagnostic methods (ZN, FM, Xpert MTB/RIF Assay and composite culture).
- Underlying diseases: Diseases that are compromise the immune system of persons or made their body weaker and more susceptible to infection or disease.
- Suitable: Easy procedure, less invasive and less cost.

Summary

Background: There are nearly 9 million new cases and 2 million deaths from tuberculosis (TB) worldwide every year. The incidence of mycobacterial lymphadenitis has increased in parallel with the increase in the incidence of mycobacterial infection worldwide. TB lymphadenitis is seen in nearly 35 per cent of extra pulmonary TB which constituted about 15 to 20 % of all cases of TB.

Extra pulmonary tuberculosis (EPTB) is a significant health problem worldwide because of difficulties in its diagnosis and in monitoring its treatment, in which Tuberculous lymphadenitis (TBLN) is high prevalent. . However, no adequate information had been made available on the tuberculous lymphadenitis in Addis Ababa. To this effect, adequate knowledge on the prevalence and better diagnosis method is required.

Objective: This study was aimed to determine the prevalence of TBLN and assesse diagnostic performance of laboratory methods in diagnosis of TBLN among presumptive TBLN patients at St. Paul's hospital millennium medical college (SPHMMC).

Materials and Methods: A cross-sectional study was conducted on tuberculosis lymphadenitis presumptive patients at SPHMMC from December 2015 to May 2016. Fine needle aspiration samples were collected from all TB lymphadenitis presumptive patients. Structured questioners were used to collect socio-demographic and clinical related data. Samples were screened for TB lymphadenitis using TB culture, cytomorphology, Xpert MTB/RIF assay and other staining techniques. The data were analyzed using software packages SPSS version 20 (SPSS Inc, Chicago, Illinois, USA). Negative predictive value, positive predictive value, sensitivity and specificity were calculated. Kappa value was calculated to see the presence of agreement. Chi-square test was done along with P-value to see the presence of associations. P-value less than 0.05 were considered as statistically significant and logistic regression analyses were used to see the association of different variables. Odds ratios and 95% confidence interval were computed to determine the presence and strength of association

Result: Of the 152 TBLN presumptive cases, 103(67.8%) were positive for TBLN by FNAC examinations. On the other hand, only 44.7% were positive by mycobacterial culture and 24.3% cases were detected by Fluorescent microscopy (FM). ziehl Neelsen (ZN) was detected in 14.5% cases while 49.3% cases were positive for mycobacteria by Xpert MTB/RIF assay. Pus (AOR

0.082, 95% CI 0.030-0.228) and caseous (AOR 0.059, 95%CI 0.020-0.169) aspirates and Previous treatment was significantly associated with TBLN (AOR 0.113, 95% CI 0.031-0.407).
The sensitivity of GenXpert compared to composite LJ culture was 78% (73.7-82.3) and specificity 74% (69.4-78.6). The sensitivity was 30.9% for ZN, 47% for FM and 94.1% for cytology compared to composite LJ culture. Cytology showed the lowest specificity (53.6%) but ZN revealed highest sensitivity 98.8% compared to composite culture. Cytology had 61.3% specificity and 87.8% sensitivity compared to against bacteriological methods. Among 49 cytological non-TBL cases, 10 were positive on GeneXpert. The highest agreement was observed between ZN and FM (*k=0.69*) and the lowest between FNAC and ZN (*k=0.199*).
Conclusion: The results of this study revealed a high prevalence of TBLN in the study sites. TBLN is an important public health problem that needs to be addressed in the area. Types of aspirate and previous treatment were significantly associated with tuberculosis lymphadenitis.
FNA cytology showed a relatively high sensitivity but a low specificity. Combining bacteriological methods with FNA cytology in an endemic region like Ethiopia improves the overall accuracy of the diagnosis of mycobacterial lymphadenitis, which in turn may lead to better patient management. Further prospective and advanced studies are recommended to determine the specific etiologic agents and contributing factors and Pathologists should be conscious of tuberculosis cases whenever they encounter enlarged lymph node with pus and/or causous aspirates to initiates immediate treatments.

1. Introduction

1.1. Background

The global burden of TB remains enormous. Globally in 2005, there are nearly 9 million new cases and 2 million deaths from tuberculosis worldwide every year (1). Globally in 2012, There are nearly 8.6 million new cases of TB (range 8.3 million–9.0 million), and there were an estimated 1.3 million people died from the disease (0.94 million deaths among HIV-negative cases and 0.32 million deaths among people who were HIV-positive) (2)

Worldwide, 9.6 million people are estimated to have fallen ill with TB in 2014: 5.4 million men, 3.2 million women and 1.0 million children. Globally, 12% of the 9.6 million new TB cases were HIV-positive (34).

Most of the estimated number of cases in 2012 occurred in Asia (58%) and the African Region (27%) (2).The five countries with the largest number of incident cases in 2012 were India (2.0 million– 2.4 million), China (0.9 million–1.1 million), South Africa (0.4 million–0.6 million), Indonesia (0.4 million–0.5 million) and Pakistan (0.3 million–0.5 million). India and China alone accounted for 26% and 12% of global cases, respectively. Of the 8.6 million incident cases, an estimated 0.5 million were children and 2.9 million (range, 2.7–3.1 million) occurred among women (2).

The infection rate with this bacteria increasing and one of the reasons for this is long process of laboratory identification, therefore establishing new diagnosis methods could decrease disease rate. WHO estimates that if the current tools for the diagnosis and treatment of TB are not improved, nearly one billion additional people will become infected by *M. tuberculosis* between the 2000 and 2020. Among these, 200 million will develop the active disease and 35 million will die from TB (3)

The incidence of mycobacterial lymphadenitis has increased in parallel with the increase in the incidence of mycobacterial infection worldwide. TB lymphadenitis is seen in nearly 35 percent of extra pulmonary TB which constituted about 15 to 20 per cent of all cases of TB. In HIV-positive patients, extra pulmonary TB account for up to 53 to 62 percent cases of TB (4).

1.1.2. Etiologic Agent

M. tuberculosis belongs to the family of Mycobacteriaceae, order Actinomycetales and genus Mycobacterium. There are three species under this genus and these include *M.tuberculosis* complex, the non-tuberculosis mycobacteria and *M. leprae (5)* Human and animal tuberculosis are caused by different members of the *Mycobacterium tuberculosis* complex (MTC), of which *M. tuberculosis* and *M. bovis* are best known and share 99.9 % of the same genome(6).

1.1.3. Transmission of Tuberculosis

M. tuberculosis infections occur by airborne transmission. The route of entry of the tubercle bacillus into the body is via the respiratory tract through the inhalation of respiratory droplet nuclei, which are few viable, virulent organisms, small enough in size to allow passage into the lower respiratory tract(7). Infection of human beings with *M. bovis* almost always occurs by inhalation of aerosols or consumption of milk/meat containing the bacillus.(8) Once organisms have made their way into the lung, they have four potential fates(9). The initial host immune reaction can be completely effective and kill all bacilli, such that the patient immune system completely controls the bacilli and has no chance of developing tuberculosis at any time in the future; if the immune system of host is undermine, the organisms can begin to multiply and grow immediately after infection, causing clinical disease known as primary tuberculosis; bacilli may become dormant and never cause disease at all, such that the patient has what is referred to as latent infection, or the latent organisms can eventually begin to grow, with resultant clinical disease, known as reactivation tuberculosis(10) . TBLN is assumed to result from lympho-haematogenous spread of tubercle bacilli from the site of primary infection in the lungs, as reactivation of previously contained foci. Hilar and mediastinal lymph nodes are initially involved. The infection then spreads through the lymphatic to the draining cervical lymph nodes (11).

1.1.4. Immunopathogenesis of Tuberculosis

Phagocytosis of *M.tuberculosis* by alveolar macrophages is the first event in the host pathogen relationship that decides outcome of infection. Phagocytized microorganisms are subject to degraded by intra lysosomal acidic hydrolases upon phagolysosome fusion. This highly regulated event constitutes a significant antimicrobial mechanism of phagocytes (12). After entry of *M. tuberculosis* into alveolar macrophages, it produces inflammatory cytokines and chemokine that serve as a signal for infection. The monocytes, neutrophils, and lymphocytes migrate to the focal

site of infection, but they are unable to kill the bacteria efficiently. During this time, the bacilli resist the bactericidal mechanisms of the macrophage (phagolysosome) by preventing phagosome-lysosome fusion, multiply in the phagosome, and cause macrophage necrosis. The released bacilli multiply extracellularly, and phagocytized by another macrophage that also fails to control the growth of *M.tuberculosis*, and likewise are destroyed (13, 14).

Within 2 to 6 weeks of infection, the specific immune response produces primed T cells which migrate back to the focus of infection, guided by the chemokines produced by the infected cells, and the accumulation of macrophages, T cells, and other host cells (dendritic cells, fibroblasts, endothelial cells, and stromal cells) leads to the formation of granuloma at the site of infection. The bacilli are contained in the caseous centers of the granuloma. The bacilli may remain forever within the granuloma, get reactivated later or may get discharged into the airways after enormous increase in number, necrosis of bronchi and cavitation. Fibrosis represents the last-ditch defense mechanism of the host, where it occurs surrounding a central area of necrosis to wall off the infection when all other mechanisms failed (12, 15).

The granuloma formation walls off tubercle bacilli from the rest of the lung tissue, limits bacterial spread, and provide microenvironment for interactions among macrophages and other cells of the immune system and the cytokines produced by these cells. The CD4+T cells producing interferon- γ (IFN- γ) recognize infected macrophages, presenting antigens from *M.* tuber contained at granuloma formation stage, it shrinks and may eventually disappear, leaving a small scar or calcification and the patient's T cells become responsive to M. tuberculosis-derived antigens. If, however, the immune response does not successfully control the bacterial replication, the granulomas increase in size and cellularity. Eventually, cell death in the granuloma leads to necrosis. In this case, if the granuloma is close to the surface of the lung, the tissue destruction caused by necrosis can breach the mucosal surface and the granuloma contents leak into the lumen of the lung – a process referred to as cavitation. This gives rise to the prototypic symptom of TB a persistent cough with blood in the sputum. At this point, the patient is highly infectious, spreading the bacteria by aerosol, even the bacilli can disseminated to other organ, like lymph node. Tissue destruction in TB is not mediated by the activities of the bacteria alone, it is primarily immunopathological in nature and the crucial point to understand is that an inflammatory immune response is critical for the survival of both the host and the bacteria (17).

1.1.5. Clinical manifestation of Tuberculosis: Tuberculosis lymphadenitis usually affects children and young adults with high female predilection. Patients usually present with gradual enlarging of lymph nodes and may otherwise be asymptomatic. Isolated cervical lymphadenopathy is most commonly seen in about 67 % HIV negative patients. The enlarged lymph nodes may be of varying size, are usually firm, painless and may be discrete or matted (18-20). Cervical lymphadenitis is the most common manifestation of mycobacterial infections encountered in the otolaryngologic practice. Cervical lymphadenopathy is generally described as painless, slowly growing neck mass or masses developing over weeks to months. The lymph nodes are usually multiple and matted but may be single, mobile, or fluctuant or with discharging sinuses. Some patients with lymph node tuberculosis may manifest systemic symptoms and these include fever, weight loss, fatigue and occasional night sweats. Cough is a less prominent feature, seen in approximately 10 percent of patients (18, 21).

1.1.6. Diagnosis and identification of Tuberculosis

Mycobacterial cervical lymphadenitis remains a diagnostic challenge in developing countries for many clinicians because of the low sensitivity of conventional methods in detecting tubercle bacilli in clinical specimens; it mimics other pathologic processes and yields inconsistent physical and laboratory findings (21).

1.1.6.1. Ziehl Neelsen (ZN) staining

The primary method for the diagnosis of tuberculosis is Ziehl Neelsen staining. Although the method is specific and rapid; the technique has low sensitivity in the detection of tubercle bacilli in various clinical specimens. Smear negative and culture positive results can occur since a significant number of bacilli must be present in the given specimen to be detected by acid fast smear examination. For instance, 5, 000 -10, 000 acid-fast bacilli per ml of sample must be present to permit detection by acid fast staining (22-25).

1.1.6.2. Fine Needle Aspiration Cytology (FNAC)

Fine-needle aspiration (FNA) has become a widely used diagnostic tool and it remains one of the most rapid and cost-effective diagnostic methods of tuberculous lymphadenitis where it is difficult to perform molecular techniques. It is being increasingly used as the main diagnostic procedure for establishing the diagnosis of tuberculous lymphadenitis. In areas where mycobacterial infections are prevalent, a diagnosis of tuberculosis can be made confidently when its cytomorphological features are met. In one study, the sensitivity and specificity of FNAC in

the diagnosis of tuberculous lymphadenitis was stated as being 88% and 96% respectively (26,27). The cytological criteria for diagnosis of possible TB-L have been clearly defined as epitheloid cell granulomas with or without multinucleated giant cells and caseation necrosis (28)

1.1.6.3. Mycobacterial culture

The identification of tubercle bacilli by culture is required for the ultimate proof of mycobacterial infection. However, due to unavailability of laboratory equipment and safety procedures, the method is not practiced in resource poor settings. Specimens collected from normally sterile body sites may be placed directly onto the culture media or can be mildly decontaminated prior to inoculation. Such specimens include FNA and biopsy materials from lymph nodes. The detection rate for *M. tuberculosis* from fine needle aspirates is low by microbiological techniques. Different media (egg based, agar based and liquid media) have been devised for cultivating of tubercle bacilli. Among the above-mentioned media, bacterial culture on egg - based Lowenstein Jensen (LJ) media are commonly used in most laboratories. Lowenstein Jensen medium enriched with sodium glycerol favors the growth of M. *tuberculosis*, while LJ medium without glycerol but containing pyruvate encourages the growth of *M. bovis*. However, bacterial culture takes at least 3 to 8 weeks to observe a positive growth after incubation at 37 ^{o}C (29-31).

1.1. 6.5. Auramine stain – FM

Fluorescence microscopy allows smears to be examined more rapidly than is possible with the basic fuchsin procedures and is particularly indicated for high-volume laboratories. It may also be more sensitive for paucibacillary specimens, since it allows examination of more fields with less effort. However, it requires a stable power supply, greater expertise in reading and microscope adjustment, and a regular supply of the costly and short-lived bulbs. Cheaper systems using halogen lamps have less stringent requirements, but performance does not entirely match that of the standard mercury vapour lamps (32).

1.1.6.6. Xpert MTB/RIF

In December 2010, WHO endorsed Xpert MTB/RIF (Cepheid, USA) for use in TB laboratories. The GeneXpert assay consists of a closed system that is based on real-time polymerase chain reaction (PCR). It can be used by operators with minimal technical expertise, enabling the diagnosis of TB and simultaneous detection of rifampicin resistance within 2 hours (33). In 2014, WHO has recommended GeneXpert over the conventional tests (including conventional

microscopy, culture or histopathology) for testing specific non-respiratory specimens (lymph nodes and other tissues) from patients suspected of having EPTB (34).

1.1.7. Treatment and prevention

M. tuberculosis is susceptible to several effective anti-microbes. Isoniazid, Ethambutol, Rifampin, Pyrazinamide, Streptomycin, and combinations of these agents constitute the primary drugs of choice for treatment of tuberculosis. Isoniazid and rifampin are active against both intra- and extracellular organisms, and pyrazinamide, Nicotinamide analog, acts at the acidic pH found within cells. Streptomycin does not penetrate into cells and is thus active only against extracellular organisms (35). *M. tuberculosis* is also susceptible to other drugs, second-line drugs, which are less efficacious and more toxic than the first-line drugs. Second-line drugs are Capreomycin, Kanamycin, Ethionamide, Thiacetazone, Aminosalicylic acid, and Cycloserine. Fluoroquinolones and Ofloxacin that may be used to replace those of the primary group if they are inappropriate because of resistance or drug toxicity (35,36). At present, the bacillus Calmette and Guérin (BCG) is the only available vaccine. It has been used for prophylaxis of tuberculosis in various countries since 1923; administration is usually intradermal. It is a live vaccine derived originally from a strain of *M. bovis* that was attenuated by repeated subculture. Although BCG can be effective in reducing the incidence of childhood TB, particularly meningitis, it is relatively ineffective in protecting against adult TB, and doesn’t prevent infection with the organism (35).

***1.2.* Statement of problem**

There are nearly 9 million new cases and 2 million deaths from tuberculosis worldwide every year (36). The incidence of mycobacterial lymphadenitis has increased in parallel with the increase in the incidence of mycobacterial infection worldwide. TB lymphadenitis is seen in nearly 35 per cent of extra pulmonary TB which constituted about 15 to 20 per cent of all cases of TB. In HIV-positive patients, extra pulmonary TB account for up to 53 to 62 percent cases of TB (4).

Cervical lymph nodes are the most common site of involvement and reported in 60% to 90% patients with or without involvement of other lymphoid tissue (37). Cervical lymphadenitis, which is also referred to as scrofula, may be manifestation of a systemic tuberculous disease or a unique clinical entity localized to neck. Mycobacterium tuberculosis is the most common causative agent in India (38-40). The incidence of mycobacterial lymphadenitis primarily

depends on the endemicity of the of Mycobacterium tuberculosis. Lymphadenopathy due to non-tuberculous mycobacterial (NTM) is uncommonly reported from India (41).

In non tuberculous adenitis, Mycobacterium avium-intracellulare complex is the most common causative agent. Mycobacterial lymphadenitis most frequently affects patients in their second decade but may afflict patients of any age. There is a female predominance (approximately 2:1) in most of the studies (35) Racial and ethnic minorities, foreign born, black and Asians are more likely than non-Hispanic white patients to develop tuberculous lymphadenitis. There is increased frequency of mycobacterial lymphadenitis in Asian population (42, 43). Infection with the human immunodeficiency virus (HIV) is associated with an increased frequency of both pulmonary and extra pulmonary tuberculosis particularly lymphadenitis (44, 45).

TB is also a major public health problem in Ethiopia. Ethiopia is among the High burden tuberculosis countries (HBTCs) with regard to the number of tuberculosis patients. The WHO TB control program estimate that the proportion of extra pulmonary TB among the total number of new TB cases (143, 503) is about 33% (2).The proportion of EPTB, with majority of TBLN among newly diagnosed TB patients has been increasing for the last two decades. In spite of these high numbers, diagnosis of tuberculosis lymphadenitis remains a challenge (44). Over a third of the population has been exposed to tuberculosis and the annual risk of tuberculosis is estimated at 2.2 %. According to the Ministry of Health statistics, tuberculosis is one of the leading causes of morbidity, the fourth cause of hospital admission, and the second cause of hospital death in Ethiopia (46, 47).

1.3 Significance of the study

Lymphadenitis is the most common extra pulmonary manifestation of pulmonary tuberculosis. It remains both diagnostic and therapeutic challenge because it mimicks other pathological processes and yield inconsistent physical and laboratory findings. Diagnosis is difficult, often requiring biopsy. A complete history and physical examination, staining for acid-fast bacilli (AFB), FNAC and polymerase chain reaction (PCR) are helpful in obtaining early diagnosis.

There should be the better diagnostic methods with minimized cost, high sensitivity and specificity. And also In Ethiopia where there is no strong surveillance system and diagnostic facilities are limited, the real burden of tuberculosis (TB) lymphadenitis is not well known. As

far as I know there was no similar study with this study in this site. Our study is on the Assessment of possible tuberculous lymphadenopathy by Xpert MTB/RIF assay compared to non-molecular methods at SPHMMC. It used different techniques to get accurate magnitude.

Therefore this study had the following benefits. One to assessed the magnitude of TB lymphadenitis and It will an input for the federal ministry of health to set new guide line and also it will an input for the researcher and reviewer to do further research and another benefit will be to found a suitable, but sensitive and specific method for diagnosis of tuberculous lymphadenitis.

2. Literature review

A prospectively study conducted in India by Pahwa R. *et al* on the Assessment of possible Tuberculous lymphadenopathy by PCR compared to non-molecular methods showed that Most of the patients (55 %) presented lymphadenopathy of variable duration of weeks to years and a few also had fever with evening rise (22 %) or fever and cough (18 %). A positive family history and positive past history were found in 13% and 12% of the patients, respectively (48).

Another Descriptive study was conducted at Jinnah Postgraduate Medical Center, Karachi from March 2007 to March 2010 by mazhar iqbal *et al*, showed that a total of 220 patients with enlarged neck lymph nodes were included. Predominantly adult males were involved. Most (n 155 - 70.45%) of the patients had tuberculous lymphadenitis. In 30 patients (13.63%) reactive hyperplasia found while 25 (11.3%) had metastatic lymph nodes and 10 (4.54%) had lymphoma. Chronic non-specific lymphadenitis was seen in five (2.27%) patients (49).

Another cross sectional retrospective study conducted in India by Nidhi P. *et al* showed that Incidence of tuberculous lymphadenitis was 55%. Overall AFB positivity was 71.0% (50).

A retrospective study was done in Germany by Geldmacher H. the result showed that The cervical lymph nodes were most frequently involved (63.3%), followed by the mediastinal lymph nodes (26.7%) and the axillary lymph nodes (8.3%). All patients (except one patient who was HIV-positive) showed a positive response to tuberculin skin testing. Lymph node excision and fine-needle aspiration (FNA) were similarly effective in obtaining sufficient material for histologic and microbiological analysis. Mycobacterium tuberculosis was identified in 43.3% of patients by microbiological testing, and culture methods showed the highest sensitivity. Despite standard treatment, the initial enlargement of the lymph nodes occurred in 20% of patients and local complications occurred in 10% (51).

A cross sectional study was done by Pathaka S. *et al* showed that Out of 249 cases of Extra pulmonary Tuberculosis,225 cases were of tuberculous lymphadenitis constituting 90.36% of cases. Remaining cases included Genito urinary tuberculosis(4.01%),Musculoskeletal tuberculosis(3.20%),Gastrointestinal tuberculosis(1.60%) and Cutaneous tuberculosis (0.80%) (53). Similar prospective study was carried out in the department of otorhinolaryngology head &

neck surgery, Kathmandu Medical College, Kathmandu, during two years, from January 2006 to January 2008 by Maharjan M, *et al* showed that 83 (54%) had tubercular lymphadenitis (52).

Fifty two (33%) cases had reactive lymphadenitis and 17 (11%) cases were diagnosed with metastatic neck nodes. Fine needle aspiration cytology was found to be highly effective in the diagnosis of tubercular lymphadenitis with 94% accuracy. Majority of patients were otherwise healthy adults, aged between 8 – 71 years.

The same cross sectional study was done by Nidh P. *et al* showed that Incidence of tuberculous lymphadenitis was 55%. Overall AFB positivity was 71.0%. Only Necrosis without epithelioid cell granulomas was the most common cytological picture and that showed highest AFB positivity also. Three fourth of the patients presented in second to fourth decade of life (53). Another retrospective study was done by Y. Ahmed N. showed that AFB were identified by ZN stain in 27/41 (65.9%) of the cases; while IHC staining was positive in 39/41 (95.1 %) of cases (54).

A Cross sectional study was done by Naveen K. showed that Of the 100 patients studied, maximum number of cases was reported in the age group of 31 to 40 years. Majority of the patients were males (81%). Most common presentations were fever in 71%, weakness in 71%, weight loss in 53% and lymph node swelling in 23% of patients. Most common site of lymph node involvement was cervical. FNAC of lymph nodes revealed that maximum number of cases had tuberculosis lymphadenitis (59%), followed by reactive hyperplasia (37%), non-Hodgkin's lymphoma (2%), Cryptococcus's (1%) and metastasis (1%) (55).

A Prospective study was conducted in Lok Nayak Hospital; by Pahwa R. showed that 74% of aspirates were positive by fluorescent stain while only 22%were positive by culture. PCR could be performed in 55 cases, out of which 22 (40 %) were positive. When compared to culture, the sensitivity and specificity of PCR were found to be 89.5% and 86.1%, respectively. Fluorescent stain was found to be the most sensitive (81.8%) of the conventional methods but showed poor specificity (28.2 %). Interestingly, PCR detected 80%of smear-negative but culture positive cases (56).

A cross sectional retrospective done by Fanny M. *et al* in Central African Republic showed that Ziehl-Nelsen staining for acid-fast bacilli was positive in 42.7% of samples, and culture

identified TB in 67.2% of cases. Of 75 samples that were stain-negative, 49 (65.3%) were culture-positive, while 12 stain-positive samples remained culture-negative. Ten of the 12 stain-positive, culture-negative samples were from patients who had received previous antimicrobial therapy. With regard to phenotypic drug susceptibility, 81/88 strains (91.1%) were fully susceptible to isoniazid, rifampicin, ethambutol and streptomycin, six (6.8%) were resistant to one drug, and one multidrug-resistant strain was found (57).

Another retrospective study was done by Smaoui S. in Tunisia showed that Demonstration of acid-fast bacilli in microscopy from either fine-needle aspirates or biopsies was done in 17.5% of cases, and cultures yielded positive results in 27%. Treatment duration was varied. Paradoxical reactions were noted in 12% and persistent lymphadenopathy after treatment completion was noted in 10% of cases (58).

A community-based cross sectional study was conducted from February to March 2009 in Gilgel Gibe Field Research area by Abebe G. *et al* the result showed that complete data were available for 27,597 individuals. A total of 87 TB lymphadenitis suspects were identified. Most of the TB lymphadenitis suspects were females (72.4%). Sixteen cases of TB lymphadenitis were confirmed. The prevalence of TB lymphadenitis was thus 58.0 per 100,000 people. Individuals who had a contact history with chronic coughers were more likely to have TB lymphadenitis. Lymph nodes with caseous FNA were more likely to be positive for TB lymphadenitis (59).

A Retrospective study was conducted by Muluye D. *et al* in Gondar University Hospital, Northwest Ethiopia A total of 3,440 lymph node aspirates were examined using fine needle aspiration cytology. Of these, 2,392 (69.5%) cases were found to have tuberculous lymphadenitis. Male 1647(47.9%) to female 1793(52.1%) ratio of all study subjects were 0.9:1. Females (54.1%) were more affected than males (45.9%) (60). A similar prospective cross sectional study was conducted from April to May 2012 by Biadglegne F. in Ethiopia showed that Out of 1070 patients attending the cytological diagnosis in the study sites 437 (41%) were positive for TBL. Of the 437 registered TBL, 59 (13.5%) were pediatric patients and 378 (86.5%) were adults. There were more females than males with a male to female ratio of 0.8:1. The cervical region had the most common group of TBL with 321 (73.2%) patients (61). The same retrospective study was conducted in Ethiopia by Ameya Buli G. the result showed that a total of 1,067 lymph nodes were aspirated in a period of five years. Tuberculosis lymphadenitis

was found to be 521(48.8%). Cervical lymph nodes were recorded to be the highest 286(54.89%) affected site with tuberculosis lymphadenitis (62).

3. Objective

3.1 General objective

The objective of this study was to determine the prevalence of TBLN and to compare diagnostic performance of laboratory methods in diagnosis of TBLN among clinically presumptive TBLN patients.

3.2 Specific objectives:

1. To determine the magnitude of tuberculous lymphadenitis.
2. To assess the agreement among different diagnostic methods (Cytology, Fluorescent microscopy, AFB and Xpert MTB/RIF assay) for diagnosing tuberculous lymphadenitis.
3. To determine the sensitivity and specificity of Cytology, Fluorescent microscopy, AFB and Xpert MTB/RIF assay for diagnosing tuberculous lymphadenitis.
4. To assess the risk factors of Tuberculous lymphadenitis
5. To Assesses the agreement between the direct and NALC- NAOH decontaminated and concentrated processing method

4. Materials and Methods

4.1 Study area

The study was conducted at SPHMMC, Addis Ababa, Capital city of Ethiopia. The town has latitude and longitude of 9.02 N and 38.74 E respectively with an elevation of 2,326 meters

above sea level. With a projected population of 2,739,551, of whom 1,305,387 are men and 1,434,164 women; all of the populations are urban inhabitants.

Addis Ababa is the center of African Union and capital city of Ethiopia. And there are 15 public and 23 private hospitals respectively. Of which (5) Federal Ministry of Health, (5) Addis Ababa Regional Health Bureau, (2) Non-Governmental Organization, (3) Defense Public Hospital, and the rest 23 are private hospitals.

SPHMMC is the referral hospital for Ethiopian and the main teaching hospital or Medical School. Accordingly, patients visiting the hospital come from all over the Northern, Western, Southern and East parts of Ethiopia. However, most patients seeking consultation at SPHMMC are predominantly residents of Northern parts of Oromia and Addis Ababa city administration. The range of monthly FNAC service users at SPHMMC is 400-500.

4.2 Study Design and Period

A cross sectional study was conducted from December, 2015 to May, 2016 to show the burden and to compare diagnostic performance of laboratory methods in diagnosis of TBLN among presumptive TBLN patients at SPHMMC.

4.3 Source Population

The source population was all patients who were visited SPHMMC.

4.4 Study Population

The study population was all patients who had an accessed to visited SPHMMC, Pathology Department during the study period.

4.5. Study subject

The study subjects were all clinically tuberculous lymphadenitis presumptive to be patients who were visited SPHMMC, pathology departments during the study period.

4.6 Sample size Calculation and Sampling Technique

4.5.1 Sample collection and transportation

The Samples from presumptive patients for tuberculous lymphadenitis were taken with fine needle aspiration (FNA) by a pathologist as routine procedure. It was preserved by normal saline and transported to the TB and pathology laboratory of EPHI and SPHMMC respectively to been investigated and the samples were performed within two hours after collection.

4.5.1.1 Biosafety

All specimens were treated as potentially infectious and wearied protective gloves and a laboratory gown while handled specimens.

Did not eat, drink or smoke in the laboratory while samples were collected and processed, used the safety cabinet while sample processed and cleaned up spills with appropriate disinfectants. All materials were Decontaminating with an appropriate disinfectant and Disposed of all waste, included all clinical material and used reagent, using an appropriate method such as placed sharp objects in a biohazard container and disposable materials in sealable waste bags for incineration.

4.5.2 Sample Size Calculation

4.5.2.1 Sample Size calculation for epidemiology study

The required samples sizes were calculated using the prevalence of Tuberculosis Lymphadenitis in Northwest Ethiopia among suspected patients 41% (56). Therefore, using the following formula

$$n = \frac{\left(\frac{Z}{2}\right)^2 pq}{d^2}$$

$where, n = sample$ ¿¿

$Z = confidence\ interval$

$p = proportion$

$q = 1 - p$

$By\ using\ 95\ confidence\ interval\ i.e.\ Z = 1.96$

P= 0.41, with an estimated prevalence of 41 % Prevalence of Tuberculosis Lymphadenitis in Northwest Ethiopia.

d= 0.05 as 95% confidence interval is taken.

Non-respondents rate=10%

Therefore, the total sample size for Prevalence of Tuberculosis Lymphadenitis survey was 408 with non-respondents' rates of 10%.

4.5.2.2 Sample size calculation for the methods evaluation

The sensitivity of the cytomorphology was 81% (46). And to measure the sensitivity within ± *0.1* then we would have chosen *n* so that the confidence interval is ± *10 %*.

Therefore the number of sample was

$$n = \frac{\left(\frac{Z}{2}\right)^2 pq}{x^2}$$

$$n = \frac{(1.96)^2 0.81(1-0.81)}{(0.1)^2}$$

n=59

Therefore, to measure the sensitivity to within ± 10% we require at least 59 samples that are positive by the 'gold standard' test. 59 infected study subjects by the reference standard test and the prevalence of infection in the study population was 41%, then there were 41 infected subjects per 100 patients seen at the hospital. Therefore, to had 59 infected subjects, needed to recruit **144** patients (100/41 × 59) (WHO, 2010) (63).

Therefore the total sample size used for both methods evaluation and epidemiology was **144**

4.6 Data Collection

A total of 152 FNAs were collected under aseptic conditions. Clinicians provided information on the clinical features and also confirmed by pathologists. Samples were prepared according to the routine procedure.

4.6.1 Administration of questionnaire

Pretest semi-structure questionnaires, which were first prepared in English and then translated into the local language (Amharic) during data collection, which were used to collect socio-demographic characteristics of the patients and other TB lymphadenitis clinical characteristics. The data collectors were trained laboratory technologists from SPHMMC pathology department.

4.6.2 Quality control

The questionnaires were pretested before the actual study began to make sure that whether the questionnaires were appropriated and understandable. The collected data were checked daily for consistency and accuracy. The appropriateness of the reagents was rechecked with a known positive and negative sample. Randomly positive and negative smears were blindedlly rechecked by an experience pathologist and microbiologist for quality assurance and smears from known positive and negative specimens were used as positive and negative controls for internal quality control.

4.7. Specimen collection, transportation and processing and laboratory investigation

The FNA were done at SPHMMC pathology department FNAC room. Three smears were made from each aspirate: Of them two were transported to EPHI TB lab and stained with, ZN, and AR and one at pathology department stained with Wright stain stains. Two sterile falcon tubes used to collected aspirate and Needle washes, one was incubated for culture over Lowenstein-Jensen medium and one was for gene Xpert analysis. If aspirates were found to be inadequate, FNA were repeated at the same time for better retrieval of aspirate. Culture over Lowenstein-Jensen medium was taken as a reference method.

4.7.1. Cytological diagnosis

One slide was air dried and stained with Wright stain and examine by experienced pathologist. The evidences for diagnosis of tuberculosis by cytological examination of FNA samples were on the presence of epitheloid granuloma with or without multinucleated giant cells and with or without caseus necrosis and / liquefied necrotic material with degenerating and viable inflammatory cells without epitheloid granuloma.

4.7.2. Ziehl Neelsen staining method

The stained smears were examined under the oil immersion objective to look for acid fast bacilli in a light microscope (Olympus C × 31- Japan). A minimum of 100 oil immersion fields were observed to declare negative smear.

4.7.3. Fluorescence microscopy

Switch on the mercury vapor lamp. The bulb takes approximately 10 minutes to reach full intensity. Using the low power objective (magnification 100-150x) first examined a known positive slide to ensure that the microscope was correctly set up. With auramine staining, the bacilli appeared as slender bright yellow fluorescent rods, standing out clearly against a dark background. Rule out any artifacts. Smear needed to be observed in “linear pattern”.

4.7.4. Xpert MTB/RIF assay

The reagent was added in a 1:1 ratio to the specimen. The sample container was agitated twice during a 15-min incubation period at room temperature. Finally, 2ml of the mixture was transferred to the Gene Xpert test cartridge. The cartridge was loaded into the Xpert MTB/RIF instrument for automated sample and real-time polymerase chain reaction (RT-PCR) processing. The instrument reported the presence or absence of M. tuberculosis complex, a semi quantitative estimate of M. tuberculosis complex concentration (high, medium, low, and very low) and the presence or absence of rifampicin resistance after 1:55 min.

4.7.5. Mycobacterial culture

After the sample directly inoculated in to two Lowenstein–Jensen (LJ) slants,which were incubated at 37 ^{0}C for 8 weeks, All the remaining specimen was fully processed by digestion decontamination and concentration. The N-acetyl-L-cysteine and sodium hydroxide method

(NALC/NaOH) was used for digestion and decontamination. Thereafter, the specimen was concentrated by centrifugation at 3500rpm for 15 min and resuspended in 1 ml of sterile phosphate buffer (pH = 6.8) . The processed specimen sediment was used to inoculate two Lowenstein–Jensen (LJ) slants, which were incubated at 37 ^{0}C for 8 weeks and both direct and decontaminated culture examined weekly until the inoculum were absorbed for eight consecutive weeks, during which time the presence or absence of growth and contamination were noted.

4.8 Eligibility

4.8.1 Inclusion Criteria

- All patients who had clinical sign and symptom
- All patients who were Presented during the study period
- All patients who were Willing to signed the consent form and
- All patients who had adequate sample volume.

4.8.2 Exclusion criteria:

- Patient who had no clinical sign and septum
- Very small or non-palpable lymph nodes, or known cases of malignant, allergic, or skin disorders
- Inadequate sample volume

4.9 Study Variables

4.9.1 Dependent variable

- Prevalence of tuberculosis lymphadenitis
- Cytology result
- Tb culture result
- Fluorescent microscopy result
- Xpert MTB/RIF assay result
- AFB result
- Risk factors

4.9.2 Independent variable

- Socio demographic characteristics
- Clinical characteristics

4.10 Authorship and Intellectual Property Right

The authorship right is for the investigators. All data that was obtained during this study period is the property of the investigators.

4.11 Statistical Analysis

The data were analyzed using software packages SPSS version 20 (SPSS Inc, Chicago, Illinois, USA). Negative predictive value, positive predictive value, sensitivity and specificity were calculated. Kappa value was calculated to see the presence of agreement. Chi-square test was done along with P-value to see the presence of associations. P-values less than 0.05 were considered as statistically significant. And Logistic regression analyses were used to see the association of different variables. Odds ratios and 95% confidence interval were computed to determine the presence and strength of association.

4.12 Dissemination of Results

The findings of this project were submitted to Addis Ababa University, college of health science, school of medicine, department of pathology. There will be defense based on the school schedule. The results of the whole study will be disseminated to the concerned health institutions. Said to be scientific the work will be published in peer review journals and will be accessible for further study.

4.13 Ethical considerations

The study was conducted after obtaining of Ethical permission from Department of pathology, School of Medicine College of Health Science, Addis Ababa University. And institutional ethical clearance from ethical review committee of Ethiopian public health institute. Informed consent and incent were also obtained from the study participants and guardian respectively. The result of the study participants were reported to the respective physicians for treatment. Information obtained at any course of the study was kept confidential. In addition, the clinical specimen collected during the study period was used for the stated objectives only and the study participants were participated once in the study period.

5. Results

5.1. Socio-demographic characteristics

In this study, 152 clinically presumed to be TBLN patients were enrolled as per the inclusion criteria. The median age of the patients was 27 (range 5–72) years. The majorities of the patients were female (55.9%), primary school (50.7%), Daily laborer (30.9%), and married (41.4%) (Table.1).

Table1.Socio-demographics characteristics of presumptive TBLN patients from SPHMMC, Addis Ababa, Ethiopia, December 2015 to May 2016 (N=152).

Socio-demographics	**Categories**	**n(%)**
Sex	Male	67(44.1)
	Female	85(55.9)
Age (Years)	0-14	18(11.8)
	15-24	44(28.9)
	25-34	41(27.0)
	35-44	24(15.8)
	45-54	14(9.2)
	>54	11(7.2)
Educational status	Illiterate	42(27.6)
	Primary school	77(50.7)
	Secondary school	25(16.4)
	Higher education	8(5.3)
Occupation	House wife	13(8.6)
	Daily laborer	47(30.9)
	Government employee	12(7.9)
	Unemployed	38(25.0)
	Farmer	36(23.7)
	Prisoner	2(1.3)
	Other	4(2.6)
Residency	Urban	84(55.3)
	Rural	68(44.7)
Marital status	Living with partner	14(9.2)
	Married	63 (41.4)
	Divorced	6 (3.9)
	Single	63 (41.4)
	Widowed	4 (2.6)
	Separated	2 (1.3)

Fine needle aspirates were collected from 152 presumptive TBLN patients. The direct and concentrated aspirates were inoculated on LJ culture media. The composite TB culture positivity rate was 68/152 (44.7%). The highest composite culture positivity rate (32.4%) was recorded among the youngest age group (25-34) years old. Most of composite culture positive patients were rural dwellers (52.9%). Sex had no association with composite LJ positivity rate. In terms of occupation, high composite LJ positivity rate (32.4%) was observed in daily laborers. However, there was no statistically significant association with occupation (Table 2).

Table 2. Association of TBLN with socio-demographic characteristics of presumptive TBLN patients from SPHMMMC, Addis Ababa, Ethiopia, December 2015 to May 2016 (N=152).

Socio-demographics	**Categories**	**TBLN (n=68), n(%)**	***P value***
Sex	Male	34(50)	0.186
	Female	34(50)	
Age(years)	0-14	8(11.8)	0.726
	15-24	19(27.9)	
	25-34	22(32.4)	
	35-44	10(14.7)	
	45-54	6(8.8)	
	>54	3(4.4)	
Educational status	Illiterate	19(27.9)	0.708
	Primary school	35(51.5)	
	Secondary school	12(17.6)	
	Higher education	2(2.9)	
Occupation	House wife	2(2.9)	0.171
	Daily laborer	22(32.4)	
	Government employee	3(4.4)	
	Unemployed	18(26.5)	
	Farmer	19(27.9)	
	Prisoner	1(1.5)	
	Other	3(4.4)	
Residency	Urban	32(47.1)	0.067
	Rural	36(52.9)	

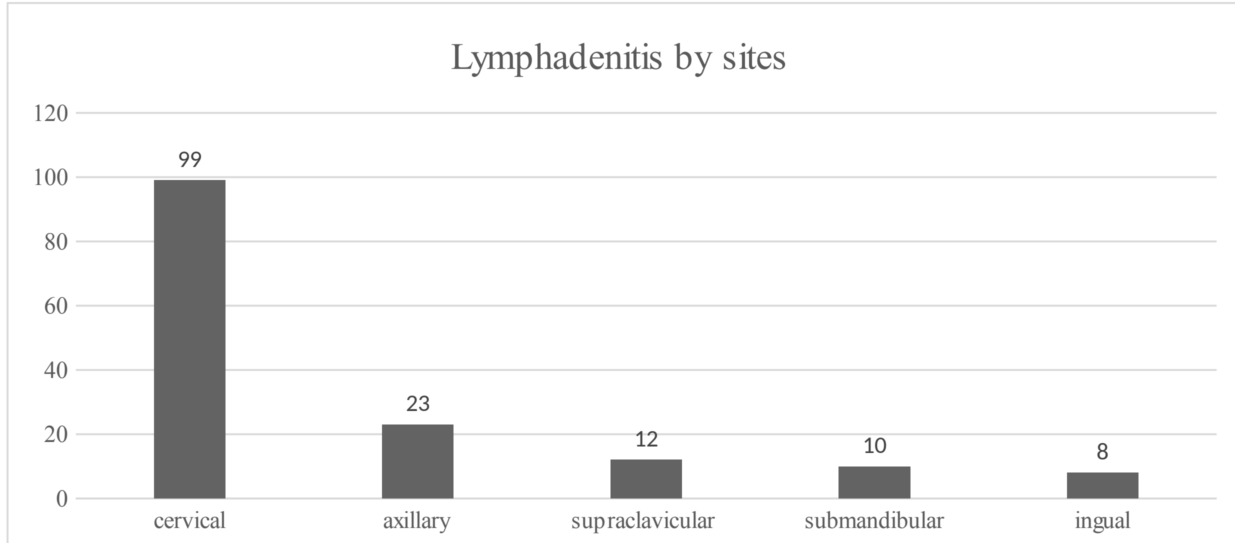
Marital status	Living with Partner	8(11.8)	0.548
	Married	31(45.6)	
	Divorced	1(1.5)	
	Single	25(36.8)	
	Widowed	2(2.9)	
	Separated	1(1.5)	

5.2. Clinical characteristics

A total of 152 presumptive TBLN patients were visited the hospital for lymph node swelling diagnosis. Among these, 25(16.4%) had previous history of PTB treatment, and of them only 4(6%) were confirmed TBLN by composite LJ culture. There was significant association with previous history of PTB treatment (*p=0.020*). As shown in Figure 1, most of FNAs were collected from cervical (65.1%) lymph node of TBLN presumed to be patients. The majority LJ

composite confirmed cases were in cervical lymph node (69%). But, there was no statistically association with the lymph node site.

Figure 1.Distribution of lymph node sites from presumptive TBLN patients at SPHMMC, Addis Ababa, Ethiopia



Pus (AOR 0.082, 95% CI 0.030-0.228) and caseous (AOR 0.059, 95%CI 0.020-0.169) aspirates were significantly associated with the TBLN by composite culture. Previous treatment was significantly associated with TBLN (AOR 0.113, 95% CI 0.031-0.407). However, diabetic mellitus was not associated with TB lymphadenitis (Table 3).

Table 3. Association of TB lymphadenitis with clinical characteristicsof presumptive TBLN patients from SPHMMMC, Addis Ababa, Ethiopia, December 2015 to May 2016 (N=152)

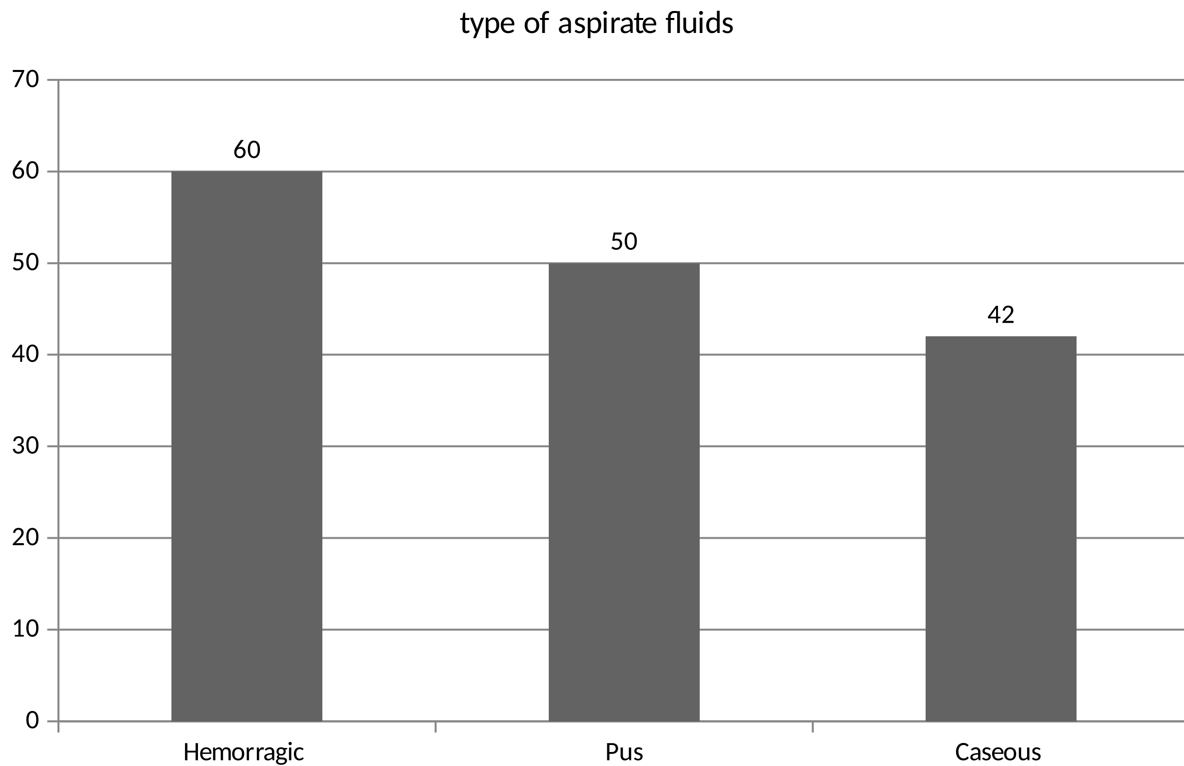
Clinical characteristics	LJ TB culture result		COR (95% CI)	AOR (95% CI)
	Positive	Negative		
Type of aspirate				

Hemorrhagic	9(15)	51(85)	Ref.	Ref.
Pus	29(58)	21(42)	0.128(0.052-0.316)	0.082(0.030-0.228)
Caseous	30(71.4)	12(28.6)	0.071(0.027-0.187)	0.059(0.020-0.169)
Previous TB treatment				
Yes	4(16)	21(84)	Ref.	Ref.
No	64(50.4)	63 (49.6)	0.188(0.064-0.557)	0.113(0.031-0.407)
Diabetes				
Yes	4(25)	12(75)	Ref.	Ref.
No	64(47.1)	72(52.9)	0.375(0.115-1.221)	0.259(0.065-1.023)

5.3. **Macroscopic Examination**

FNAs gross examination was performed for 152 samples. Hemorrhagic or blood mixed (39.5%) was the predominant type of FNA, followed by purulent or pus (32.9%) and caseous (27.6%) (Figure 2).

Figure 2. Distribution of FNA types obtained from presumptive TBLN patients at SPHMMC, Addis Ababa, Ethiopia December 2015 to May 2016 (N=152).



Cytological examination was identified TB lymphadenitis in 40/42 (95.2%) of caseous aspirates; however, *M. tuberculosis* was detected in 30(71.4%), 28(66.7), 15(35.5%), and 8(19%) of the caseous aspirates using LJ culture, GeneXpert, Fluorescence Microscopy (FM), and ZN Microscopy (ZN), respectively. Cytology revealed TB lymphadenitis in 40 out of 50 (80%) of pus aspirates. But, *M. tuberculosis* was confirmed in 29(58%), 35(70%), 20(40%) and 13(26%) of the pus aspirates using LJ culture, GeneXpert, FM and ZN, respectively. Cytology has identified 23/60(38.3%) of TBLN; however, *M. tuberculosis* was detected in 9/60 (15%),

12/60(20%), 2/60(3.3%) and 1/60 (1.7%) by TB culture, GeneXpert, FM and ZN, respectively. There was significant association between the FNA types and confirmed TB lymphadenitis utilizing different diagnostic modalities (*p<0.001*) (Table 4).

Table. 4. Comparison of diagnostic yield of different laboratory methods for diagnosis of TBLN with aspirate types at SPHMMC, Addis Ababa, Ethiopia, December 2015 to May 2016 (N=152)

Types of aspirate	**Laboratory methods**										
	Culture		**FNAC**		**GeneXpert**		**FM**		**ZN**		**total**
	Positive (n,%)	Negative (n,%)	Positive (n,%)	Negative (n,%)	Positive (n,%)	Negative (n,%)	Positive (n,%)	Negative (n,%)	Positive (n,%)	Negative (n,%)	
Hemorrhagic	9(15)	47(85)	23(38.3)	37(61.7)	12(20)	48(80)	2(3.3)	58(96.7)	1(1.7)	59(98.3)	60
Pus	29(58)	21(42)	40(80)	10(20)	35(70)	15(30)	20(40)	30(60)	13(26)	37(74)	50
Caseous	30(71.4)	12(28.6)	40(95.2)	2(4.8)	28(66.7)	14(33.3)	15(35.7)	27(64.3)	8(19)	34(81)	42

5.4. Laboratory result

One hundred fifty two fine needle aspirates (FNAs) were collected at pathology department of SPHMMC from eligible patients. Cytological analysis indicated that 103 out of 152 (67.8%) of the presumed to be TBLN patients had TB lymphadenitis. As shown in table 4, TB positivity rate was 14.5%, 24.3%, 44.7%and 49.3% using ZN, FM, LJ culture and GeneXpert, respectively.

Table 5. TBLN positivity by different diagnostic methods (N=152).

Methods	Positive	Negative
Cytology	103(67.8%)	49(32.2%)
Gene Xpert	75(49.3%)	77(50.7%)
Culture	68(44.7%)	84(55.3%)
FM	37(24.3%)	115(75.7%)
ZN	22(14.5)	130(85.5%)

5.4.1. Cytology Result

Cytological diagnoses of FNA direct smears were available for all collected specimens. The criteria for cytological diagnosis were epithelioid cell granulomas with or without multinucleate giant cells, with or without necrosis and caseous necrosis without granuloma. Thus, cytomorphological features of FNAC consistent with tuberculosis lymphadenitis were reported in (67.8 %) of the examined smeared specimens, whereas the remaining 49/152(32.2%) smeared specimens showed different kinds of lymphadenopathies and malignancy.

A comparison of FNAC and GeneXpert showed that both diagnostic modalities diagnosed TBLN in (42.8%). When compared with LJ culture both combined together dictated 64/152(42.1%). When it compared with ZN stain both methods diagnosed (13.2%) and with FM (22.4%). and the agreement of the cytology with GeneXpert was fair (*k=0.371*), however, with FM and ZN to diagnosis TBLN were slight (*k=0.199*) and (*k=0.107*) respectively (Table 6). Moreover the sensitivity and specificity of the cytology were 94.1% and 53.6% respectively when composite culture used as the gold standard (Table 7) and when we compared cytology against bacteriological MTB result the sensitivity and specificity of the cytology was 87.8% and 61.3 % respectively (Table 8).

5.4.2. GeneXpert

One hundred fifty two FNAs were analyzed by Gene Xpert. The positivity rate was 49.3%. A comparison of Gene Xpert with LJ culture (34.9%), ZN (13.8%) and FM (22.4%) microscopies indicated that both methods to diagnosed TBLN. And the agreement with FM was moderate **(***k= 0.417***)**. Whereas, with ZN was slightly lower (*k=0.269*). The diagnostic sensitivity and

specificity of Gene Xpert was 78% and 74% respectively when composite culture used as the gold standard (Table 6 & Table 7).

5.4.3. LJ TB Culture

A total of 152 FNAs were processed for bacterial growth on Löwenstein–Jensen (L-J) culture media. The composite culture positivity rate was 44.7%. 64(42%) samples were grew on direct LJ culture but 48 (32%) were on concentrated. The high agreement was observed between direct and composite LJ culture (*k=0.92*). From the total confirmed cases in composite LJ culture, 21 (30.9%) cases were grew on direct LJ culture but not grew on concentrated LJ culture whereas, 3(4.4%) cases were grew on concentrated but not on direct culture media.

5.4.4. Ziehl Neelsen microscopy

All collected specimens were processed to look the bacilli by Ziehl-Nelson staining. ZN smear positivity rate was 14.5%. And also compared with FM both techniques showed that the same rate (14.5%) that alone. Moreover the agreement between these two techniques in diagnosis of TBLN was moderate (*k=0.689*). The overall FNA, ZN smears detected 32.4% of culture proven TBLN cases. The diagnostic sensitivity and specificity of ZN was 30.9% and 98.8% respectively when composite culture used as the gold standard (Table 6 & Table 7).

5.4.5. Fluorescence Microscopy (FM)

One hundred fifty two FNAs were diagnosed by direct FM. The smear positivity rate was 24.3%. The direct FM has detected 54.4% of culture confirmed TBLN cases (Table 6**)**. The diagnostic sensitivity and specificity of direct FM was 47% and 94%, respectively, against LJ culture (Table 7).

Table 6. Comparison and kappa values of diagnostic yield of different laboratory methods used for diagnosis of TBLN with each other's at SPHMMC, Addis Ababa, Ethiopia.(N=152).

Diagnostics	**Cytology**		***Kappa value***
	Positive	**Negative**	
GeneXpert			
Positive	65	10	
Negative	38	39	*0.371*
ZN microscopy			*0.107*
Positive	20	2	
Negative	83	47	
FM			*0.199*
Positive	34	3	
Negative	69	46	
	ZN microscopy		
	Positive	Negative	
Xpert			*0.269*
Positive	21	54	
Negative	1	76	
FM			*0.689*
Positive	22	15	
Negative	0	115	
	FM		
	Positive	Negative	
Xpert			*0.417*
Positive	34	41	
Negative	3	74	

Table 7. Sensitivity, specificity, positive and negative predictive values of the laboratory methods against composite reference standard: direct and/ or concentrated LJ culture for the diagnosis of presumptive TBLN patients SPMMC, Addis Ababa Ethiopia.

Laboratory Methods	**Sensitivity (95%CI)**	**Specificity (95%CI)**	**PPV (95%CI)**	**NPV (95%CI)**
Cytology	94.1%(91.5-96.5)	53.6%(48.4-58.8)	62.1%(57.1-67.1)	91.8%(89-94.6)
GeneXpert	78%(73.7-82.3)	74%(69.4-78.6)	71%(66.3-75.7)	81%(76.9-85.1)
ZN	30.9%(26-35.7)	98.8%(97.8-99.8)	95.5%(93.5-97.5)	63.8%(58.8-68.8)

FM	47%(941.8-52.2)	94%(91.5-96.5)	86.5%(83-90)	68.7%(63.9-73.5)

Table 8. Sensitivity, specificity, positive and negative predictive values of the cytology against bacteriological methods for the diagnosis of presumptive TBLN patients SPMMC, Addis Ababa Ethiopia.

Operation	Cytology
Sensitivity (95%)	87.8% (84..8- 90.8)
Specificity (95%)	61.3% (57.1-65.1)
PPV (95%)	40% (32.1- 48.3)
NPV (95%)	77.5% (72.9-82.1)

6. Discussion

In this study, the prevalence of TBLN was 44.7%, which is near to half of all lymph node swelling caused by tuberculosis. Which is lower than study done by Ketema A. et al in Ethiopian with prevalence of 78% (64). This higher prevalence could be due to the sample size, study area and the time of study where awareness of TB was poor years ago, scanty bacilli in glands (65). In contrast to this study, the prevalence of TBLN was lower in northern Ethiopia (33%) (66). This difference could be due to study area, sample size, where small sample size was used in their study.

In our study, females were slightly more common than males (55.9% vs. 44.1%). Similar observation was noted by Ergete et al and Purohit et al while male predominance was noted by Rajsekaran et al (67,68,69). Reason of this high number of females to be suffering from lymph node swelling is that the females in developing countries have weak immune system as they usually do more work, consume less and low quality food and bear the high nutritional and physical burden during repeated pregnancies and lactation (70,71,72).

The age group of patients with TBLN exposed involvement of younger patients with 25–34 years old being affected accounting 32% followed by 15–24 years old accounting 29.2% of the cases.

This finding is inconsistent to other studies in Ethiopia, where younger than 30 years old are the commonest age group affected by this disease (73,74,75).An explanation for this finding remains unclear, but it is suggested that endocrine factors may play a key role in their behavioral changes in this age group thus increase the chance of exposure to infection. Furthermore, this could be due to fact that, the increased exposure of the young adult and adolescent to the environments as they have active social life and also the most affected age group of HIV.

Tuberculosis lymphadenitis confirmed cases were higher in daily laborer (27%) followed by students (18.9%).This might be due to fact that the daily laborer live with low nutritional and economic background and possible also be due to occupational background. A considerable number of TBLN cases were students. This might be due to, this category of patients are among young reproductive age and more vulnerable to HIV infection with high prevalence than other age group (74,76). But occupations were not statically significant (*p=0.370*). And assessed the HIV sero-satatus of individuals, the TBLN manifestation of TB was prevalent in 36.4 % of non HIV cases while it occurred in 53.3% of patients co-infected with HIV this might be due to HIV favor infection of TB.

Majority of TBLN confirmed cases 45.6% was married this could be more than a person live together and followed by single 36.8% ,if single get together with divorced and widowed they represented the high risk group, prone to HIV and TB infections.

The cervical lymph nodes were the most affected anatomical site in TB confirmed cases (65.1%) compared to other affected sites. This finding is in agreement with the previous study done in Ethiopia where cervical regions (74.2%) being mostly affected site (77).

This could associated with the physical closeness of the cervical lymph nodes to the route of infection, as the bacilli can easily picked up by macrophages or dendritic cells which facilitate the transportation of the bacilli in the cervical lymph nodes where they can cause disease (66)

Previous history of PTB treatment had statically significant association with TBLN (AOR 0.113, 95% CI 0.031-0.407) (*P=0.020).*This might related to inadequate treatment, too short administration period, receiving inappropriate dosage so that the organism are getting time to develop resistance to anti-TB drugs or re infection (79,80). And also Pus (AOR 0.082, 95% CI 0.030-0.228) and caseous (AOR 0.059, 95%CI 0.020-0.169) aspirates were significantly

associated with the TBLN by culture. However, diabetic mellitus was not associated with TB lymphadenitis.
Caseous specimens were more likely to be suggestive for TB by FNA cytology as compared to purulent type specimens, supporting previous reports that caseation is strongly indicative for TBLN (81,82). And purulent specimens were more likely to be positive on AFB microscopy. This suggests that combining AFB microscopy with FNA cytology may increase the specificity of the diagnosis.

The diagnosis of TBLN has been a true challenge solely by clinical evidence both in developed and developing countries, particularly in developing countries due to limited diagnostic facility on hand (83). TBLN positivity was confirmed by FNAC in 95.2%, by culture in 71.4% of caseous material and by Gene Xpert in 70% by Ziehl Neelsen staining in 26% and by FM in 40% of pus material. We tried to correlate different gross appearance aspirates with different diagnostic modalities. The highest positivity rate was recorded in caseous material by cytology and culture and in pus material by Gene Xpert, FM and ZN diagnostic methods. Our finding is comparable with studies done by others (81,82). This might be due to the fact that the caseous aspirate believed to contain large numbers of bacilli in advanced stage of the disease (74). This suggests that combining one of the three methods (AFB microscopy, FM or Gene Xpert) with FNA cytology may increase the specificity of the diagnosis if culture is not found. Besides the high positivity of caseous and pus aspirates among different diagnostic modalities were statically significant ($p < 0.05$).

Diagnosis of TBLN mainly relies on FNA cytology, as it is simple, cost effective and less invasive as compared to excision biopsy. Additionally, it is sensitive and requires minimal laboratory infrastructure (83), However, since it depends on cytological changes but not on bacterial detection, its specificity is confounded by inflammatory reactions other than those caused by tuberculosis (84). Moreover, it is not possible to differentiate tuberculous and non-tuberculous mycobacterial causes (85). Our present finding showed that 68.7% was confirmed as positive with cytology. Study in our county, Ethiopia by Dagnachew et al (86) is in consistent with our finding (69.5%). But others study in southern Ethiopia showed low number (48.8%). The reason might be the kind of patients seen at different geographical location, in our study low

sample size used; they used retrospective study method and the stage of disease at the period of data collection. A comparison of FNAC and Genxpert showed that both diagnostic modalities diagnosed TBLN in (42.8%). 10 samples were detected in Gene Xpert but negative in FNAC. this might be due early stage of disease there is no adequate cellular change that can see in light microscope. When compared with LJ culture both combined together dictated 64/152(42.1%) but FNAC alone 68. 7 %. This might be due to other inflammatory reaction increased false FNAC diagnosis or there may be dead bacilli after initiated treatment not grew on culture but the cellular change may persist. FNAC had not good agreement (*k=0.371*) with Gene Xpert.

A study in Ethiopia by Derese et al has shown the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of FNAC against culture to be 81%, 50%, 54.2%, and 78.6% respectively. In our study, the sensitivity, specificity, PPV, and NPV of FNAC was 94.1%, 53.6%, 62.1% and 91.8% respectively *(*73*)*. These results show that cytology had the highest sensitivity (94.1%), which may lead to yielding many false positives. Furthermore, cytology also had low specificity (53.6%) compared to against composite culture and When compared cytology against bacteriological MTB result the sensitivity and specificity of the cytology was 87.8% and 61.3 % respectively. As TBLN were diagnosed among 39 culture negative samples. In general, cytological morphology in FNA lacks specificity but has higher sensitivity because non-tuberculous granulomatous patients could also be diagnosed as TBLN. Therefore, trusting on cytology alone could lead to false case reporting to policy makers as well as wrong patient management and improper use of anti-TB drugs.

Gene Xpert positivity rate was 49.3%. 22 cases were detected from the culture negative cases. This could be the bacilli die due to different factors. In other hand 15 cases negative in Gene Xpert but the positive in culture. This might be due to limited bacilli found in FNA sample.

In the present study, the sensitivity of Gene Xpert was 78%. A systematic review and meta-analyses conducted by Denkinger et al showed that Gene Xpert test has a sensitivity ranging from 50% to 100% with pooled sensitivity of 83% (87). However, the sensitivity of Gene Xpert in the current study is higher than the study done by Mulualem et al (sensitivity, 66.7 %) (88) . The specificity (74%) of the X pert in the current study was found to be higher than the study done by Biadigilegn et al (specificity, 69.2%)(89). And lower than with previous studies

reported by others (specificity, 89–99%) (90). The agreement of Gene Xpert with FM was fair **(***k= 0.417***)**. Whereas, with ZN was slightly lower (*k=0.269*).

The presence of 10-100 live bacilli/ml of sample is enough for positive culture result. 44.7% cases of composite culture proven TBLN patients were identified. This finding is in agreement with 10-60% reported by different investigators (73,91,92,93-97).We observed as low as 45% of culture positive rate. Several reasons can support these low findings: Previous treatment by one or more course of anti-biotic before they visit the health institution could suppress and inhibit bacterial growth. Besides, scanty nature of the bacteria in the FNA sample (98,99). Of our study participants, 16.5% were previous treated with one or more course of antibiotics. As it was seen in our study high culture positivity rate (71.4%) was recorded in caseous aspirates materials. This is might be high number of bacterial load in caseous aspirates with advanced stage of disease, as it was reported by others (102). 64(42%) samples were grew on direct LJ culture but 48 (32%) were on concentrated. The high agreement was observed between direct and composite LJ culture (*k=0.92*). From the total confirmed cases in composite LJ culture, 21 (30.9%) cases were grew on direct LJ culture but not grew on concentrated LJ culture this could be bacilli die due to harsh decontamination process whereas, 3(4.4%) cases were grew on concentrated but not on direct culture media probably not inoculated adequate bacilli on direct LJ culture or limited bacilli found in FNA sample.

There is convincing evidence of increased effectiveness of using aura mine stain to demonstrate AFB as compared to the ZN method.
In our study FM gave higher positivity 24.3 % especially in paucibacillary cases, hence it is the most sensitive of the conventional methods but certainly a less specific technique as the number of false positivity is quite high. Direct FM has detected 47% of culture confirmed TBLN cases this is probably low bacilli found in FNA sample. And 5 cases were positive in FM but culture negative this probably dead bacilli could not grew on culture. 15 cases were positive in FM but negative in ZN this may be due to FM has higher sensitivity. The diagnostic specificity was observed (94%). and sensitivity of direct FM was 47% and respectively, against LJ culture (100). The high frequency of FM-positive, culture-negative, Gene Xpert-negative samples in this study

raises the possibility that the frequency of lymphadenitis due to non-tuberculous mycobacteria may be higher than previously appreciated.

If cytological diagnosis by FNAC is confirmed by ZN stain for AFB detection then it becomes an reliable and valuable diagnosis of tuberculous lymphadenitis and treatment can be initiated without any doubt. In our study, maximum positivity of AFB (59%) was observed in pus aspirates well supported by another study conducted by Nidhi et al where they found 85.5% AFB positivity in the smears showing necrosis. The reason is that AFB concentration is more in the aspirates showing purulent or necrotic material both by gross or microscopic examination (101,102). Ziehl Neelsen staining to investigate the AFB in FNA is a simple and affordable method which can be done in determination of lymph node enlargement. Our study showed that 22/152(14.5%) was confirmed as TBLN from the FNA sample. This finding has low positive rate ,as investigating bacilli in specimen by Ziehl Neelsen staining requires more than 10,000 organisms/ml of sample ,thus in FNA sample the expected bacilli is very low. Other investigator also agreed with our present study (74), but different from other studies (73, 94, 98), which consisted 37%-42.7% of efficiency. Different reason might be a raised: the method and the reagent used, the sample size and the distribution/homogensity of bacilli in aspirate can be making difference between our finding and their findings. Even though the sensitivity of this method was found to be relatively low (30.9%), the high specificity (98.8%) was reported. Acid fast bacilli alone give enough confident to the clinician in determination of initiation of anti-TB treatment. This in turn very important to eliminate antibiotic and anti-TB trials so that the patient to get the treatment early. This low degree of sensitivity is in line with the findings of other studies with sensitivity ranging from 20% to 43 % (103). In another study, AFB smear positivity was 27.1 % (73). The quality of the smear as well as the scanty bacilli found in the FNA could be the main factor for decreased sensitivity and, as expected, the specificity was the highest of the four methods. However, one case of the FNA culture negative smear sample was positive by ZN stain. This result may be due to the presence of dead bacilli that failed to grow on culture.

7. Conclusion and recommendation

7.1. Conclusion

The results of this study revealed a high prevalence of TBLN in the study sites. TBLN is an important public health problem that needs to be addressed in the area.

A significant percentage of enlarged lymph nodes were caused by Tuberculosis lymphadenitis. Types of aspirate and previous treatment of PTB were significantly associated with Tuberculosis lymphadenitis. Pathologists should be conscious of tuberculosis cases whenever they encounter enlarged lymph node with pus and/or caseous aspirates to initiates immediate treatments.

Supplementing FNA cytology with conventional diagnostic technique in an endemic region like Ethiopia helps to increase the specificity of the diagnosis of TBLN. Moreover, culture allows drug resistance testing which was previously given less attention in this form of extrapulmonary TB. All this may substantially improve the management of patients that present with lymphadenopathy. And Molecular technique like Gene Xpert may be employed in case with strong clinical suspicion and confusing result, especially at early stage of disease for better diagnosis, management and treatment. From this finding we conclude that the current prevalence

of TBLN with the gold standard technique tool was 44.7%. Genetically MTC are highly related species causative agent for tuberculosis, particularly *M tuberculosis* and *M.bovis*. In our knowledge majority of the causative agent of TBLN was *M.tuberculosis*, though the contribution of *M.bovis* is not excluded.

FNA cytology showed a relatively high sensitivity but a low specificity however ZN revealed high specificity but low sensitivity. Combining bacteriological methods and GeneXpert with FNA cytology in an endemic region like Ethiopia improves the overall accuracy of the diagnosis of mycobacterial lymphadenitis, which in turn may lead to better patient management.

7.2. Recommendations

Based on the findings the following recommendations are forwarded.

1. Because of a true diagnostic challenge nature of TBLN, introduction of easy, cost effective, rapid, more efficient and time saving methods should be applicable.

2. The prevalence of TBLN still high in our study area, so early diagnosis and treatment should be implemented.

3. This study was conducted in the capital city only in one hospital such not representative to the national wide, thus we strongly recommend that further study on epidemiology of *M.tuberculosis*, representative with better and more discriminative power of molecular technique should be implemented.

4. This study was done on small sample size so further prospective and advanced studies are recommended to determine the specific etiologic agents and contributing factors of TBLN in the study area.

8. Strength and limitation of the study

8.1. Strength

1. Culture, cytology and Gene Xpert tests have been done within two hours after samples were collected
2. It's tried to show the prevalence of TBLN in the study area and evaluate most conventional TBLN diagnosis methods.

8.2. Limitation

1. The study used small sample size, this not enough to show the prevalence of TBLN and its risk factors
2. We could not determine the specific etiologic agents of TBLN

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Annex I: Physical examination form

1. Position ____

2. Location A) uni later right side B) uni later left side C) Bilateral

3. Tenderness A) tender B) non tender

4. Number of node: A) single B) few (2-4) C) multiple (>5)

5. Size _________(cm)

6. Mobility A) mobile B) non mobile

7. Condition of the node A) discrete B) matted C) Firm D) soft E) hard F) fluctuant G) draining sinus

8. Clinical diagnosis ____

Annex II : Laboratory examination form

1. Information of aspirated sample and laboratory result

1.1 Aspirated sample information

Date of sample collection / day / month / / / year__

Time of sample collection ____________________

Total number of sample received

1. Smear on slides

AFB dry | FM dry | wright dry

2. FNA suspended in normal saline A) yes B) No

3. Adequate number of sample collected A) Yes B) No

4. If no, why? A) Withdrawal B) Insufficient amount of aspirate

1.2 laboratory results

1.2.1 ZN staining A) NEG

If positive

B) POS 0. Scanty 1. 1+ 2. 2+ 3. 3+ 4. 4+

1.2.2 Aura mine o staining (LED FM)

1. NEG

If positive 2. | Pos | Scanty | 1. 1+ | 2. 2+ | 3. 3+ | 4. 4+ |

1.2.3 LJ culture result at 8th weeks

1. NEG
2. POS
3. Decontaminate

1.2.4 Gene Xpert

Detected

Not detected

1.2.5 Gene Xpert Rifampicin test result

1. A. Susceptible B. Resistance c. intermediate

1.2.6 Cytology result

1.2.6.1 Microscopy for wright smears report

1. Abscess __________
2. Necrosis _________
3. Necrosis + granuloma ___________
4. Granuloma (epitheloid + giant cell) ______

5. Others ________Specify ___________________________

1.2.6.1 Conclusion

1. TB ______

2. Non TB _________

3. Malignancy _________Specify __________________________________

4. Others _______Specify ____

Annex III: Patient Information, Consent and assent Forms

1. Purpose of the study

I have been informed about a study that plans to investigate the Assessment of possible tuberculous lymphadenopathy, which will help in understanding the magnitude of M. tuberculosis in lymph node other than the lung in developing better ETB control method in the country.

We can detect the tuberclosis lymphadenitis infection using cytology from fine needle asparate here at the SPHMMC but there are deferent methods will be done at EPHI. It may take a few days to get the results back to you. We would like to ask for your help in comparing different test methods. In the future we will be able to chosen the best diagnostic method from the same sample.

2. Procedures to be followed

If you agree to participate in the study, the pathologist will give you a routine medical examination and ask you some questions according to standard clinic procedure. He/she will take sample using FNA as is the normal procedure. We will use your sample for different test to diagnosis TB lyphadenitis. You will receive treatment or intervention based on the standard laboratory-based test results.

3. Voluntary participation

During the study, you can choose not to answer any particular question or provide the FNA specimens. A decision not to participate or to withdraw from participation will not affect the care you will receive at the hospital in any way. If you do agree to become a study participant, you can withdraw from study at any time (verbally).

4. Discomfort and risks

You may feel a small amount of discomfort while your FNA is taken and you may have some bruising at the place where the sample is taken. The bruise should disappear in a short time.

5. **Benefits**

There will be no immediate benefits in your participation in the study. When the study results are known the patient will receive the treatment and also may benefit from having better method available to diagnose extra pulmonary tuberculosis.

6. Compensation

There will be no monetary compensation for this study, but routine medical consultation and appropriate treatment services will be provided.

6. Confidentiality statement

The records concerning your participation are to be used only for the purpose of this research project. Your name will not be used on any study forms or labels on laboratory specimens or in any report resulting from this study. At the beginning of the study, we will give you a study identification number and this number will be used on the forms and on the laboratory specimens. Any information obtained in connection with this study will be kept strictly confidential. Only members of the study team will have access to information linking your name with your study number.

8. Questions and freedom to withdraw from the study

You may withdraw from the study at any time without affecting your present or future medical care at the hospitals. You may contact any of the study staff if you have questions about the

research. You may speak with the staff at the hospital. You can also call the hospital during working hours at tel.: +25192284471 Ato Mengistu Fantahun.

9. Results publication

Data from the study will be kept for a minimum of one year after publication of its results.

10. Participant statement

I have been informed verbally and in writing about this study and understand what is involved. I also know whom to contact if I need more information. I understand that confidentiality will be preserved. I understand that I am free to withdraw from the study at any time without affecting the care I normally receive at the hospital. I agree to participate in this study as a volunteer subject and will be given a copy of this informed consent to keep.

________________	______________________	________________________
Date	Name of volunteer	Signature of volunteer
________________	______________________	________________________
Date	Name of witness	Signature of witness

11. Investigator's statement

I, the undersigned, have defined and explained to the volunteer in a language he/she understands, the procedures of this study, its aims and the risks and benefits associated with her participation. I have informed the volunteer that confidentiality will be preserved, that she/he is free to withdraw from the study at any time without affecting the care she/he will receive at the hospital. Following my definitions and explanations the volunteer agrees to participate in this study.

______________	______________________	________________________
Date	Name of investigator	Signature of investigator

12. Certificate of Assent

I have been informed about a study that plans to investigate the Assessment of possible tuberculous lymphadenopathy, which will help in understanding the magnitude of M. tuberculosis in lymph node other than the lung in developing better ETB control method in the country.

We can detect the tuberculosis lymphadenitis infection using cytology from fine needle asparate here at the SPHMMC but there are deferent methods will be done at EPHI. It may take a few days to get the results back to you. We would like to ask for your help in comparing different test methods. In the future we will be able to chosen the best diagnostic method from the same sample.

I have read this information (or had the information read to me) I have had my questions answered and know that I can ask questions later if I have them.

I agree to take part in the research. Or I do not wish to take part in the research and I have <u>not</u> signed the assent below.___________ (initialed by child/minor)

<u>Only if child assents:</u>

Name of child ___________________

Signature of child: ____________________

Date:________________

If illiterate:

I have witnessed the accurate reading of the assent form to the child, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

Name of witness (not a parent)_________________ AND Thumb print of participant
Signature of witness ______________________
Date ________________________

I have accurately read or witnessed the accurate reading of the assent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given assent freely.

Name of researcher_________________

Signature of researcher___________________

Date__________________

Day/month/year

Statement by the researcher/person taking consent

I have accurately read out the information sheet to the potential participant, and to the best of my ability made sure that the child understands that the following will be done:

1. Taking FNAS

2. Filling the questionnaire

I confirm that the child was given an opportunity to ask questions about the study, and all the questions asked by him/her have been answered correctly and to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.

Name of Researcher/person taking the assent________________________

Signature of Researcher /person taking the assent __________________________

Date ___________________________

Day/month/year

Parent/Guardian has signed an informed consent ___Yes ___No _____(initialed by researcher/assistant)

Annex IV-Questionnaire

This questionnaire form is intended to Assessment of possible tuberculous lymphadenopathy by Xpert MTB/RIF assay compared to non-molecular methods at SPHMMC hospital. The study will be conducted through analysis of FNA sample from patients who are suspected of having tuberculosis-lymphadenitis (TB-L)

Date of interview____________ (EC)

A. identification of the patient

Name_____________________________

Hospital number __________

Study code number______________

Age:__________ Sex ____________Weight______ Height __________

Address: Region __________________ Wereda ___________ Kebele______House No_____ Phone No________________

1 **Educational status:** A) primary school B) secondary school C) higher education

2. **Occupation:** A) house wife B) daily laborer C)government employee D) unemployed E) farmer F) Other___________

3. **Marital status**; A) living with partner B) Married C) Divorced

D) Single E) widowed F) Separated

4. **Living area** A) Urban B) Rural

Date FNAC Collection: __________________

B. current clinical presentation

1. Manifestation of patient

A. sweating B. Fever C. pain D) others (specify) ___

2. Duration manifestation _____________ (in weeks)

C. predisposition for tubeclues lymphadenitis

3. Diabetes; A) Yes B) No

If yes, is it being treated? A) Yes B) No

4. Cigarette smoking; A) Yes B) No

If yes, how many cigarettes per week do you smoke? ___________________________________

If yes, when was the last time you smoked? ____________________________________

How long have you smoked? ____

5. Do you drink alcohol? A) Yes B) No

If yes, when was the last time you drank alcohol? _____________________________________

6. Type of the alcohol (circle it)

A) Tella B) Tej C) Araki D) Beer/Drought E) Other (specify)____________

7. Amount of alcohol in average (per week)………………………

How long have you taken alcohol? ____

8. Do you know your HIV status A) yes B) No

9. Your result A) positive B) Negative

10. Time since diagnosis of HIV ___________________________

11. Any treatment for HIV A) yes B) no

12. If any, what type? ______________________

13. Do you drink raw milk? A) Yes B) no

D. Hospitals and prisons are places where ETB is acquired.

14. Do you have Previous admitted for any disease? A) Yes B) No

If your answer is yes,

a. When was it? Month/year ___________________

b. Which ward was you admitted? ___________________

c. How long were you admitted? ___________________

d. Were there chronically coughing individual? A) Yes B) No

E. history on previous PTB

15. Previously treated for PTB? A) Yes B) No

F. Standardized history

a. How long have you been sick? __________________Weeks

b. Have you had the same symptoms prior to this episode? A) Yes B) No

c. Have you had other symptoms of lung disease prior to this episode A) Yes B) No

d. Have you had X-ray examinations prior to this episode? A) Yes B) No

e. Have you had sputum examinations prior to this episode? A) Yes B) No

f. Have you had drug treatment for PTB? A) Yes B) No

g. what were the names of the drugs ___________________________________

Signature of Responsible Professional Health worker ______________________

Thank you!

Annex V

የዉል ስምምነት

ቅጥያ I: ስለጥናቱ ማስተዋወቂያና በጥናቱ ለመሳተፍ ፈቃደኝነት መጠየቂያ የአማርኛ ቅጽ
በንፍፊት ዕባጭ ላይ የቲቢ ስርጭትና መጠን

1. <u>ስለጥናቱ ማስተዋወቂያ ቅፅ</u>

ጤና ይስጥልኝ? እኔ ስሜ_____ ይባላል። የአዲስ አበባ ዩኒቨርቲ ህክምና ትምህርት ቤት ተማሪ ሥሆን ጥናቱን የምሰራው በንፍፊት ዕባጭ ላይ የቲቢ ስርጭትና አጋላጭ ምክንያቶች የሚል ነው፤ የጥናቱ አላማ በንፍፊት ዕባጭ ላይ የቲቢ በሽታን መጠን፤ስርጭትናአጋላጭ ምክንያቶችን ማጥናት ነው።ጥናቱ የሚካሄደው በጥቁር አንበሳና በቅዱስ ጳውሎስ ማሰተማሪያ ሆስፒታሎች ይሆናል። ጥናቱ ለእርሰዎ ቀጥተኛ የሆነ ጥቅም ባይኖረውም ለፖሊሲ አውጭዎችናአስፈጻሚዎች እንዲሁም ለማህበረሰቡ ስለአጋላጭ ሁኔታዎችና ስለመከላከያ መንገዶች ለማወቅ ይረዳል።በሌላ በኩልም ስለ በሽታው ግንዛቤና ጥንቃቄ ለማገኝት ይረዳል። ናሙናዎ በላብራቶሪ ሲመረመር ምንም አይነት ችግር ካሳየ የመድሃኒት ትእዛዝና የባለሙያ ምክር ይሰጥዎታል።

እርሰዎንም በዚህ ጥናት እንዲሳተፉ በትህትና እንጠይቀዎታለን።በዚህ ጥናት በመሳተፈዎ የምናገኘው መረጃ ለጥናታችን ውጤታማነት እነዲሁም በጥናቱ ውጤት ላይ ከፍተኛ አስተዋፅዎ ይኖረዋል።ስለዚህም በዚህ ቃለ-መጠይቅ በመሳተፈዎ ምስጋናዬ የላቀ ነው። በጥናቱ በመሳተፈዎ ምክንያት የሚመጣበዎት ምንም አይነት ችግር አይኖርም።ነገር ግን ናሙናዉን ለመዉሰድ መርፌ ሲገባ ከሚፈጥረዉ የቅጽበት የህመም

ስሜት በስተቀር የጎላ ችግር አያመጣም ፤ምቾት ካልተሰማዎት ሀኪም እንዲያይዎት ይደረጋል።

በጥናቱ ውስጥም
ስምዎ በማንኛውም ሁኔታ አይገለጽም፤ስለሆነም የሚሠጡት መረጃ ሙሉ በሙሉ ሚስጢራዊነቱ

የተጠበቀ
ነው።ስለዚህ በጥናቱ ለመሳተፍ የእርሠዎ ሙሉ ፈቃድ አስፈላጊ ነው።በተጨማሪም ለመመለስ

የማይፈልቸው
ጥያቄዎች ካሉ ጥያቂዎችን ለመመለስ አይገደዱም።እንዲሁም በጥናቱ ላለመሳተፍ ከፈለጉ

በማንኛውም ጊዜ
ማቋረጥ ይችላሉ።በጥናቱ ባለመሳተፊዎ በርሰዎ ላይ የሚያስከትለው ወይም የሚያመጠው ምንም

አይነት ጉዳት
የለውም። ቃለመጠየቁን ለማካሄድ 30-45 ደቂቃዎች ይወስዳል።
ቃለ መጠየቁን በተመለከተ ወይንም አጠቃላይ ስለጥናቱ ማንኛውንም አይነት ጥያቂ ና አስተያየተ

ቢኖረዎት
በሚከተሉት አድራሻዎች መጠቀም ይችላሉ።
መንግስቱ ፋንታሁን
ስልክ፡ 0922844171

<u>II ከመጠየቁ በፊት የተጠያቂውን ስምምነት ማረጋገጫ ቅፅ</u>

ከላይ በመግቢያው ላይ የተጠቀሰውን መረጃ አንብቢያለሁ ወይም በቃል የተሰጠኝን ማብራሪያ

ተረድቻለሁ።
በዚህ መሰረት ከእኔ የሚጠበቅብኝን ድርሻ በሚገባ አውቂያለሁ እናም በዚህ ጥናት ላይ በመሳተፌ

ሊከሰቱ
የሚችሉትን ሁኔታዎች ተገንዝቢያለሁ። ከዚህ ጥናት በማንኛውም ሠዓት ያለምንም ቅድመ ሁኔታና

ምክንያት
እራሴን ከተሳታፊነት የማግለል ሙሉ መብት እንዳለኝ ተረድቻለሁ። ይህን ውሳኔዬን ተከትሎ በእኔም

ሆነ
በቤተሰቦቼ ላይ በምንፈልገው የጤና አገልግሎት ላይ ምንም አይነት አሉታዊ ተጽዕኖ

እንደማይደርስብኝ ተረድቻለሁ።

ስም (ተሳታፊ)________________ፊረማ ______________ቀን _________________

የዋስ ስም _______________________________________ፊርማ ___________ቀን_________

የአጥኝዉ ስም ____________________________ፊርማ_______________ቀን_________________

ፎርም 1፡ ክሊኒካል መረጃ

ጥናቱን በተመለከተ የቃል ማብራሪያ የተሰጠ መሆኑን የሚያረጋግጥው የቃለ መጠይቁ አድራጊ ስምና ፊርማ

የጠያቂው ስም-- ፊርማ --------------- ቀን------------------------ ኮድ-------------------------- መጠይቁን እንድቀጥል ፈቃደኛ ነዎት;

1. አዎ ፈቃደኛ ነኝ ---------------------- ቃለ መጠይቁ ይቀጥላል።
2. አይ ፈቃደኛ አይደለሁም ------------------ ቃለመጠይቁን በማቆም አመስግነው ይለያዩ።

<u>የመጠየቁ ውጤት መግለጫ</u>

ሀ.ሙሉ በሙሉ የተሞላ ለ. በከፊል የተሞላ ሐ.ተጠያቂው ፈቃደኛ አይደለም መ. ሌላ ካለ---------

መጠየቁን የሞላው ሰው ስም---------------ፊርማ-------------------ቀን--------------------የቃለመጠይቅ ቀን _______________(ዓ.ም)

ሀ) የህሙማን መረጃ

ስም ___________ እድሜ _____ ፆታ ____ክብደት____ቁመት_________

አድራሻ፡ ወረዳ_____ቀበሌ_________________________የቤት ቁጥር___________________________
ስልክ ቁጥር ....

1.የትምህርት ደረጃ __________ ስራ --------------------------__________________

2. የጋብቻ ሁኔታ ሀ) ያላገባ ለ) ያገባ ሐ) የፈታ

ኤፍ ኤን ኤ የተሰበሰበበት ቀን ....

ለ) የአሁኑ ክሊኒካል ሁኔታ

3. የህመምተኛዉ የሚሰማዉ ስሜት ሀ) ማላብ ለ) ትኩሳት ሐ) ህመም መ) ሌላ ካለ ጥቀስ.......

4. የሁኔታዉ ቆይታ.........................(በሳምንት)

ሐ) ለቲቢ የሚጋልጡ ሁኔታዎች

5. የስካር በሽታ አለብዎት ሀ) አዎ ለ) የለበኝም

6. አዎ ከሆነ መልስዎ መታከም ጀምረዋል ? ሀ) አዎ ለ) አልጀመርኩም

7. ሲጋራ ያጨሳሉ ? ሀ) አዎ ለ) አላጨስም

8. አዎ ከሆነ መልስዎ በቀን ምንያክል ያጨሳሉ ?...................

9. አዎ ከሆነ መልስዎ ለመጨረሻ ጊዜ ያጨሱት መቼ ነዉ ? ....

10. ለምንያህል ጊዜ አጨሱ ?..................

11. መጠጥ ይጠጣሉ ? ሀ) አዎ ለ) አልጠጣም

12. አዎ ከሆነ መልስዎ ለመጨረሻ ጊዜ የጠጡት መቸነዉ ? ....

13. ምን አይነት አልኮል ነዉ የሚጠጡት ? ሀ) ጠላ ለ) ጠጅ ሐ) አረቄ መ) ቢራ/ድራፍት ሠ) ሌላ ከሆነ................

14. የምትጠጣዉ አማካይ አልኮል መጠን በሳምንት? ....

15. አልኮል ለምን ያክል ጊዜ ጠጡ ? …………..

16. ያልፈላ ወተት ይጠጣሉ ? ሀ) አዎ ለ) አልጠጣም

ሠ) ሆስፒታል እና ማረሚያ ቤት

17. ሆስፒታል አልጋ ይዘዉ ያዉቃለ ? ሀ) አዎ ለ) አላዉቅም

18. አዎ ከሆነ መልስዎ መቼ ነበር ?………………

19. የትኛ ዋርድ ነበር ?……………

20. ለምን ያክል ጊዜ ነበር ?………….

21. የተኛችሁበት ክፍል አክታ ያለዉ ህሙማን ነበር? ሀ)አዎ ለ) የለም

22. ካሁን በፊት ቲቢ ታመዉ ያዉቃሉ ? ሀ) አዎ ለ) አላዉቅም

ሀ. ለምን ያክል ጊዜ ታመሙ ?………………(በሳምንት)

ለ. ካሁኑ ጋር ተመሳሳይ ምልክት ነበረዉ? ሀ) አዎ ለ) የለዉም

ሐ. ካሁን በፊት ሌሎች የሳንባ ህመም ምልክቶች ነበሩህ? ሀ) አዎ ለ) የሉም

መ. ካሁን በፊት የጨረር ምርመራ አርገዉ ነበር ? ሀ) አዎ ለ) አላዎቅም

ሠ. ካሁን በፊት የአክታ ምርመራ አርገዉ ነበር ? ሀ) አዎ ለ) አላዎቅም

ረ. የቲቢ መድሀኒት ወስደዉ ያዉቃሉ ? ሀ) አዎ ለ) አላዎቅም

ሸ. ከወሰዱ ለምን ያክል ጊዜ ?………….

ቀ. ምን አይነት መድሀኒት ነበር የወሰዱት ?………………..

መጠይቁን ያስሞላዉ ባለሙያ ፊርማ....................

አመሰግናለሁ!!!

Annex VI . Standard operating procedures

1. Standard operating procedure for aura mine stain (*LED)*

Principle

The property of acid-fastness is based on the presence of mycolic acids in the mycobacterial cell wall. Primary stain (auramine) binds cell-wall mycolic acids. Intense decolourization (strong acids, alcohol) does not release primary stain from the cell wall and the mycobacteria retain the fluorescent bright yellow colour of auramine. Potassium permanganate is used to quench fluorescence in the background; however, it provides little contrast for focusing and stains are therefore sometimes preferred, of which blue ink may be the best.

Fluorescence microscopy allows smears to be examined more rapidly than is possible with the basic fuchsin procedures and is particularly indicated for high-volume laboratories. It may also be more sensitive for paucibacillary specimens, since it allows examination of more fields with less effort. However, it requires a stable power supply, greater expertise in reading and microscope adjustment, and a regular supply of the costly and short-lived bulbs. Cheaper

systems using halogen lamps have less stringent requirements, but performance does not entirely match that of the standard mercury vapour lamps.

Specimens

Any incoming specimen must be properly labeled, as a minimum with a unique identification number. This identification is also written on the request form .

Equipment and materials

Alcohol sand jar (only if a loop is used, not needed with disposable sticks).

Bunsen burner or spirit lamp

Diamond pencil or lead pencil (if frosted-end slides are available)

Filter paper, appropriate for funnel size

Funnels, small, for filtering solutions in use

Forceps

Lens paper or soft tissue paper

Plastic bag for waste disposal

Bamboo or wooden sticks or wire loops

Fluorescence microscope with objectives of 20x or 25x, and 40x (ideally specific for fluorescence microscopy), and eyepieces of 10x

Slide staining rack

Slide boxes

New, clean slides (rinse in alcohol and dry if necessary)

Timer

Staining reagents

Staining bottles, 250 ml, with spout

Beaker for rinsing water

Sink and water supply

Disinfectant solution

Reagents and solutions

Auramine staining solution, 0.1%

Acid-alcohol decolourizing solution, 0.5%

Counterstaining solution

Potassium permanganate, 0.5%, or blue ink, 10%

Staining method

- Place the slides, smear upwards, on the staining rack over a sink, about 1 cm apart.
- Place a new filter paper in a small funnel, keep it over the first slide and fill it up with auramine staining solution.
- Let the solution filter through the paper, covering each slide completely. *Do not heat.* Leave for 20 minutes.
- Using forceps, tilt each slide to drain off the stain solution. Rinse the slides well with distilled water or clean tap water from a beaker (not directly from the tap).
- Pour the acid solution over the smears, covering them completely, and allow to act for 3 minutes.
- Using forceps tilt each slide to drain off the acid-alcohol solution. Gently rinse each slide again with distilled water or clean tap water from a beaker (not directly from the tap).
- Flood smears with potassium permanganate or blue ink solution for 1 minute. Time is critical because counterstaining for longer may quench the AFB fluorescence.
- Using forceps, tilt each slide to drain off the counterstain solution. Gently rinse each slide again with distilled water or clean tap water from a beaker (not directly from the tap).
- Using forceps, take each slide from the rack and let the water drain off. Stand the slide on edge on the drying rack and allow to air-dry.

Reading, recording and reporting

Reading

- Keep stained smears in the dark (in a box or folder) and read as soon as possible – fluorescence fades quickly when exposed to light.

- Switch on fluorescent lamp 5 minutes before use; leave the lower ordinary lamp off.
- Rotate the nosepiece so that the 20x (or 25x) objective is in the light path.
- Select the filter set position suitable for auramine stain (see manufacturer's manual)
- Check that there is a strong blue light; if not, open shutters and/or the fluorescent light beam diaphragm
- Load the positive control slide on the stage and move the stage to position the slide under the objective.
- Use the coarse adjustment first, and then the fine adjustment, to focus the objective. If this fails (i.e. in thin negative smears), turn the filter set to transmitted light, switch on the lower normal lamp and focus as with a light microscope. Then switch off the lower lamp and return to the required filter position. The field should now be in focus..
- Check that bright yellow fluorescent AFB are clearly seen. If not, adjust the lamp and/or the mirror position. Check that the whole field is evenly lit. If not, centre the diaphragm after partially closing it (see manufacturer's manual).
- Exchange the positive control for the first routine smear without changing focus or rotating the objective. Repeat the procedure with each smear to be examined.
- Using the 20x (or 25x) objective, scan the stained smear systematically from one side to the other and back again – at least one length must be scanned before reporting a negative. At 200x magnification, this corresponds to three lengths or 300 high-power fields (HPF) using the oil-immersion 100x objective; at 400x it equals two lengths or 200 HPF with the oil-immersion objective. The process will take 1–2 minutes.

  Acid-fast bacilli appear bright yellow against the dark background material.

  Tubercle bacilli are quite variable in shape, from very short fragments to elongated types, and may be uniformly stained or with one or many gaps, or even granular. The typical appearance is of bacilli that are rather long and slender, slightly curved rods. They occur singly or in small groups, and rarely in large clumps. With good staining (always check a freshly stained positive control first), there may also be fluorescing (sometimes green) artefacts, which do

not have the typical shape. Non-fluorescing bacillary shapes must also be considered as artefacts.

- Use the 40x objective for confirmation of AFB
- Store the slides in a slide box in order of the numbers of the laboratory register; they will be needed for external quality assessment. Do not write results on the slides.
- When finished, turn the power off. When work needs to be interrupted for just a few minutes only, block the light using the shutter but do not switch off the light source. After switching off a mercury lamp, wait at least 15 minutes before switching it on again. Other types of lamps for short periods of time without problem.

Recording

Because fluorochrome-stained smears are examined at magnifications of 200x to 400x, the number of AFB can roughly be divided by a factor 10 or 5, respectively (depending on the objective) to make them equivalent to fields seen on examination of fuchsin-stained smears at 1000x.

IUATLD/WHO scale (1000x field = HPF) **Result**	**Microscopy system used**		
	Bright-field (1000x magnification: 1 length = 2 cm = 100 HPF)	**Fluorescence** (200–250x magnification: 1 length = 30 fields = 300 HPF)	**Fluorescence** (400x magnification: 1 length = 40 fields = 200 HPF)
Negative	Zero AFB / 1 length	Zero AFB / 1 length	Zero AFB / 1 length
Scanty	1–9 AFB / 1 length or 100 HPF	1–29 AFB / 1 length	1–19 AFB / 1 length

1+	10–99 AFB / 1 length or 100 HPF	30–299 AFB / 1 length	20–199 AFB / 1 length
2+	1–10 AFB / 1 HPF on average	10–100 AFB / 1 field on average	5–50 AFB / 1 field on average
3+	>10 AFB / 1 HPF on average	>100 AFB / 1 field on average	>50 AFB / 1 field on average

4.6.3 Reporting

Results must be reported in a special register of TB laboratory examination. Use red ink for positive results. Reports must be provided *as soon as possible*.

- For a negative result report: "Acid-fast bacilli were not seen."
- For a positive result: report quantification of AFB seen. (It should not be assumed that AFB are tubercle bacilli.)
- *Never report "No TB" (or equivalent wording).*

2. Standard operating procedure for LJ culture

Inoculation of diagnostic samples on media

Prior to culture of mycobacteria the modified Petroff homogenization and decontamination method is performed to remove bacteria and fungi (e.g. living as commensals on the human skin) from specimens. As M. ulcerans is a slow growing mycobacterium it would be otherwise overgrown by these organisms.

Precautions

Decontamination is performed in the culture laboratory. The laboratory must be equipped with lab coats, gloves and all necessary laboratory items exclusively used in this room.

Before handling of diagnostic material the laminar flow is allowed to run for 15 minutes.

All steps of homogenization and decontamination are performed under sterile conditions under the laminar flow.

Reagents

- 0.9 % Sodium Chloride (NaCl)
- 4 % Sodium Hydroxide (NaOH)

NaOH and NaCl can be kept at room temperature.

Material & Instruments

- Lab coats
- Gloves (disposable, non sterile)
- Scalpel, sterile (e.g. Cutifix #11)
- Small scissors, sterile
- Mortar & pestle (for grinding tissue samples)
- Falcon tubes, 50 ml
- Rack for Falcon tubes
- Marker (for labelling)
- Laminar Flow
- Centrifuge
- Waste disposal bottle

The supernatant is gently discarded into the waste bottle under the laminar flow.

15 ml of sterile 0.9 % NaCl is added to each falcon tube and subjected to another centrifugation step at 3000 rpm for 15 minutes.

Afterwards the supernatant is gently discarded. To dissolve pellets, 0.5 ml 0.9 % NaCl is added and the falcon is tapped from outside where the pellet is located, which is thereby well dissolved.

Solutions are now decontaminated and ready for inoculation on LJ medium.

Inoculation of culture

Reagents

- Loewenstein-Jensen culture media

LJ is stored at 4°C

Materials & Instruments

- Disposable plastic pipettes, graduated to 3 ml

- Inoculation loops 10µl/200mm, disposable, sterile (e.g. Greiner Bio-one)
- Incubator
- Racks for LJ slant tubes

Inoculation of primary culture

A decontaminated material is transferred to the LJ culture tube labelled with the patient ID and the inoculation date of the primary culture. Inoculated media are incubated at 31°C.

Reading of cultures

Inoculated LJ tubes are checked weekly for growth of M. ulcerans within the first 6 months.
If no growth is detected after 6 months the inoculated medium is then checked for growth monthly for an additional six months while cultures are stored at room temperature, if no spare space is available in the incubator.
Results are reported after 6 months (if positive before 6 months, report at the time detected).
If results change between month 6 and month 12, the changed results are reported.
Negative cultures are discarded after 12 months.

3. Standard operating procedure for ZN

Principle

This procedure is used to stain mycobacterium tuberculosis and mycobacterium leprae. These bacteria are also called acid fast bacilli. They stain with carbol fuschin, which is a red dye. They retain the dye when treated with acid, which is because of the presence of mycolic acid in their cell wall.

Reagents

1. Carbol fuschin (basic dye)
2. Mordant (heat)
3. 20% sulphuric acid (decolorizer)
4. Methylene blue (counter stain) or Malachite green

Procedure

1. Fix the smear of the specimen over the glass slide, either by heating or alcohol fixation.
2. Pour carbol fuschin over smear and heat gently until fumes appear. Do not overheat and allow it to stand for 5 minutes, then wash it off with water.
3. Pour 20% sulphuric acid, wait for one minute and keep on repeating this step until the slide appears light pink in color. Wash off with water.
4. Pour methylene blue, wait for two minutes, again wash with water
5. Allow it to air dry and examine under oil immersion lens.

Result

Acid fast bacilli stain pink, straight or slightly curved rods, at times having beaded appearance. The background appears blue due to methylene blue.

Interpretations

If definite bacilli are seen, report as AFB positive. However, it is better to report the result quantitatively as follows:

- > 10 AFB/high power field –>+++
- 1-10 AFB/high power field –> ++
- 10-100 AFB/100 high power fields –> +
- 1-9 AFB/100 high power fields –> exact number

4. **Standard operating procedure for Gene Xpert Procedure**

Principle

WHO has issued policy recommendations for the use of Xpert MTB/RIF in the diagnosis of extrapulmonary TB and rifampicin resistance detection geneXpert MTB/RIF should be used in preference to conventional microscopy and culture as the initial diagnostic test in testing cerebrospinal fluid specimens from patients presumed to have TB meningitis (strong recommendation given the urgency of rapid diagnosis, very low quality of evidence); Gene Xpert MTB/RIF may be used as a replacement test for usual practice (including conventional microscopy, culture, and/or histopathology) for testing of specific non-respiratory specimens (lymph nodes and other tissues) from patients presumed to have extra pulmonary TB.

Specimen processing

The Xpert MTB/RIF assay can be used directly for FNA samples or on decontaminated specimens if culture is performed concurrently. Whenever possible, specimens should be transported and stored at 2 to 8°C prior to processing (a maximum of 7 days).

1. The reagent was added in a 1:1 ratio to the specimen
2. The sample container was agitated twice during a 15-min incubation period at room temperature.
3. Finally, 2ml of the mixture was transferred to the Gene Xpert test cartridge.
4. The cartridge was loaded into the Xpert MTB/RIF instrument for automated sample and real-time polymerase chain reaction (RT-PCR) processing.
5. The instrument reported the presence or absence of M. tuberculosis complex, a semi quantitative estimate of M. tuberculosis complex concentration (high, medium, low, and very low) and the presence or absence of rifampicin resistance after 1:55 min.

5. Standard operating procedure for FNAC Wright's stain

Wright stain named after Homer Wright, who devised the stain, a modification of the Romanowsky stain.

Staining solutions:

Wright's ready to use stain solution or Wright's stain solution can be prepared

Ph 6.8 Phosphate Buffer solution

Wright's Stain solution Preparation:

Wright's stain commercially available powder = 0.3 grams

Dissolve the Wright' stain powder in absolute methanol=100ml in a closed container at room temperature for 24 hours and filter before use

pH 6.8 Phosphate Buffer Solution:

In a 250ml Erlenmeyer or flat bottom flask weigh/measure the following:

Sodium phosphate dibasic=0.3 grams

Sodium phosphate monobasic=0.7 grams

Distilled water=100ml

Wright's Staining Procedure:

a. If need be fix the wet air waved smeared slides in absolute methanol for 5-10 mts and then air dry
b. Flood the fixed and air dried smears with Wright's stain(about 15 drops) and leave for 1 mt
c. Add twice the volume(30 drops) with distilled water or 1: 6 diluted pH 6.8 Phosphate buffer solution-2mts
d. A metallic sheen should be seen over the smear
e. Drain off and rinse with diluted pH 6.8 Phosphate buffer solution or distilled water until the thinner portions of the blood film are pink
f. Allow to stand in a vertical position to air dry (do not blot dry)
g. After the smears have dried, clear in xylene and mount in DPX

The evidences for diagnosis of tuberculosis by cytological examination of FNA samples were on the presence of epitheloid granuloma with or without multinucleated giant cells and with or without caseus necrosis and / liquefied necrotic material with degenerating and viable inflammatory cells without epitheloid granuloma.