MYCOBACTERIUM AND OTHER BACTERIA POSITIVITY IN LYMPHADENOPATIES WITH PURULENT ASPIRATES, PATHOLOGY DEPARTMENT IN JUMC

JIMMA, SOUTH WEST ETHIOPIA

BY WUBISHET ASSEFA (MD, PATHOLOGY RESIDENT)

A RESEARCH THESIS TO BE SUBMITTED TO JIMMA UNIVERSITY MEDICAL CENTER, DEPARTMENT OF PATHOLOGY, FOR PARTIAL FULLFILMENT OF

SPECIALITY TRAINING IN ANATOMIC PATHOLOGY

JANUARY, 2018

JIMMA ETHIOPIA

MYCOBACTERIUM AND OTHER BACTERIA POSITIVITY IN LYMPHADENOPATIES WITH PURULENT ASPIRATES, PATHOLOGY DEPARTMENT IN JUSH

JIMMA, SOUTH WEST ETHIOPIA

BY WUBISHET ASSEFA

ADVISORS:

MESELE BEZABIH (Epidemiologist,Asso. professor of pathology)

GEMEDA ABEBE (Asso. Professor, Director, Institute of Biotechnology)

MULUALEM TADESSE (PHD candidate)

JANUARY, 2018

JIMMA, ETHIOPIA

ABSTRACT

Background: Tuberculosis is the biggest health challenges of the world. Tuberculous lymphadenopathy is an important of extrapulmonary tuberculosis. Fine needle aspiration cytology (FNAC) is the cost effective and quick method for diagnosis of such lesions. Its specificity and sensitivity can be improved by using conventional Zheil neelson staining and fluorescent LED microscopy techniques.

Objectives: To assess mycobacterium and other bacteria positivity in lymphadenopathies (LAP) with purulent aspirates in patients visiting Jimma University Medical Center (JUMC).

Methods: The study was conducted in department of pathology, cytology unit at Jimma medical center, from august to December 2017 GC. Patients with peripheral lymphadenopathy attending cytology department at JUMC having clinical suspicion of tuberculosis and showing purulent aspirates were enrolled for the study. Samples was collected by pathology residents and interpreted by pathologists and senior laboratory technologists.

Result and Discussion: 53 TB suspected LAP cases with purulent aspirates underwent cytomorphologic LED and ZN staininig for AFB out of which mycobacteria infection detected in LAP 49%, 43.4% and 9.4% cases respectively. Combining cytomorrphology with LED techniques increases the detection rate of FNAC by 15% while AFB staining increases this detection rate by 7.6%. Gram reaction observed in 17% of cases.

Conclusions: This study reaffirms the usefulness of FNA cytology in the diagnosis of TB lymphadenitis. We recommend the combined use of routine FNAC with AFB staining and LED techniques to increases the detection of myocobacterial infection in purulent aspirates .Performing Gram stain further help to reveal other bacterial causes of suppurative inflammation.

ACKNOWLEDGEMENTS

First of all, I would like to thank Jimma University for arranging this program, funding and giving me the opportunity to go through identification of the health problems and take part in problem solving activities.

My most sincere thanks go to my advisors Dr. Mesele Bazabih, Dr Gamada Abebe and Mr. Mulualem for their encouragement, guidance and expertise advises.

TABLE OF CONTENTS

Contents **Page**

LIST OF TABLES

ACRONYMS

- MTB – Mycobacterium Tuberculosis
- TB – Tuberculosis
- TBL- Tubercoulous Lymphadenitis
- EPTB- Extra-Pulmonary Tuberculosis
- AFB – Acid Fast bacilli
- JUSH- Jimma University Specialized Hospital
- FNAC – Fine needle aspiration cytology
- LED – Light Emitting Diode
- ZN – Ziehl – Neelsen
- WHO – World health Organization

1. INTRODUCTION

1.1. Background

Tuberculosis (TB) is a serious chronic pulmonary and systemic disease caused most often by *Mycobacterium tuberculosis* (MTB) **(1)** that has been present in humans since antiquity **(2).** TB ranks as the second leading cause of death from a single infectious agent, with an estimated 9 million people developed the disease in 2013 **(1).** According to world health organization (WHO) report, Ethiopia is among the 30 high TB burden countries in the word **(3).** Despite efforts made by international community targeting to end global TB epidemic by 2030, the TB burden still remains high. **(4)**

TB lymphadenitis (TBL) is a chronic specific granulomatous inflammation which causes necrosis in a lymph node **(5).** It is the most common clinical presentation of extra pulmonary TB which most frequently involve the cervical lymph nodes (LN) followed by mediastinal, axillary and mesenteric LNs **(6).**

Suspected TBL cases may be difficult to diagnose clinically and remains expensive when applying surgical procedures **(5).** Thus, fine needle aspiration cytology (FNAC) technique is quick, safe, cheep and effective for the diagnosis of TBL with rare complications **(7).**

Although definite cytological diagnosis of TBL can be offered in smears with caseating granulomas with or without giant cells, those cases with necrotizing suppurative smears would be dismissed as suppurative lymphadenitis in the absence of Zeihl-Neelsen stain **(8).**

Only the presence of acid fast bacilli (AFB) confirms presence of TB. However, some features observed on the FNAC which are associated with TB includes: caseous necrosis, presence of Langerhans giant cells and granulomas on a necrotic background **(9).**

This research was aimed to evaluate comparative advantages of using FNAC with AFB staining, LED microscopy and gram staining techniques to detect presence of TB and differentiate it from other bacteria causes in patients with chronic LAP showing purulent features.

1.2. Statement of the problem

The burden of TB poses a great health and economic impact particularly in developing countries including Ethiopia. Despite many efforts undertaken, the 2016 global tuberculosis report by WHO showed that prevalence of Tuberculosis to be remained as one of the top 10 causes of death worldwide in 2015 **(3).**

Ethiopia ranks 3rd in terms of the number of ETB cases globally, most of which are TBL **(10)**

The WHO estimate that proportion of EPTB among the total number of new TB cases to be about 36.6% **(3)**

In Ethiopia, EPTB accounted for 34.8% of TB cases with the largest group being TBL (80%) **(11).** This contrasts with proportion of EPTB in other high TB burden countries in Asia, such as India (14.9%), China (4%), Indonesia (2.5%), and in Africa such as South Africa (17.5%), Nigeria (4.3%) or Kenya (16.6%) **(12).**

The diagnosis of TBL can be established using FNAC method which shows a reliable cytomorphological features with clearly defined criteria sufficient for diagnosis of TBL even with negative ZN stains for AFB **(13).**

According to a research done in Jimma, South West Ethiopia, out of 13 cases classified as suppurative abscess on cytology, TBLN was diagnosed in 7 cases by the concentration method and in 4 cases by direct smear microscopy. The possible explanation for the misdiagnosis of the specimens as suppurative abscess on cytology was explained by absence of characteristic features within abundant mixed inflammatory cells and other bacterial supper infections **(14).**

Researches show statistically significant correlation between the gross appearance of an aspirate and its cytologic and ZN staining features. Those aspirates with cheesy gross appearance demonstrate higher positivity for TBL on cytology (100%) and lower positivity of AFB on ZN (only 33.33%) when compared to those with purulent aspirates which show only 86% positivity on cytology and 53% AFB positivity on ZN. (15). Hence, this study is conducted to facilitate appropriate and accurate detection TBL cases especially on those cases presented with supportive features by incorporating other diagnostic tools together with cytology.

1.3. Significance of the study

By investigating the socio-demographics, cytomorphologic, mycobacterial and other bacteria positivity of suspected TBL cases with purulent aspirate, the result of this study believed to have a paramount importance for policy makers to help them design evidence based strategies on preventing, early case detection and treatment of TBL.

For the health professionals, particularly for clinicians, it will provide a reliable clinical clue in diagnosing cases and initiating appropriate individualized management.

For practicing pathologists of developing countries it will provide scientific basis to diagnose TBL cases with purulent features using the limited resource available.

This research result will also serve as a basis for future researches on TBL cases.

2. LITERATUE REVIEW

2.1 Etiology:

TB is caused by *M. tuberculosis* complex (MTBC) bacteria. The MTBC comprises closely related species responsible for strictly human and zoonotic TB. The complex consists of seven species and subspecies including *M. tuberculosis, M. canetti, M. africanum, M. pinnipedii, M.microti, M. caprae* and *M. bovis*. Despite different species tropisms, the MTBC is characterized by 99.9% or greater similarity at the nucleotide level and possess identical 16S rRNA sequence **(16).**

The genus *Mycobacterium* is non-motile, non-capsular and non-spore-forming. It is obligate aerobic thin rod usually straight or slightly curved having 1-10μm length and 0.2-0.6μm width and is facultative intracellular mostly of macrophages and has a slow generation time about 15-20 hours. Its cell wall is rich in lipids that provide it the thick waxy coat which is responsible for acid fastness and hydrophobicity. This waxy coat is also greatly contributing for the bacterium resistance to many disinfectants, common laboratory stains, antibiotics and physical injuries. **(17).**

Once stained, the rods cannot be decolorized with acidic solutions; hence the name acid-fast bacteria. Because the mycobacterial cell wall is complex and this group of organisms is fastidious, most *Mycobacteria* grow slowly, dividing every 12 to 24 hours. Isolation of *MTBC* may require 3 to 8 weeks of incubation. This slow growth further complicates diagnosis and makes long-term drug treatment necessary **(18).**

2.2 Epidemiology of extra pulmonary tuberculosis

In countries where the incidence of TB is high, Extra-pulmonary TB constitutes 15-20% of all cases of Tuberculosis. Out of which TBL comprises nearly 35% cases among which cervical lymph nodes are the most common site of involvement (60-90%) **(19).** There are various risk factors associated with EPTB like age, gender and HIV/AIDS **(20)**. In Ethiopia, TBL is the most common type of EPTB accounting for 80% cases **(3)**.

2.3 Pathogenesis

Extra-pulmonary TB can occur in isolation or along with a pulmonary focus as in the cases of patients with disseminated TB. TBLN is considered to be the local manifestation of a systemic disease.*M. tuberculosis* gains entry into the body via the respiratory tract and undergoes haematogenous and lymphatic dissemination in to other organs and the host eventually develops granulomas, foci fibrose, scar and calcification **(21).**

2.4 Clinical features

EPTB patients show constitutional symptoms like low grade fever, anorexia, weight loss, malaise, fatigue and occasional night sweat **(22).** In addition other symptoms and signs related to the organ system involved may be manifested **(23)**. Multiplicity, matting and caseation are the three important physical findings in patients with TBL **(24).** The lymph nodes are not tender unless secondary bacterial infection has occurred **(25).** According to study done on Pakistan TBL presenting clinically as cold abscess accounts for 31.51% **(26).**

2.5 Laboratory diagnosis

Identification and treatment of affected patients is the primary strategy for the control of TB **(27).** But, diagnosis of TBLN is a formidable challenge in developing countries where there is high rate of human immunodeficiency virus infection. It is estimated that only 50-60% of all patients with TB worldwide are actually diagnosed. Conventional methods, cytology and culture are still the methods of choice in most mycobacteriological laboratories **(28).**These diagnostic techniques have varied sensitivity, specificity, speed and cost.

2.5.1 Microscopic staining and culture:

The definitive diagnosis of TB depends on the isolation and identification of the etiological agent responsible for the disease. This can only be made by culturing *M. tuberculosis* organism from a specimen obtained from the patient. However diagnosing EPTB remains challenging as the samples obtained from relatively inaccessible sites may be paucibacillary, decreasing the

sensitivity of diagnostic test. Since the conventional smear microscopy has a low sensitivity with a range of 0%–40%, negative results cannot exclude the presence of TB. Up on mycobacterial culture the yield varies from 30-80%. However, it is not convenient to make it a routine diagnostic technique as it usually takes 2–8 weeks to receive the laboratory results and made a treatment decision **(29).** Moreover, due to the absence of laboratory equipment and safety procedures in areas with resource poor settings made the mycobacterial culture inconvenient method **(30).**

2.5.2 Fine needle aspiration cytology (FNAC):

FNAC plays a vital role in diagnosing enlarged lymph nodes in resource limited set up due to its cost effectiveness, simplicity, accuracy, completely safe and quick method for diagnosis of lymphadenopathy and it reduces the need for surgical biopsy **(31).** To define a positive test of EPTB up on this technique, histological evidence of presence of granulomas and caseation have been commonly used **(29).** Cautious interpretation should be done in immunocompromised individuals as FNAC can result in histopathologic findings with greater suppurative response and less well-formed granulomas **(32).** The Ethiopian national guideline recommends use of FNAC and tissue biopsy as routine diagnostic methods among patients having high index of clinical suspicion for TBL **(33).** Research conducted on south west part of Ethiopia showed that from gross examination aspirates taken from 154 presumptive TBL cases, purulent aspirates were observed in 50.7% of the cases, followed by caseous in 41% and blood-stained aspirates in 8.3% **(34).**

2.5.3 Molecular/ Nucleic acid amplification tests:

One of the advances in molecular epidemiology is the widely use of PCR molecular tools for the diagnosis and study of EPTB. The major advantage of this is, it's a rapid diagnostic technique **(29).** Because EPTB is a paucibacillary disease, the sensitivity could be improved by PCR, as it can detect as few as 10 mycobacteria **(35).**

Recently, there is a fast, sensitive and automated cartridge based molecular test called Xpert MTB/RIF which is considered to be useful for rapid molecular diagnosis of EPTB **(36).**

2.5.4 Fluorescent Light Emitting Diode (LED) Microscopy:

kLED is documented to have higher sensitivity than ZN and can reduce laboratory workloads (**37**). WHO recommends LED as an alternative for conventional ZN light microscopy for the diagnosis of pulmonary TB with 84% sensitivity and 98% specificity against culture as the reference standard (38). It is statistically significantly more sensitive by 6% with no appreciable loss in specificity, when compared with direct Ziehl-Neelsen microscopy **(39).**

2.6. Extra Pulmonary Tuberculosis in Ethiopia

Ethiopia has one of the highest incidence rates of human EPTB in the world. It is also noteworthy to mention that 36% of incident of TB cases in Ethiopia are extra pulmonary (WHO, 2008). TB lymphadenitis in cervical lymph nodes (TBLN) accounts for ≈33% of all new cases, which is greater than the global average of ≈15% (WHO, 2011).

3. Objective

3.1.General Objectives:

To asses mycobacterium and other bacteria positivity in lymphadenopathies with purulent aspirates in Jimma University Medical center from August to December 2017

3.2. Specific Objective

- To assess sociodemographic patterns of LAP with purulent aspirates
- To identify possible factors associated with LAP
- To evaluate cytologic features of LAP with purulent aspirates
- To Evaluate zehil nelseen positivity of LAP with purulent aspirates
- To Evaluate LED microscopy positivity of LAP with purulent aspirates
- To determine Gram stain positivity of LAP with purulent aspirates

4. METHODOLOGY

4.1.Study area and study period

The study was conducted in J USH which is found in Jimma town, Oromia regional state. Jimma town in located in South western part of Ethiopia 356KM away from Addis Ababa.

JUMC is a teaching University hospital serving as a specialized referral area for most of south western Ethiopia including Jimma town. Estimated catchment area of the hospital is 17, 500km With 15 million people is believed to get the service.

The pathology department is JUMC has two pathologists, 13 pathology residents, one general practitioner along with 10 technical assistant workers. The department activities subdivided in to Histopathology, hematopathology and cytopathology units.

The cytopathology unit (the area where this research was conducted) is the division where FNAC and fluid cytology samples prepared and reported.

The study was conducted from August 2017 till December 2017

4.2. Populations

4.2.1. Target population: All patients in JUSH

4.2.2. Source population: it includes all patients with lymphadenopathy who came to cytopathology department during the study period

4.2.3. Study population

It include those patients with lymphadenopathy which show purulent aspirate on aspiration

4.2.4. Sample size and sample technique

All the study population that came within the time of the study period was included.

NO sample size calculation and sampling technique employed

4.3. Inclusion and Exclusion Criteria

4.3.1. Inclusion criteria - All patients with lymphadenopathy who came to cytopathology department during the study period showing purulent aspirate on gross examination of the aspirate

4.3.2. Exclusion Criteria - Those patients with lymphadenopathy having other kind of aspirates such as scant, hemorrhagic, fluidy, caseous. Patients on anti-tuberculosis treatment at the time of the lymph node aspiration were excluded from the study.

4.4. **Study design** – Facility based cross sectional study was conducted

4.5. Data collection instrument

4.5.1. Recruitment of study participants

Clients with suspected TBLN was subjected to FNAC from which only those cases which show gross appearance of purulent aspirates were included

Demographic and clinical information of the participants including HIV status collected using a checklist.

4.5.2. Collection and processing of samples

At Jimma University Pathology department; FNAC of Lymph node was done as follows:

Lymph node aspirate collected after receiving consent from the study participants.

First FNAC techniques are performed on a swollen superficial, lymph node by using a sterile 21-gauge needle with an attached syringe. The overlying area cleaned with 70% alcohol. Then the node punctured by developing a negative pressure in the syringe. At least six in and out passes will be made by the needle without exiting the node. After removing the needle gross appearance of aspirate characterized as caseous for cheese like or yellow-White aspirate and purulent/non-caseous for greenish yellow or yellow aspirate, scant and hemorrhagic. Those aspirates with characteristically purulent gross appearance were enrolled in to the study as follows.

Two drops of aspirate was placed on three clean slides for FNA cytology. The rest of specimen transferred into a falcon tube for direct AFB smear, gram staining and LED fluorescent staining at Jimma University Laboratory of Mycobacteriology.

The FNA smears were prepared on clean slides on the spot. The slides were air dried and flooded with freshly filtered Weltered Wright's stain and buffered will clean tap water. The buffered slides then continuously stained with Wright's stain for 10 minutes and with tap water and air died.

Finally, the slides examined by a pathologist and final year pathology residents to evaluate for presence of the following cytomorphologcic features of TBLN: epithelioid cell aggregate with or without Langerhans giant cells and necrosis, epithelioid cell aggregate without necrosis, necrosis without epithelioid cell aggregate or polymorphocytes with necrosis **(40)**

At Jimma University Mycobacteriology Research Center: Direct smear microscopy for the detection of AFB with ZN and conventional LED method as well as gram stain for other bacterial infection was done as follows:

Two smears were made of each specimen. One stained using the hot ZN method and examined under light microscopy (Olympus XC31 light microscope; Olympus, Tokyo, Japan) following the standard procedure. The stained smears will be examined for AFB under oil immersion (1000 magnification). At least 100 fields were examined before reporting the smear as no acid-fast bacilli observed. The other smear stained with auramin 0 and examine under LED microscopy (Primko Star iLED, Caarl Zeiss, Gottingen, Germany) with 4009 magnification and 40 fields was examined. Briefly, the smears covered completely with auramine 0 solution (Sigma-Aldrich, USA). After 20 min, the slides washed with running water and decolorized by 0.5% acid alcohol solution for 3 min and counterstained with 0.5% potassium per manganate for 1 min. Stained smears was then examined on the date of staining. Blind reading of the slide was performed by two independent laboratory technologists. All AFB smear-positive slides graded based on the International Union Against Tuberculosis and Lung Diseases (IUATLD) scale. The time required to read individual slide documented for all smear-positive slides. Immediately after the specimen collected, a drop of aspirate placed on a clean slide to make a direct smear. The standard Ziehl-Neelsen staining procedure will be applied

To exclude any bacterial infection, Gram stain was performed for every case

4.6. Data collector

Final year resident and one 2nd year resident and two technical assistants participated in specimen collection and preparation process. One porter enrolled for routine transportation of specimens from cytopathology to microbiology unit where ZN staining, LED microscopy and GM staining performed. Two experienced senior laboratory technicians included for reporting these slides

4.7. Study Variable

Independent variables

- Age
- Sex
- HIV Status
- Sites of Lypmphadenopathies
- LAP with purulent aspirates

Dependent Variable

- Cytologic feature
- AFB staining positivity
- LED microscopy positivity
- Gram stain positivity

4.8. Data analysis, interpretation and dissemination

Immediately after the data collection is completed, data was coded, edited and entered into computer software of SPSS version 20 for analysis. Descriptive analysis was done to describe number and percentages of the variables in the study. The result of logistic regression analysis reported. Data was cleaned, edited, compiled and described. Analysis was done using SPSS 20 version applied and result presented using ration, frequency tables and graphs.

4.9. Data quality control

Was used to evaluate completeness of the aspirates

The principal investigator followed and supervised the sample collection and preparation using check lists. Consultation by senior pathologist was sought at time of technical difficulties and the pathologist performed or repeated the procedure if necessary.

Team approach involving at least one senior resident and one pathologist was used for microscopic characterization of the cytopathologic smears

4.10. Ethical consideration

Permission to conduct the study was obtained from the Ethics Committee/Institutional Review Board of Jimma University. In each of the procedure information describing the nature and purpose of the study was briefly explained to individual patients.

5. RESULTS

5.1. Sociodemographic and clinico-epidemiology features of patients

This Study was conducted on 53 clinically suspected tuberculous lymphadenitis cases who attended cytopathology unit of JUMC, Jimma from August to December 2017. A total of 53 lymph node aspirates were taken from 53 patients with gross characteristics of purulent appearance. The study population age group ranges from 6 to 67 years with a male to female ratio of 2.05.With respect to serologic status for HIV 26 (419.1%) have unknown status while 23(43.4%) HIV negative, and 4(7.5%) were HIV positive. Among the total study population 31(58.5%) cases have 1 or more of the constitutional symptoms, only 15(28%) cases have cough as a complaint as shown in Table 1. Chi-square analysis was performed to see the association between selected characteristics of the patients and the FNAC status. However, no significant association was observed as shown in the table below.

Table 1: Sociodemographic profile and FNAC result of 53 patients attending in cytopathology department, Jimma University Medical Center, 2017

Variable		**Number examined (%)**	**Number with FNAC positive (%)**	**$\chi 2$ (p-value)**
Sex				0.15 (0.46)
	Male	34 (64.2)	16(61.5)	
	Female	19(35.8)	10(38.5)	
				2.13(0.14)
Age (year)	<15	2(3.80)	1 (3.84)	
	15-60	48(90.50)	24(92.32)	
	>60	3(5.70)	1(3.84)	
Address				2.34(0.11)
	Urban	24(45.3)	9(34.6)	
	Rural	29(54.7)	17(65.4)	
HIV status	Unknown	26(49.1)	13 (50)	4.3 (0.1)
	Positive	4(7.5)	0(0)	
	Negative	23(43.4)	13 (50)	
Constitutional symptoms				0.18(0.44)
	Present	29(54.7)	15(57.7)	
	Absent	24(45.3)	11(42.3)	
TB contact history				2.54(0.14)
	No	45(84.9)	20(76.9)	
	Yes	8(15.1)	6(23.1)	
History anti-TB treatment				2.12(0.17)
	No	48(90.6)	22(84.6)	
	Yes	5(9.4)	4(15.4)	

5.2. Site of distribution of affected lymph nodes

Based on their site of distribution 35 (66%) were cervical lymph nodes; 5(9.4%) Axillary Lymph nodes, 3.8% (2/53) were inguinal and 9 (17%) of them involve multiple site. Submandibular and posterior auricular each observed in one case.

Table 2: Distribution and frequency of affected lymph nodes in 53 patients attending in cytopathology department, Jimma University Medical Center, 2017

Lymph node affected	Frequency	Percent
Axillary	5	9.4
Cervical	35	66.0
Generalized	9	17.0
Inguinal	2	3.8
Posterior auricular	1	1.9
Submandibular	1	1.9
Total	53	100.0

5.3. Cytological, LED and AFB staining findings

Cytologic, LED and AFB techniques were employed to each of the 53 samples taken from the study participants. Accordingly, cytologic examination showed cytodiagnosis of tuberculous lymphadenitis in 26 (49.1%) cases while 27 (50.9%) cases as chronic abscess. Similarly, all of the 53 smears were also examined for the presence of mycobacterium using ziehl-Nelson stain and conventional LED fluorescent microscopy and the results are depicted in table table 3.

Table 3: Cytological, LED and AFB staining findings in 53 cases of Lymphadenopathies with purulent aspirates attending in cytopathology department, Jimma University Medical Center, 2017

Diagnostic method	Test result	Frequency	Percent
FNAC	Suppurative	27	50.9
	TB	26	49.1
LED	Negative	30	56.6
	Positive	23	43.4
AFB	Negative	48	90.6
	Positive	5	9.4

Combining the different techniques found to have an increase sensitivity of detection of presence of MTB by individual techniques alone. Combining AFB staining with cytomorphology raised detection of TB

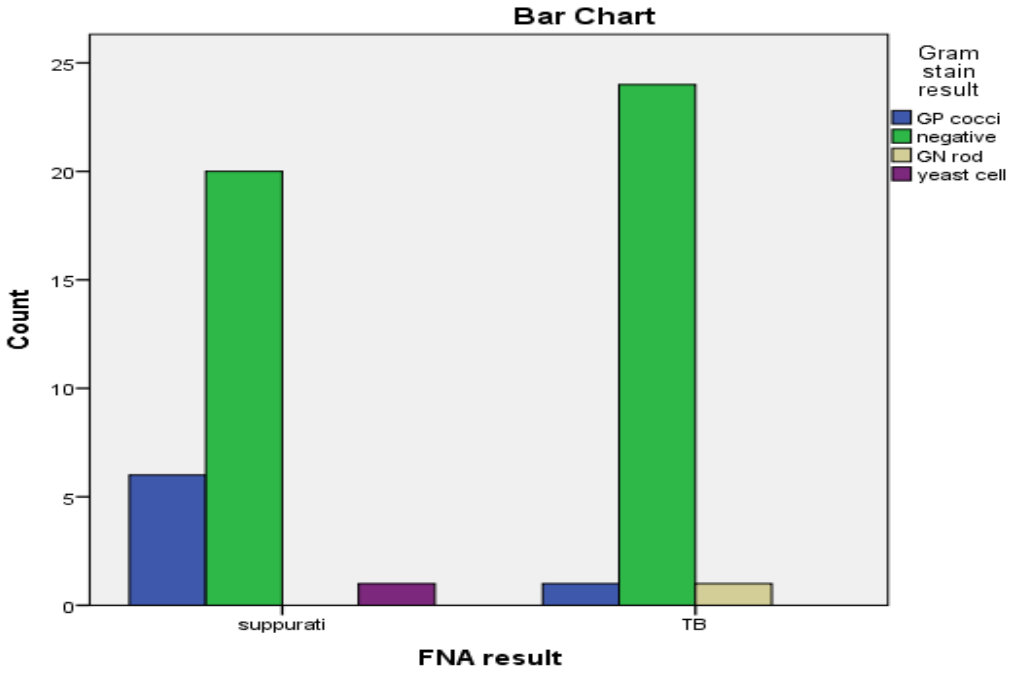
cases to 30 (56.6%) when compared with cytomorphology alone 26 (49%). Using LED techniques with Cytomorphology raised the detection rate by 7.6% .Up on combined use of all the three techniques 2 cases were detected that otherwise missed by LED and cytology together .Taking FNAC as reference the Sensitivity is 58 % for LED and 3.9% for ZN while the specificity is 70% and 85% for LED and ZN respectively as shown in Table 4.

Table 4: Cross-tabulation of FNAC result with LED and AFB results

		LED result		ZN staining	
		Negative	Positive	Negative	Positive
FNAC Result	Suppurative (% of Total)	19 (35.8%)	8 (15.1%)	23(43.4%)	4 (7.5%)
	TB (% of Total)	11 (20.8%)	15 (28.3%)	25 (47.2%)	1 (1.9%)
Total (% of Total)		30 (56.6%)	23 (43.4%)	48 (90.6%)	5(9.4%)

5.4. **Gram staining results**

Finally all the 53 smears were also gram stained for presence of other bacterial infection and in 6 (11.3%) of cases gram positive cocci, in 2 (3.8%) gram negative rods and, in 1 (1.9%) yeast cell were identified as shown in the graph below. A total of 7 (26%) of suppurative cases by FNAC show gram reaction.



5. DISCUSSION

TB lymphadenitis is the most common form of EPTB. Cervical lymph nodes are the most common site of involvement 35 (66.0%) in agreement with other study **(16)**

TBL clinically present with cough in only 15 (28.3%) patients and constitutional symptoms such as low grade fever, weight loss and night sweat seen in 29(54.7%) cases comparable with other similar study**18).**

The three important physical findings in TBL patients were Multiplicity, matting and fistula tract formation in one study in contrast to our finding of discrete and fluctuant mass **(19)** the difference in this finding is due to exclusion of patients with non-purulent aspirates.

Purulent aspirates show only 86% positivity on cytology and 53% AFB positivity on ZN in contrast to our finding of 49% and 9.5% respectively (15).

Cytomorphology shows more sensitivity to detect mycobacteria infection in LAP with purulent aspirates followed by LED and AFB staining with case detection rate of 49%, 43.4% and 9.4% respectively. Combining cytomorrphology with LED techniques increases the detection rate of FNAC by 15% while AFB staining increases this detection rate by 7.6%.

Gram reaction observed in 17% of cases. Twenty two percent of supurrative cases by FNAC yield gram positive cocci while only 2(7.7%) of TB cases were superinfected by a gram negative rod and yeast cell.

In conclusion, this study reaffirms the usefulness of FNA cytology in the diagnosis of TB lymphadenitis. We recommend the combined use of routine FNAC with AFB staining and LED techniques increases the detection of myocobacterial infection in purulent aspirates .Perform Gram stain further could help to exclude other bacterial infections that cause suppurative inflammation.

7. REFERENCES

1. Robbins and Cotran pathologic basic of disease/ Vinay Kumar, Abul K. Abbas, Jon C. Aster; Ninth edition., 2015

2. Roaeon J, Horne N, Miller F (1993) Tuberculose Clinique. ULCTMR : 150 – 164.

3. World Health Organization, Global Tuberculosis Report. Switzerland : Geneva, 2016

4. United Nations, Sustainable Development Goals (SDGs) New York 2015.

5. Brizi MG, Celi G, Scaldazza AV, Barbaro B. Diagnostic imaging of abdominal tuberculosis: gastrointestinal ttract, peritioneum, lymph nodes. Rays. 1998; 23: 115-125

6. Nayak S, Mani R, Kavatkar An, Puranik SC. Holla VV (2003) Fineneedle aspiration cytology in lymkphadenopathy of HIV – positive patients. Diagn Cytopathol 29: 146-148.

7. Saboorian MH, Ashfaq R (2001) ie juse of fine needle aspiration biopsy in the evaluation of lymphadenopathy. Semin Diagn Pathol 18: 110-123.

8. Shenoy R, Kapadi SN, Pai KP et al. Fine needle aspiration diagnosis in HIV-related lymkphaden opathy in Mangalore, India. Acta Cytol 2002; 46 (1) : 35-9

9. Gaque MA, Talukder Si (2003) Evalumation of fine needle aspiration cyrology (FNAC) of lymph node in Mymensingh. Mymensingh Med J 12 : 33 – 35).

10. WHO, "Global Tuberculosis Control : a Shourt Update to the 2009 Report," (2009) World Health Organization, Geneva,

11. Biadglegne F, Tesfaye W, Anagaw B, Tessema B, Debebe T, et al. (2013) Tuberculosis lymphadenitis in Ethiopia. Jpn J Infect Dis 66: 263 – 268.

12. WHO, "Global Tuberculosis Control, 2008," World Health Organization, Geneva, 2008.

13. A Sulaiman, S. Afshan, Tazeen, et al., " A Comparision mof Fine Needle Aspiration Cytology With Ziehl Neelson Staining Technique in Diagnosis of Tuberculosis Lymphadenitis," A Comprehensive Cytopathology, Vol. 16 No. 4, 2010, pp. 707-709

14. Taadesse M, Abebe G, Abdissa K, Bekele A, Bezabih M. et al. (2014) Concentration of Lymph Node Aspirate Improves the Sensitivity of Acid Fast Smear Microscopy for the Diagnosis of T Uberculous L ymphadenitis in Jjimma, Southwest Ethiopia. PLoS One 9 (9):

15. Mistry Y. Ninama G. Mistry K.., Rajat R., Parma R, Godhani A. Efficacy of fine niddle aspiration cyrology ziehl-neelsen stain and culture in diagnosis of TBL, India Volume 2 Issue 1 Jan March 2012.

16.Dye C. (2006). Global epidemiology of tuberculosis. *Lancet*, 367: 938-940.

17.Brosch R, Gordon SV, Marmiesse M, Brodin P, Buchrieser C, Eiglmeier K, Garnier T,Gutierrez C, Hewinson G, Kremer K, Parsons LM, Pym AS, Samper S, Soolingen VD,Cole ST. (2002). A new evolutionary scenario for the Mycobacterium tuberculosis complex. *Proc Natl Acad Sci*, 99: 3684-3689.

18. Cole ST. (2002). A new evolutionary scenario for the *Mycobacterium tuberculosis* complex. *Proc Natl Acad Sci*, 99: 3684-3689.

19. Jones PG, Campbell PE. Tuberculous lymphadenitis in childhood: the significance of anonymous mycobacteria. Br J Surg. 1962;50:302-314.

20.Kruijshaar ME, Abubakar I (2009). Increase in extrapulmonary tuberculosis in England and Wales 1999-2006. Thorax;64:1090-5.

21.Sharma SK and Mohan A. (2004). Review article: Extra pulmonary tuberculosis. *Indian. J. Med Res,* 120:4316 – 4353.

22. Dandapat MC, Mishra BM, Dash SP, Kar PK. Peripheral lymph node tuberculosis: a review of 80 cases. *Br J Surg*. 1990;77: 911-9112)

23. Kvaerner KJ, Kvestad E, Orth M. Surgery required to verify atypical mycobacterial infections. *Int J Pediatr Otorhionolstyngol*. 2001:61:121-128.

25.Fauci A, Braunwald E, Kasper D L, Hauser S, Longo D, J. Jameson L, and J Loscalzo. (2008). Harrison's Principles of Internal Medicine, 16th Edn, 1732-1750, McGraw-Hill Professional publisher.Fine Needle Aspiration cytology. *Indian J Pathol Mcrobiol* 26:273-278

26. Pakistan Oral & Dental Journal Vol 33, No. 3 (December 2013)

27.Frieden TR, Sterling TR, Munsiff SS, Watt LJ, Dye C. (2003). Tuberculosis. *The Lancet, 362*: 887- 99.

28.Daniel TM. (1989). Rapid diagnosis of tuberculosis: Laboratory techniques and application in developing countries. *Review. Infec. Dis.* 11 Suppl, 2: 471- 8.

29.Lee J Y (2015). Review: Diagnosis and Treatment of Extrapulmonary Tuberculosis. *Tuberc Respir Dis*. 78:47-55.

30.Hung J, Shen M, Sun Y. (2000). Epidemiological analysis of extra pulmonary tuberculosis in Shanghai. *Article in Chinese*, 23: 606 – 8

31. Goroll AH, May LA, Mulley Ag. Primary care medicine: office evaluation and management of the adult patient. 2nd ed. Philadelphia: Lippincott; 1987, P. 150-76.

32.de Noronha AL, Bafica A, Nogueira L, Barral A, Barral-Netto M (2008). Lung granulomas from Mycobacterium tuberculosis /HIV-1 co-infected patients display decreased in situ TNF production. *Pathol Res Pract* 2008;204:155-61.

33. Federal Misistry of Health of Ethiopia. Guidelines for Clinical and programmatic Management of TB, Leprosy and TB/HIV in Ethiopia. Addis Ababa, Ethiopia. 2013.

34. Bezabih M., Abdissa K., Tadesse M., Abdella K., Bekele A., Abebe G. (2015) Diagnostic performance of fluorescent light-emitting diode microscopy for tuberculous lymphadenitis in a high-burden setting, Jimma, south west Ethiopia.

35. Hanseheid T. The future looks bright: low-cost fluorescent microscopes for detection of Myc obacterium tuberculosis and Coccidiae. *Trans R Soc Trop Med Hyg* 2008: 120: 520-521)

36. World Health Organiztion. For Diagnosis of Tuberculosis: Policy Statement. 2011.

37.Manjunath N, Shankar P, Rajan L, Bhargava A, Saluja S, Shriniwas (1991). Evaluation of a polymerase chain reaction for the diagnosis of tuberculosis. *Tubercle*;72:21-7.

38.Denkinger CM, Schumacher SG, Boehme CC, Dendukuri N, Pai M, Steingart KR (2014). Xpert MTB/RIF assay for the diagnosis of extrapulmonary tuberculosis: a systematic review and metaanalysis. *Eur Respir J* 2014;44:435-46.

40. Fluorescent light-emitting diode (LED) microscopy for diagnosis of tuberculosis, WHO, 2011, printed by the WHO Document Production Services, Geneva , Switzerland)