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Diagnosis of Tuberculous Lymphadenitis Combining IOSR JDMS Cytomorphology and Microbiological Methods

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Abstract: *In developing countries like India, detection of Tuberculous lymphadenitis with traditional diagnostic tools is a major challenge. Sputum microscopy is the simplest and rapid test currently available to detect acid fast bacilli (AFB) by Ziehl –Neelsen staining method. But, it requires at least 5000 Bacilli per ml culture and it takes at least 3 weeks and is too long for initiating the treatment.Over the past decade,fine needle cytology has assumed an important role in the evaluation of peripheral lymphadenopathy. Most often superficial lymphnodes are affected in tuberculous lymphadenitis and are accessible for fine needle aspiration without radiological guidance.The presence of epithelioid cells is the first step in the diagnosis of tuberculous lymphadenitis in countries such as India , where TB continues to be the most common cause of lymphadenopathy compared to other non- TB causes of granulomas.Fine needle aspiration is a simple ,less expensive procedure to obtain material for examination. Cytological examination together with microbiological examination of the fine needle aspirates is a reliable investigation to diagnose tuberculous lymphadenitis .The presence of epithelioid cells either with caseation or positive Ziehl Neelsen stain for acid –fast bacilli appears to be the best criterion to diagnose tuberculous lymphadenitis by fine needle aspiration.Cytomorphology can be supplemented with AFB smear and culture wherever required and PCR should be kept as a reserve method for equivocal cases.*

Key Words: *Mycobacterium, Tuberculosis, Microscopic analysis, Staining, PCR*

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I. Introduction

Tuberculosis (TB) is a chronic infectious disease caused by *Mycobacterium tuberculosis*. It may present with many clinical -PathologicPatterns. While pulmonaryTB is the most common presentation, tuberculous lymphadenitis is most frequent presentation of extrapulmonary tuberculosis (EPTB) (Robin and Cotrans., 1999)To make global situation worse , Tuberculosis has formed a lethal combination with HIV .[1] India accounts for nearly one third of the global burden of TB . Every day more than 20,000 people become infected with tubercle bacillus , more than 5000 develop the disease and more than 1000 die from Mycobacterium tuberculosis.[3]Extra pulmonary tuberculosis is on the increase world over. In India 10 – 20 % of new TB cases may be extra pulmonary , while amongHIV positives it could be 50 %.[4] In. India and other developing countries almost two thirds of the cases of lymphadenopathy are due to tuberculosis (TB) . Fine needle aspiration is a simple , painless , less time consuming and inexpensive outpatient procedure to obtain material for examination. Most often superficial lymphnodes are affected in tuberculous lymphadenitis and are accessible for fine needle aspiration without radiological guidance. The aspirates obtained from the affected lymphnodes can be used for cytological assessment , Ziehl Neelsen stain and culture.[5] The sensitivity of direct smear microscopy for tubercle bacilli is not optimal ,as the minimum number of tubercle bacilli necessary to produce a positive smear result has been estimated to be minimum 5,000 to 10,000 per ml. [6] Mycobacterial culture is the gold standard method for detection of tubercle bacilli.However , Mycobacteria are slow growing and culture is not routinely done as it takes 6 to 8 weeks to grow on conventional Lowenstein-Jensen medium .[7] Hence the present study was performed to assess the diagnostic role of cytology of fine needle aspirations performed on clinically suspected cases of tuberculous lymphadenitis. We intended to determine the agreement between the cytological and microbiological criteria in the diagnosis of tuberculous lymphadenitis .

II. Materials And Methods

Source of data**:** Out patients ,inpatients and referral cases with clinical suspicion of tubercular lymphadenitis sent for FNAC to department of Pathology ,Government Medical College, Government General Hospital , Ongole..

Study period: July 2017 to June 2019.

Sample size :

This study was carried out on 65 patients who were clinically suspected of having Tuberculous lymphadenitis, referred to the department of cytopathology. Clinical suspicion was based on chronic, low grade fever, weight loss, anorexia and enlarged palpable lymphnodes with history of TB contact. Cases with cytomorphological features of granulomatous lymphadenitis were taken in our study. Study included cases of clinically suspected tuberculous lymphadenitis of all age groups.

Methods of collection of data

Methods

Patients presenting with lymphadenopathy were subjected to brief clinical examination. Data regarding age, sex,duration,description,details of swelling like site ,number,size and association with HIV were documented for each patient. An informed consent was taken from the patient after explaining the procedure.

FNAC was performed under strict aseptic precautions. Aspiration was carried out with a disposable syringe of 10 ml with attached needle of No. 22 gauge

After fine needle aspiration of lymphnode, sample was processed for direct microscopy by staining with H & E, Giemsa and conventional Ziehl –Neelsen stains. Z-N stained smears were examined for presence of tubercle bacilli. Other smears were studied for cytomorphological evidence of tuberculosis (Sinha et al., 2010)

Aspirates were subjected to culture in conventional Lowenstein – Jensen media.Each aspirate was inoculated in two L-J slants, incubated at 37^{0} c in an atmosphere of 5 – 10 % co_2 and high humidity.One L-J slant was incubated in the dark by wrapping the L-J slant by dark paper.These were examined weekly for growth.After 8 weeks of incubation ,negative cultures were reported and cultures were discarded.when growth appeared , rate of growth, pigmentation ,and colonial morphology were recorded (Bhatial et al., 2003)

Polymerase chain reaction (PCR) *for Mycobacterium tuberculosis* was done on the positive cultures to confirm the species..The oligonucleotide primers had the following primers.The sense primer has the following sequences :5 `GCT TGC TTT TAA AAG GTT AGC GGC 3` and the antisense primer has the following sequences: 5` GCA CTC ATC GTT TAC GGC GTG GAC 3 `. (Brown et al., 2003)

Inclusion criteria:

Clinically suspected cases of tubercular lymphadenitis of all age groups.

Exclusion criteria :

Lymphadenopathy which was not suspected to be due to tuberculosis.

Statistical methods

1.Diagrammatic representation

2.Sensitivity, Specificity and Positive predictive value.

III. Results

The present study was undertaken to emphasize the role of FNAC in diagnosis of tuberculous lymphadenitis .. The aspirates obtained by FNAC can be used for detection of tubercle bacilli by conventional direct smear microscopy , culture and molecular methods.

This study was carried out on 65 patients with clinically suspected tuberculous lymphadenitis, referred to the cytology department.These 65 cases are evaluated and the results were analysed as follows.

Majority of the patients were in the age group of 21 – 30 years(constituting 43 %). followed by age group of 11 – (constituting 26 %).The youngest patient was 3 years old, and the oldest patient was 80 years old .A Female preponderance was seen in the study(63%) .Most patients 26 (40 %), had lymphadenopathy of less than one monthduration followed by 1- 3 months duration in 23 (35.3%) patients and 6 months in 8 patients (12.3%).

TABLE -1 LOCATION OF LYMPHADENOPATHY

Location	No. of cases	Percentage
Cervical	43	66%
Supraclavicular	4	6%
Submandibular	7	10.7%
Axiliary	5	7.6%
Inguinal	1	1.5%
Generalised	3	4.6%
Others (Intrabdominal)	2	3%
Total	**65**	**100%**

Majority of the patients presented with lymphadenopathy of cervical region,seen in 43 patients (66%) followed by submandibular ,supraclavicular and axillary seen in 6 - 10%.Generalised lymphnode involvement was seen in 3 patients. (4.6 %) 3% of cases involvement of intra abdominal lymphadenopathy was seen.

Majority of patients (53.8 %) presented with lymphnodes of 1- 3 cm in size. 15 patients (23 %) had lymphnodes of size more than 3 cm. 15 patients (23 %) had lymphnodes of size less than 1 cm.32 patients (49 .23 %) had multiple matted lympnodes, all of them were diagnosed as granulomatous lymphadenitis on cytology and 18 patients (27.6%) had Solitary lymphadenopathy.15 patients (23.07%) had multiple, discrete lymph nodes.

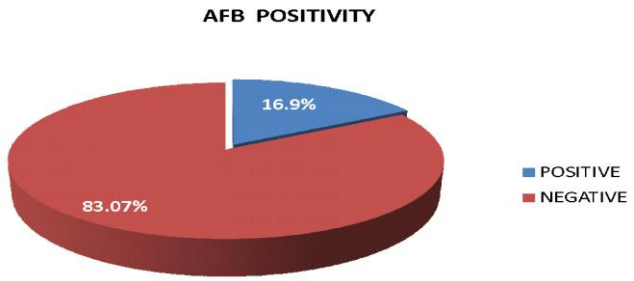
TABLE-2 CYTOMORPHOLOGICAL DIAGNOSIS

Diagnosis	**No. Of cases**	**Percentage**
Granulommatous Lymphadenitis	21	32%
Caseating granulommatous lymphadenitis.	23	35.38%
Gr. LN with superaddedSuppuration	4	6.1 %
Gr.LN with fungal infection.	2	3.0 %
Chronic Nonspecific Reactive Lymphadenitis.	15	23.07 %
Total	**65**	**100%**

Majority of cases i.e 23 (35.38 %) were cytomorphologically diagnosed as Caseating Granulomatous lymphadenitis followed by 21 cases (32%) Granulomatous lymphadenitis (Gr.LN) . 4 cases (6.1%) were associated with superadded suppuration and 2 cases (3%) showed associated fungal infections.

15 cases were diagnosed as chronic non specific reactive lymphadenitis constituting 23.07%.

CONVENTIONALZ-N METHOD FOR AFB.



GRAPH NO. 10

In the present study a total of 11 cases were AFB positive and 54 cases were AFB negative by conventional Z – N method., Majority (7) of them were of grade 1 +, 3 cases of 2 + and only one case of grade 3+.

TABLE 3 CULTURE POSITIVITY- L-J MEDIUM

Culture	**No . of cases**	**Percentage**
Positive	16	24.61 %
Negative	49	75.38 %
Total	65	100 %

In the present study 16 cases (24%) were culture positive in conventional Lowenstein – Jensen medium.

TABLE -4 CONFIRMATION OF CULTURES BY PCR

Culture positive	Confirmed by PCR	Percentage
16	16	100%

Growths from the colonies are confirmed as *Mycobacterium tuberculosis* by PCR (100 %) (Fig. 5).

IV. Discussion

Tuberculosis (TB) is a contagious disease caused by *Mycobacterium tuberculosis*, which mainly affects the respiratory tract . But extrapulmonary TB is also common.[34]The clinical parameters for the diagnosis of tuberculosis in lymphnodes are neither specific nor do their absence exclude tubercular involvement. [32] Early diagnosis of tuberculosis and initiating optimal treatment would not only enable cure of an individual patient but will curb the transmission of infection and diseases to others in the community.[33]Diagnostic modalities must also be tailored to the needs of the population and epidemiology of TB in that region.These include microscopy , usage of liquid culture for childhood and extrapulmonary TB , chemical and physical detection of mycobacterial antigen in paucibacillary condition , antigen capture , antibody detection , cellular immune recognition ,nucleic acid amplification and phage assay.[33]Pulmonary TB is diagnosed by demonstration of acid –fast bacilli in the sputum.In tuberculous lymphadenitis cytomorphological features of lymphnode aspirates together with demonstration of AFB in aspirate smears is diagnostic. However a trained technician should carefully examine the smears to detect the few bacilli in the smears.[34]

Though culture and isolation of the organism is considered the gold standard in the diagnosis of most infections, culturing Mycobacterium tuberculosis as a diagnostic test is often not feasible in the clinical setups as the organism takes about 6 – 8 weeks to grow in conventional Lowenstein –Jensen medium [34].

Although Middlebrook medium recovers mycobacterium rapidly ,it takes a few weeks.The mean duration to give a positive culture in the present study was 5 weeks. This is too long as it is necessary to commence treatment as soon as possible.Therefore , quicker methods need to be established to diagnose tuberculous lymphadenitis.[34]

In developing countries ,where tuberculosis is still rampant , fine needle aspiration cytology (FNAC), as a primary diagnostic tool has provided an efficient alternative to excision . Cytologic diagnosis can be made with cytomorphological features of well – formed epithelioid granulomas and the presence of caseous necrosis (Fig.1).However, bacteriological confirmation is essential because of the presence of various etiological agents of granulomatous inflammation.

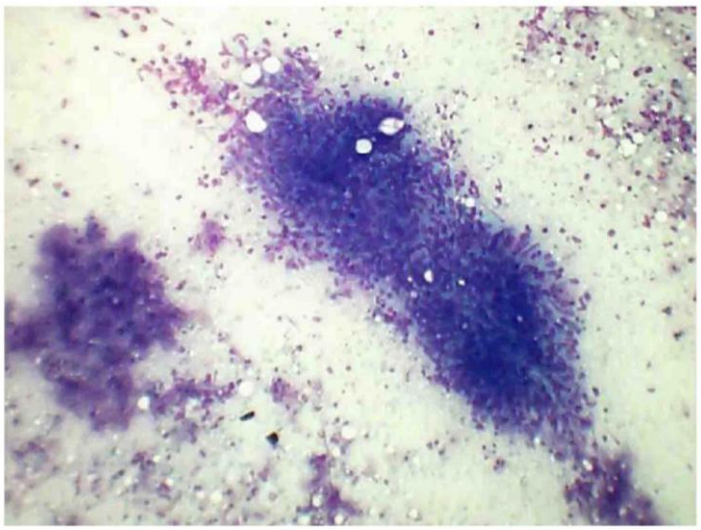


Fig 1. Caseating necrosis with granuloma

This study was performed to evaluate and compare the role of FNAC , mycobacterial culture and PCR in diagnosing tuberculous lymphadenitis (Fig. 2).

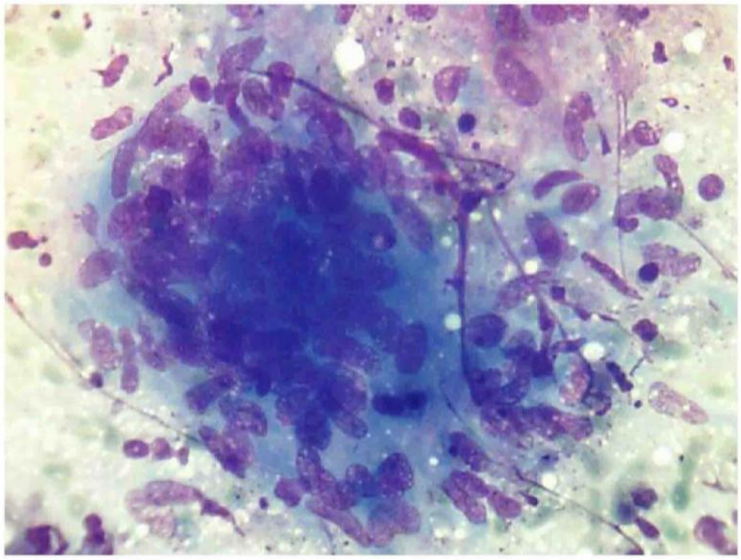


Fig. 2 Epitheliod Granuloma

In the present study , acid –fast bacilli were identified in 11(16.92 %) cases of the total sample and in 100 % of all culture positive aspirates (all smear positive cases are also culture positive in conventional L-J medium) . Both culture and smear were positive in 16 (24.61 %) cases Fig. 3). It was observed that AFB positivity was higher in untreated patients and with HIV positive cases. All the culture positive cases were confirmed as Mycobacterium tuberculosis by PCR.

Fig 2 . Mycobacterial Colonies On L-J Medium

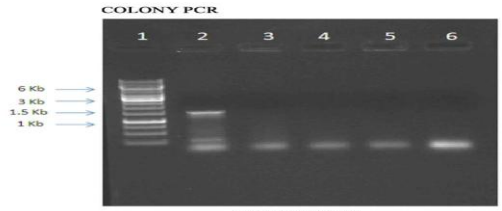


FIGURE NO. 21

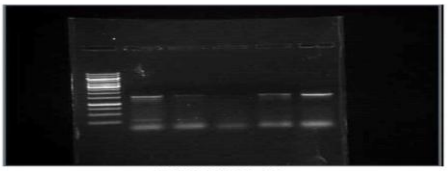
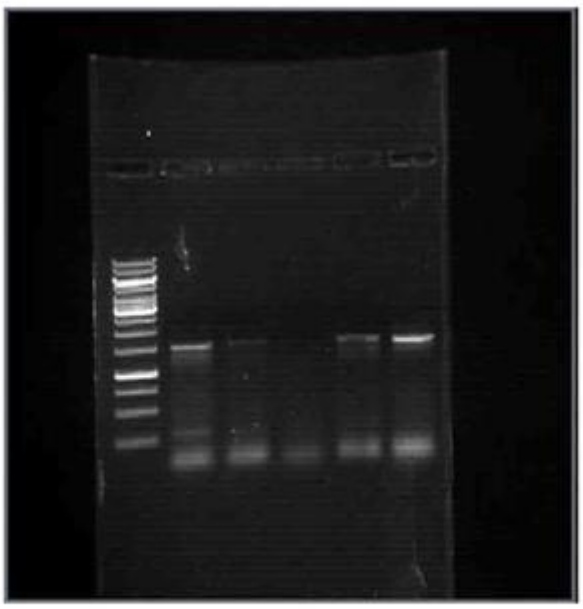


FIGURE NO. 22
LANE 1, 1 kb LADDER, 2: STRAIN NO.1,
3: S.N.3, 4: S.N.15, 5: S.N.19, 6: S.N.25

Mudduwa et al. studied 43 aspirates from patients who were clinically suspected to have tuberculous lymphadenitis.Out of 43 aspirates , acid –fast bacilli were identified in 7 aspirates and 13 yeilded mycobacterial growth in Middlebrook media. The time taken to give a positive culture was 2 to 3 weeks. All of them were confirmed by PCR (Fig 5). 34

Sinha SK et al. studied 143 cases . FNA cytology in 102 cases (71.3%) had caseating epithelioid granulomas (Fig. 2)while smear for AFB was positive in 57 cases (39.8%). Both culture and smear were positive in 29 cases (20.2%). Combining both smears and culture yielded positive results in 47.5 %[35].

A.S. Aljafari et al. studied 60 cases , aspirates were used for cytological examination , Z-N staining , mycobacterial culture and PCR. Of them 25 cases (41.6%) were treated and responded well to anti –tuberculosis therapy. Among them 17 cases (68 %) were diagnosed by FNAC, eight by microbiological methods (32%) and 24 by PCR (96%).[36](Fig 4)



LANE 1, 1 kb LADDER, 2: STRAIN NO.1,
3: S.N.3, 4: S.N.15, 5: S.N.19, 6: S.N.25

Fig. 4 Identification of Mycobacterium by PCR

Mittal et al. studied 50 cases ,out of which 36 cases showed cytological features consistent with TB. 26 cases were correctly diagnosed by acid –fast bacilli (AFB) smear, by culture in 30 cases and by PCR in 30 cases.The overall sensitivity of AFB smear was 76.47 % and that of culture as well as PCR was 88.23%[37]

Comparison of cytology , AFB positivity & culture in different studies.

Authors	Cytology	AFB positive	Culture
Mudduwa et al.	78%	16.2 %	30 %
Sinha SK et al.	71 .3 %	39.8 %	47.5%
S. Aljafari et al.	68 %	32 %	32 %
Mittal et al.	72%	52 %	60 %
Present study	76 %	16.92 %	24.61 %

In our study, 50 of 65 cases showed the cytomorphological pattern consistent with those of granulomatous inflammation with or without necrosis. As the commonest cause of caseating granulomatous lymphadenitis in India is tuberculosis, tuberculous lymphadenitis can be confidently diagnosed. If the caseation is minimal in the affected lymphnode ,it could be absent in the smears.Follow up of these cases showed improvement with antitubercular therapy (ATT). When our study was correlated with other studies , cytomorphologic pattern alone could diagnose tubercular lymphadenitis in 68 – 88% of the aspirates.

In the present study acid-fast bacilli were identified in 16.92 % of the total sample and in 100 % of all culture positive aspirates. The overall AFB positivity in aspiration smears can vary from 16 – 52 % in correlation with other studies.In HIV patients with tuberculosis the detection of AFB is higher.

24.61 % 0f the cases yielded positive culture in our study which ranges between 30 -60% in other studies. Although 24.61 % of aspirates are culture positive ,AFB were demonstrated in 16.92 % of the smears (Fig. 5). This is probably due to the low concentration of mycobacteria in the aspirates.

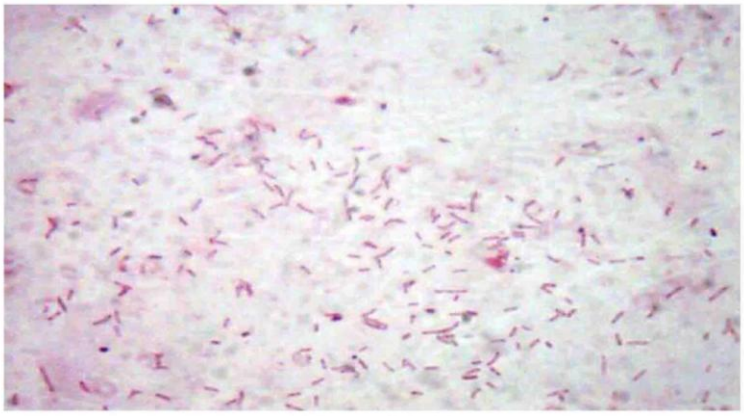


Fig 5. AFB positive smears from colonies of LJ medium

The concentration of organisms in the sputum in pulmonary tuberculosis has a direct relationship to the sensitivity of the Ziehl Neelsen stain and a concentration of $> 10^4$ organisms /mL would guarantee a positive smear. This is applicable to the aspirates from tuberculous lymphadenitis as well.The overall acid- fast Bacilli positivity in fine needle aspiration smears can vary from 37.4 % to 59.4%.

AFB Smear Positivity As The Sole Diagnostic Criteria, with Culture As Gold Standard.

	Culture	
Afb Smear	**Positive**	**Negative**
Positive	11 (A)	0(B)
Negative	5 (C)	49(D)

In our study , positive Ziehl Neelsen stain as the sole diagnostic criteria had a sensitivity of 68.7 %,with a specificity of 100 % with positive culture as gold standard.The positive predictive value is high (100%), and the negative predictive value of 90.7 %.

Epitheloid Cells With Positive Z-N Staining As Diagnostic Criteria

	CULTURE	
CRITERIA	**POSITIVE**	**NEGATIVE**
Epitheloid cells & AFB positive	11 (a)	0(b)
Epitheloid cells positive & AFB negative	5 (c)	34(d)

Sensitivity =68.7% , Specificity=100%,
Positive Predictive Value =100%, Negative Predictive Value =87.17%.
When presence of epithelioid cells together with positive Ziehl Neelsen stain is taken as criteria ,the positive predictive value was again 100%.,with a lower negative predictive value (87.7 %).

Only Cytomorphology As Diagnostic Criteria.

	CULTURE	
CYTOMORPHOLOGY	**POSITIVE**	**NEGATIVE**
Granulomatouslymphadenitis	16 (a)	34 (b)
Reactive lymphadenitis	0 (c)	15 (d)

Sensitivity =100 % ,Specificity=30.16% ,Positive Predictive Value =32 ,Negative Predictive Value =100 %

The presence of epithelioid cells alone had a good agreement with gold standard ,demonstrating a higher negative predictive value (100%) when compared to its positive predictive value (32%).This is due to the fact that, presence of epithelioid cells signifies only the presence of granulommatous inflammation where tuberculosis is one possibility.

The presence of epithelioid cells either with caseation or positive acid –fast stain had high positive(100%) and negative predictive (87.17%) values in our study. Therefore, it is the best diagnostic criterion to be used in the diagnosis of tuberculous lymphadenitis on fine needle aspirates.

The major limitation in the FNAC of suspected lymphadenitis was the limited volume of material that can be aspirated.This can limit the sensitivity of detection of caseation and Acid-fast Bacilli.However,when the lymphadenitis was associated with suppuration ,the volume of material that could be aspirated was more.

V. Conclusion

The presence of granulomas and caseous necrosis are highlysuggestive of tubercular etiology, especially in scenario of developing countries where incidence of TB is high.Cytomorphology can be supplemented with AFB smear and culture wherever required and PCR should be kept as a reserve method for equivocal cases.

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