

A STUDY ON PERIPHERAL LYMPHADENOPATHY SUSPICIOUS FOR TUBERCULOSIS BY CYTOMORPHOLOGY, AFB POSITIVITY AND CBNAAT IN SOUTH ODISHA

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BACKGROUND:

Tuberculosis (TB) is a major cause of ill health and is one of the top 10 causes of death worldwide. Although pulmonary involvement is the most common presentation of TB, extra pulmonary TB constitutes 10-15% of total TB cases which primarily involve the pleura, lymph nodes, gastrointestinal tract, central nervous system, genitourinary system, bones/spine and other organs with a significant case mortality rate (25 to 50%). Cytology and ZN stain for AFB have been used as the initial diagnostic tools for tuberculosis lymphadenitis in resource poor settings. However, the newer technique like Gene Xpert utilizes a DNA-PCR technique for simultaneous detection of M. tuberculosis and rifampicin resistance-related mutations.

AIM AND OBJECTIVE:

Correlation of cytomorphological pattern and AFB positivity of FNAC based cytospreads with CBNAAT result from all lymphadenitis suspicious of TB lesion.

PATIENTS AND METHODS:

This is a comparative study conducted in Department of Surgery in collaboration with Department of Pathology M.K.C.G. M.C.H., Berhampur from September 2021 to January 2023. Out of total of 398 cases, 354 cases were included in the study in which cytomorphology of aspirated material were correlated with AFB stain positivity and CBNAAT from lymph nodes suspicious for tuberculosis.

RESULTS:

The cytological diagnosis made most frequently was granulomatous lymphadenitis (64%), followed by caseating granulomatous lymphadenitis (14%), reactive lymphadenitis and suppurative lymphadenitis (11% each). The presence of acid-fast bacilli by ZN staining was seen in 17 samples (5%). 88 samples (25%) were positive in CBNAAT. CBNAAT positivity was seen in 36 cases (72%) of caseating granuloma, 46 cases (20%) of granulomatous lesions and 6 (15.3%) suppurative lymphadenitis cases; all cases diagnosed as RHL were negative. Out of the total 88 patients 3 patients (4%) were Rifampicin resistant, one case each belonging to cervical, axillary and inguinal lymph nodes

CONCLUSION:

Employment of a molecular technique like CBNAAT in FNAC aspirate helps to obviate the need of excision biopsy for diagnosis of tuberculous lymphadenitis especially when FNAC does not show caseous necrosis in a granulomatous lesion and AFB is not detected by Ziehl Neelsen stain.

KEYWORDS: CBNAAT, TUBERCULOSIS, FNA, LYMPHADENITIS.

INTRODUCTION:

Tuberculosis (TB) is a major cause of ill health and is one of the top 10 causes of death worldwide¹. Tuberculosis (TB) is a bacterial disease caused by Mycobacterium tuberculosis; the causative organism was identified 130 years back². It is a chronic infectious disease and for a very long period, has caused considerable morbidity and mortality³. Major suffering due to tuberculosis is attributable to appearance of virulent strains, resistance to multiple drugs and steady increase in Human Immunodeficiency Virus (HIV) infection⁴.

An estimated 10 million (range, 9.0– 11.1 million) people fell ill with TB in 2018, with 1.2 million (range, 1.1–1.3 million) TB deaths among HIV-negative people in 2018 and an additional 2, 51,000 deaths (range, 223000–281000) among HIV-positive people¹. India has the highest burden of tuberculosis worldwide (27%), having an estimated incidence of 26.9 lakh cases in 2019⁵.

Pulmonary involvement is the most common presentation of TB, although it can potentially affect any organ or system of the body. Extra pulmonary tuberculosis is defined, according to WHO classification criteria, as an infection by *M. tuberculosis* which affects tissues and organs outside the pulmonary parenchyma⁶. In India, EPTB constitutes 10-15% of total TB cases which primarily involve the pleura, lymph nodes, gastrointestinal tract, central nervous system, genitourinary system, bones/spine, and other organs with a significant case mortality rate (25 to 50%)⁷.

Cytology and conventional smear microscopy have been used as the initial diagnostic tools for tuberculous lymphadenitis in resource poor settings⁸. Fine needle aspiration cytology (FNAC) is a simple and rapid diagnostic technique where presence of epithelioid granuloma with caseous necrosis on cytosmears is considered as an evidence of Tubercular lymphadenitis^{9, 10}. However this method has low sensitivity and specificity due to the absence of diagnostic findings in all cases and presence of similar cytomorphological features in lesions other than those associated with TB¹¹. Conventional smear microscopy lacks sensitivity due to the pauci-bacillary nature of fine needle aspirates (FNA)⁸. Other tests like serological assays (both antigen and antibody detection) and Mantoux test have variable sensitivity and specificity. Mycobacterium culture is a gold standard for diagnosis of TB and because drug susceptibility testing is not always available in resource poor settings, their results may take 4 to 8 weeks or even longer⁶. Considering these limitations, more rapid and reliable methods are needed.

In December 2010, WHO endorsed CBNAAT or Gene Xpert MTB/RIF1 for use in TB laboratories and in India it was adopted by RNTCP in 2012. The CBNAAT assay consists of a closed system that is based on real time polymerase chain reaction (RT-PCR). The Gene Xpert utilizes a DNA-PCR technique for simultaneous detection of *M. tuberculosis* and rifampicin resistance-related mutations. Patients with a high risk of tuberculosis-like HIV-associated TB patients and extra pulmonary cases in whom Ziehl-Neelson (ZN) stain smear examination is usually negative are the most likely to be benefited from GeneXpert^{11, 12}.

In the present study conducted in Department of Surgery in collaboration with Department of Pathology, M.K.C.G. Medical College, Berhampur from 2021 to 2023, cytomorphology of aspirated material from lymph nodes suspicious for tuberculosis were correlated with AFB stain positivity and CBNAAT result.

AIM AND OBJECTIVE OF THE STUDY:

Correlation of cytomorphological pattern and AFB positivity of FNAC based cytosmears with CBNAAT result from all lymphadenitis suspicious of TB lesion.

MATERIALS:

A total of 398 patients of both sexes with peripheral lymphadenopathy and different age groups attended to the Department of Surgery MKCG MCH Berhampur Odisha were studied, out of which 354 met the inclusion criteria.

Inclusion criteria:

1. All patients presented with peripheral lymphadenopathy suspicious for Tubercular aetiology irrespective of age and sex.
2. HIV positive patient with lymphadenopathy.

Exclusion criteria:

1. Patient with history of TB or on ATT.

METHODS:

This study focused on lymph node tuberculosis and involved patients attending the outpatient department and inpatients. The researchers obtained proper consent and recorded demographic details such as age, sex, and outpatient/inpatient status. They also gathered relevant medical history, including information on the duration, site, and associated symptoms of the swelling, as well as any history of tuberculosis or upper respiratory tract infections. The researchers noted available blood tests, Mantoux test results, and radiological investigations. They also examined the patient, noting the location, size, mobility, consistency, tenderness, and skin condition of the swelling. Fine Needle Aspiration (FNA) was performed in a procedure room in the pathology department using sterile equipment and a 22-25 gauge needle attached to a 5ml syringe. The aspirate was examined for its nature, amount, and appearance and divided into four parts. One part was fixed in isopropyl alcohol for Haematoxylin & Eosin staining, another was air-dried for diff quick staining, a third was dried for special staining for acid-fast bacilli by Ziehl-Neelsen method, and the fourth was transferred to a falcon tube containing sterile normal saline for detection of Mycobacterium tuberculosis by Polymerase Chain Reaction. The falcon tube was then refrigerated and later sent to the CBNAAT procedure room at a regional diagnostic center. A pre-designed Performa was used to record the patient's details at M.K.C.G medical college.

RESULTS AND DATA ANALYSIS:

IBM Statistical Package for the Social Sciences software version 23.0 was used for performing the statistical analysis. The presence of acid-fast bacilli in cytological smears and detection of mycobacterium tuberculosis by CBNAAT method were correlated and studied using **Chi-Square test**.

A total of 398 cases were recruited for the study out of which 354 met the inclusion criteria and were included for the study. The results of these 354 cases are described as below.

Table 1: Frequency of eligible cases

Criteria	Frequency	Percentage
Inclusion	354	89
Exclusion	44	11
Total	398	100

Table 2: Frequency of PLHA Cases

Criteria	Frequency	Percentage (%)
PLHA	13	4
Non PLHA	341	96
Total	354	100

All AIDS patients with lymphadenopathy were included in the present study as per the inclusion criteria. Out of the total 354 cases, PLHA patients amounted to 4% (13 / 354).

Table 3: Age wise distribution of cases

Age Group	Frequency	Percentage (%)
0-10 Year	31	9
11-20 Year	112	32
21-30 Year	98	28
31-40 Year	53	15
41-50 Year	25	7
51-60 Year	16	5
More than 60 Year	19	5
Total	354	100

Maximum cases belonged to the age group of 11-20 years (32%) followed by 21-30 years (28%).

Table 4: Sex wise Distribution of cases

Sex	Frequency	Percentage (%)
Male	148	42
Female	206	58
Total	354	100

There was a female preponderance with about 58% of the study population while males accounted for 42% of the total.

Table 5: Frequency of site of lymphadenopathy

Site Of Lymphadenopathy	Frequency	Percentage (%)
Cervical	252	71
Axillary	41	12
Inguinal	12	3
Sub Mental	5	1
Supraclavicular	22	6
Submandibular	20	6

Infraclavicular	1	0
Preauricular	1	0
Total	354	100

Cervical area (71%) was the most common site for lymphadenopathy followed by axillary (12%), supraclavicular and submandibular (6% each) and inguinal (3%). Only one patient each reported with infraclavicular and pre-auricular lymphadenopathy.

Table 6: Frequency of Cytological Diagnosis

FNAC	Frequency	Percentage (%)
Granulomatous	227	64
RHL	38	11
Suppurative	39	11
Caseating Granuloma	50	14
Total	354	100

Majority of the patients were diagnosed as Granulomatous lesions (64%) in FNAC whereas 14% of the patients had caseating granuloma. RHL and Suppurative lesions were found in about 11% of the patients each.

Table 7: Frequency of Ziehl- Neelsen stain positivity

Acid Fast Bacilli	Frequency	Percentage
Negative	337	95
Positive	17	5
Total	354	100

The presence of acid-fast bacilli was seen in 17 samples (5%). In the rest 337 samples AFB was not detected by ZN stain.

Table 8: Frequency of CBNAAT results

CBNAAT Results	Frequency	Percentage (%)
Detected	88	24.85%
Not Detected	266	75.15%
Total	354	100

In 24.85% (88) of the cases, TB was confirmed by Cartridge based Nucleic Acid Amplification test while rest were negative.

**Table 9: Comparison of CBNAAT with cytology.**

Cytology	CBNAAT		Total
	Detected	Not Detected	
Caseating granuloma	36	14	50
Granulomatous	46	181	227
RHL	0	38	38
Suppurative	6	33	39
Total	88	266	354
Chi Square = 73.8755, P Value = <0.00001			

88 cases were confirmed to be tubercular by CBNAAT. This included 36 cases (72%) of caseating granuloma by FNAC, 46 out of 227 (20.26%) granulomatous lesions and 6 out of 39 (15.3%) suppurative lymphadenitis cases. None of the cases diagnosed as RHL were positive in CBNAAT. This was statistically significant (Chi square =73.8755, p<0.00001).

Table 10: Comparison of CBNAAT with ZN Stain.

ZN Stain	CBNAAT		Total
	Detected	Not Detected	
Positive	16	1	17
Negative	72	265	337
Total	88	266	354
Chi square = 45.858, p<0.00001			

There were 17 cases in which AFB was detected by ZN stain out of which in 16 cases, there was CBNAAT positive while in 1 case CBNAAT was negative. This difference was significant statistically (Chi square = 45.858, p<0.00001).

DISCUSSION:

A total of 398 cases were evaluated out of which 354 cases (including 13 cases of PLHA) met the inclusion criteria and were included in the study.

Maximum cases (31.63%, 112/ 354) belonged to the age group of 11-20 years followed by 21-30 years (28%, 98/354). In a study conducted by Vishnu Kumar Goyal et al ¹³ most of the patients (40.9%) were also from the same age group. The results of this study were also in accordance with various authors like Ahmad et al. ¹⁴, Kalra SK et al. ¹⁵ and Lokeshwaran RS et al. ¹⁶ who also reported 2nd decade to be the most common age group in their studies.¹⁴ However Dr Heena Gupta et al ¹⁷ found 21-30 years age group (40%) were to be most commonly affected.

There was female preponderance among the cases in this study with the female to male ratio being 1.38:1. Similar results with female preponderance were also found in the studies of Dr Heena Gupta et al ¹⁷(F: M-1.08:1),

Vishnu Kumar Goyal et al¹⁸ (F: M-1.15:1), Dr Khiste J.A.¹⁹ (F: M-1.3:1). While on contrary Prakashan M et al.²⁰ and Ishar T et al.²¹ reported male predominance in their studies.

The present study shows cervical group of lymph nodes to be the most commonly affected accounting for about 71% of all cases. Dr Khiste J.A.¹⁹ (68.8%), Sanjiv Goyal Locham²²(60.6%) , Vimal S et al²³ (50.8%) and M Pradeep Reddy , Nekhiel Moor Chung et al²⁴ also found the cervical group of lymph nodes to be most commonly affected.

Majority of the patients in the present study were diagnosed to be Granulomatous lesions (64%) followed by caseating Granuloma (14%). RHL and Suppurative lesions were found in 11% of the patients each. Lighthelm et al²⁵ also found granulomatous lesions in 66.7% of their cases. Similar findings were observed by Bhavani et al²⁶(42.26%) and Subham Ali et al²⁷ (39.4%) with maximum number of granulomatous cases.

The presence of acid-fast bacilli with ZN staining was seen in only 17 samples (5%). Similar low ZN stain positivity were also found in the studies of Bajrami R et al²⁸ (14.6%) and Chandrasekhar et al³⁴ (12.5%).

CBNAAT detected 88 samples out of 354 (24.85%) for TB out of which maximum (46) were from granulomatous lesions. CBNAAT detected 72% cases of caseating granuloma (36 out of 50). Out of 39 cases of suppurative lymphadenitis 6 cases were CBNAAT positive. No cases of RHL were positive in CBNAAT. A similar CBNAAT positive ratio of positivity (17.6%) was found in a study carried out by Gour Sanjay M et al²⁹. However, several other studies had a much higher rate of CBNAAT positive cases. Low CBNAAT positivity may be due to various technical reasons like amount of material sent as well as the second or third pass of FNAC sent for CBNAAT yielding blood mixed material.^{27,29,30}

Out of 88 positive cases for tuberculosis in CBNAAT, in 72 cases AFB was not detected by ZN stain. Similar results were found in a study conducted by Bajrami R et al²⁸ in which M. tuberculosis was detected in 29.3% cases by CBNAAT as compared to Z-N staining alone which was positive in only 14.6% cases. Although Ziehl – Neelsen method for Acid Fast Bacilli plays a key role in the diagnosis of Tuberculosis, it carries a major disadvantage of low sensitivity ranging from 20% to 43%.³⁴

One case out of 17 AFB positive cases by ZN stain was detected negative by CBNAAT in the present study. Dr Heena Gupta et al¹⁷ did a study in which 2 cases positive for Z.N. were negative for CBNAAT. The reason for this is that CBNAAT, though very specific, does not have 100% sensitivity. According to WHO Xpert guidelines those patients who were cytologically positive and clinically suspicious of tuberculosis should receive TB treatment. So, this explains that CBNAAT negative result can still have TB.³⁵ On comparison, CBNAAT (24.85%, 88/354) proves to be much more sensitive than Z.N. stain (5%, 17/354) in detection of Tuberculosis.

Thus, the present study highlights the impact of CBNAAT in diagnosis of tubercular lymphadenitis cases in resource poor countries like India, where tuberculosis is so prevalent, so as not to miss even a single case of Tuberculosis. Recently WHO recommends CBNAAT to be used as the initial diagnostic test in patients of suspected extra pulmonary tuberculosis. With early detection of these cases proper timely management could be done and further spread can be prevented.

CONCLUSION:

Tuberculosis is a systemic disease, commonly affecting lymph nodes. Early diagnosis and management are crucial to reduce morbidity and mortality. FNAC is a reliable initial diagnostic tool for suspected lymph node TB. CBNAAT is a more sensitive and effective diagnostic tool, detecting drug resistance. All available parameters should be used and results correlated with clinical findings to diagnose TB.

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