Original Research Article

Role of CBNAAT (Cartridge based nucleic acid amplification test) in early diagnosis of tuberculous lymphadenopathy and drug resistant tuberculous lymphadenopathy with the help of fine needle aspiration cytology

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Abstract

Introduction: In the India, we had about a quarter of the world's TB Cases with a very high incidence of MDR TB and HIV associated TB and high mortality; mostly because of lack of early diagnosis and treatment. The Extra-Pulmonary Tuberculosis (EPTB) is challenging due to the pauci-bacillary nature of the disease and limited tests. It is also necessary to rule out other causes of granulomatous inflammation on FNAC and confirmation by CBNAAT. In Dec 2010, WHO recommended CBNAAT to be used as the initial diagnostic test in suspected EPTB cases. **Aim:** To assess the applicability of CBNAAT in early diagnosis of TB lymphadenopathy and early identification of drug resistant TB lymphadenopathy with the help of FNAC. **Material and method**: A hospital based retrospective study carried out over a period of 3 years (Jan2018 to July2021) in Pathology dept, GMERS Medical College, Gandhinagar. All presumptive cases of tubercular lymphadenopathy and purulent aspirates from the lymph nodes of various sites were included in the study. Smears were made after FNA and stained with H & E stain and FNAC aspirates was collected in Falcon tube and sent tube for CBNAAT in all cases of lymphadenopathy. **Results:** The total number of cases with presumptive tubercular lymphadenitis was 475. Majority of the aspirates are from posterior triangle of neck lymph node accounting for 56.42% (268 cases). FNAC has detected tuberculosis in 281 (59.15%) cases. CBNAAT has detected 99 (20.84%)) cases, among them 12 cases (2.52%) which were not detected by FNA. The sensitivity of FNAC in our study was 95.9% and specificity was 100% while the sensitivity of XPERT was 29.53% and specificity was 93.4%. **Conclusion:** CBNAAT can be added with FNAC to get more specific results. CBNAAT is less sensitive for blood stained samples than purulent samples and hence FNA still remains as the cheapest and first line test to diagnose in cases suspected of tubercular lymphadenopathy. The present study highlights the utility of CBNAAT from FNAC material as one of the rapid and adjuvant diagnostic tool in tuberculous lymphadenopathy.

Keywords: Tuberculous lymphadenopathy, extrapulmonary TB, CBNAAT, TB abscess, granulomatous lymphadenitis

Introduction

Lymph node tuberculosis is commonest form of extra pulmonary tuberculosis accounting 58% of extra pulmonary lesions[1].As per the Global TB report 2020 incidence of TB in India was 25,90,000MDR TB was 1.25 lakh and 4,93,000 died because of tuberculosis alone, incidence of HIV-TB 53,000; mortality due to HIV-TB co-morbidity 11,000[2].This high mortality is due to lack of early diagnosis, particularly in HIV co infected persons[3,4]. The samples from extrapulmonary infection will have low bacterial count as compared with sputum specimens[5]. Gold standard for diagnosis of TB is culture but it takes 4-6 weeks, hence other modalities are needed for rapid diagnosis. FNAC is an efficient primary diagnostic tool but Mycobacterial confirmation by CBNAAT[6].CBNAAT is a fully automated rapid molecular assay based on closed system real-time PCR that detects M. tuberculosis[7].

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In 2014, WHO recommended CBNAAT over conventional tests (conventional microscopy, culture or histopathology) in patients suspected of having extra pulmonary TB particularly in HIV-associated TB and MDR TB for early diagnosis and treatment[8,9].MDR-TB diagnosis is offered to patients initiated on re-treatment or who remain smear positive on any follow up and those at high risk like contacts of MDR-TB cases.

Aims and objectives

1) To evaluate the role of FNAC as a diagnostic tool in suspected cases of tuberculous lymphadenopathy
2) To assess the applicability of CBNAAT in early diagnosis of TB lymphadenopathy and early identification of drug resistant TB lymphadenopathy.

Materials and methods

A hospital based retrospective study carried out over a period of 3 years (Jan 2018 to July 2021) in the Department of Pathology, GMERS Medical College, Gandhinagar.

Inclusion Criteria

PLHA presenting with lymphadenopathy, all clinically suspicious cases having TB, patients already taken ATT presenting with lymphadenopathy, patients reported to have drug resistant TB presenting with lymphadenopathy, children with lymphadenopathy suspected of having TB and any purulent aspirate from lymph node irrespective of the clinical suspicion

Exclusion Criteria

Scant aspirate in whose case material could not be sent for CBNAAT and patients who did not give consent for the procedure. Cases that were already diagnosed with CBNAAT before were excluded from the study.

Procedure

Before doing any type of FNAC, consents from all the patients and guardians in case of children less than 10 years were taken.

FNAC was performed on a total of 475 patients presenting with lymphadenopathy presumed of having tuberculosis using 22 - 24 gauge needles with 5-10 ml syringes.4-5 smears were made, 2-3 for conventional cytology with H and E staining and2 for AFB staining using standard protocol, remaining aspirate aspirate was taken into normal saline containing falcon tube and sent for CBNAAT testing. (XPERT MTB/RIF CEPHEID, USA)- Figure- 1. It is a rapid, fully automated test and based on PCR technique that detects DNA directly from the clinical specimens along with Rifampicin resistance detection. Cytomorphological findings were categorized into tuberculous lymphadenitis, granulomatous lymphadenitis, cold abscess, reactive lymphadenitis, suppurative lesion, chronic non specific lymphadenitis, malignancy (Lymphoproliferative disease). Even a single rod seen on AFB staining was taken as a positive result. Demographic details, clinical details along with results were tabulated and statistically analysed.

Fig 1:XPERT MTB/RIF CEPHEID, USA

Result

In Our study, we had included a total of 475 patients with lymphadenopathy and The age group range was from 1-70 yrs with most common age group was young adults between 21-30 years of age (121cases) – Table-2.

The various cytological pattern seen in our study as per Table 1 composed of TB lymphadenitis ,granulomatous lymphadenitis, Chronic non-specific lymphadenitis and TB abscess, Reactive lymphadenitis, Suspicious of TB and Malignancy.

Table-1.Results of FNAC diagnosis(n=475).

Sr. No.	Diagnosis	No. Of Cases	Percentage
1.	TB lymphadenitis	220	46.32%
2.	Granulomatous lymphadenitis	86	18.10%
3.	Chronic non-specific lymphadenitis	68	14.32%
4.	Reactive lymphadenitis	8	1.68%
5.	TB abscess	61	12.84%
6.	Malignancy	21	4.42%
7.	Suspicious of TB	11	2.32%
	TOTAL	475	100%

Table-2.Age distribution of total cases(n=475).

Sr.No.	DIAGNOSIS	AGE IN YEARS							
		<1	1 to 10	11 to 20	21 to 30	31 to 40	41 to 50	51 to 60	61 to 70
1.	TB lymphadenitis	0	28	56	61	37	21	10	7
2.	Granulomatous lymphadenitis	0	22	19	22	14	6	1	2
3.	Chronic non-specific lymphadenitis	1	27	12	17	6	2	3	0
4.	Reactive lymphadenitis	0	4	1	0	3	0	0	0
5.	TB abscess	2	3	13	19	11	6	7	0
6.	Malignancy	0	0	1	0	1	6	9	4
7.	Suspicious of TB	0	3	3	2	2	0	0	1
	Total	3	87	105	121	74	41	30	14

Table-3.Sex distribution of total cases(n=475).

Sr. No.	Diagnosis	MALE	FEMALE	Total
1.	TB lymphadenitis	111	109	220
2.	Granulomatous lymphadenitis	35	51	86
3.	Chronic non-specific lymphadenitis	31	37	68
4.	Reactive lymphadenitis	4	4	8
5.	TB abscess	38	23	61
6.	Malignancy	17	4	21
7.	Suspicious of TB	5	6	11
	TOTAL	241(50.73%)	234(49.26%)	475

Table 4 Clinical presentation of cases(n=475).

Sr. No.	CLINICAL FEATURE	NO. OF CASES	PERCENTAGE

1.	Swelling	475	100%
2.	Fever	132	27.79%
3.	Cough	79	16.64%
4.	Weight loss	92	19.37%

Table 5 Site wise distribution of total cases(n=475).

Sr. No.	SITE	NO. OF CASES	PERCENTAGE
1.	Post auricular	5	1.05%
2.	Pre auricular	6	1.26%
3.	Posterior triangle	268	56.42%
4.	Anterior triangle	58	12.21%
5.	Supraclavicular	42	8.85%
6.	Sub mandibular	67	14.10%
7.	Sub mental	5	1.05%
8.	Axillary	16	3.37%
9.	Inguinal	8	1.69%

In the present study, majority of the cases had fever as associated symptom along with lymphadenopathy followed by weight loss and cough.[Table-4].

Most common site of lymphadenopathy was posterior triangle of neck, cervical lymph node accounting for total 268 cases (56.42%) – Table-5.

Table-6. Distribution of type of FNAC aspirates along with CBNAAT results(n=475). FNAC: Fine Needle Aspiration cytology

Sr. No.	Type of aspiration	No of cases	CBNAAT positive	CBNAAT negative
1.	**Blood mixed**	338 (71.16%)	39 (11.54%)	299 (88.46%)
2.	**Purulent**	111 (23.37%)	52 (46.85%)	59 (53.15%)
3.	**Cheesy**	26 (5.47%)	8 (30.77%)	18 (69.23%)
4.	**Total**	475	99	376

Table 7 Comparison of Cytomorphological diagnosis(FNAC) with CBNAAT(n=475).

Sr. No.	Diagnosis	No of patient	CBNAAT positive	CBNAAT negative
1.	**TB lymphadenitis**	220	49(22.27%)	171(77.73%)
2.	**Granulomatous lymphadenitis**	86	9(10.46%)	77(89.54%)
3.	**Chronic non-specific lymphadenitis**	68	2(2.94%)	66(97.06%)
4.	**Reactive lymphadenitis**	8	1(12.5%)	7(87.05%)
5.	**TB abscess**	61	34(55.73%)	27(44.27%)
6.	**Malignancy**	21	0(0%)	21(100%)
7.	**Suspicious of TB**	11	4(36.36%)	7(63.64%)
	Total	475	99(20.84%)	376(79.15%)

Most common aspirate encountered while doing FNAC was blood mixed accounting for 71.16% of the total out of which 11.54% cases were CBNAAT positive while cheesy aspirate signifying tuberculosis was seen in 5.47% of the total cases out of which 30.77% cases were CBNAAT positive–Table-6**.**

All clinical suspected tuberculosis cases were sent for CBNAAT in which majority of cases showed tuberculous lymphadenitis pattern on cytology with 49 cases (22.27%) showed CBNAAT positive, 34 cases (55.73%) of TB abscess showed CBNAAT positive as shown in Table 7. **Rifampicin Resistance was seen in 8 cases out of the total 99 XPERT positive cases and one cases of isoniazid resistance was found.**

XPERT was positive in a total of 99 cases (20.84%) of which 83 cases fell under the reference standard of our study. There were 171 cases in whom FNAC was positive for tuberculosis

While XPERT showed negative result.

There were 12 cases that were negative for tuberculosis on cytology and reported as Granulomatous and chronic non specific lymphadenitis but positive on XPERT for Mycobacteria; this is one reason why we recommend the use of XPERT in any purulent aspirate irrespective of clinical suspicion as the epithelioid granulomas and caseation may not be visible in the presence of suppuration.

There were 8 cases out of the total 475 who were reactive for HIV, of whom, 4 (50%) showed TB positive on FNAC and CBNAAT. There were 19 patients of the total patients who gave history of taking ATT in the past (probably MDR cases). The sensitivity of FNAC in our study was 95.9% and specificity was 100% while the sensitivity of XPERT was 29.53% and specificity was 93.4%. The positive predictive value and negative predictive value of FNAC were 100% and 93.44% while the positive predictive value and negative predictive value of CBNAAT were 87.36% and 46.34%.

Discussion

The present study is a hospital based retrospective study in the diagnosis of suspected tubercular lymphadenopathy by CBNAAT than we compare with FNAC results. This study aimed to assess the applicability of CBNAAT in early diagnosis of TB lymphadenopathy and early identification of MDR-TB. FNAC being an OPD procedure, inexpensive and minimally invasive, it is routinely used now-a-days for diagnosing extrapulmonary TB[10].

WHO recommended the use of CBNAAT for the following (2014):

1. Diagnosis of drug resistant TB among previously treated TB patients.
2. Patients with smear microscopy positive result at any time during treatment.
3. HIV-TB patients.
4. Diagnosis of TB among children.
5. Extra pulmonary presumptive TB patients.
6. Person with Chest X-Ray suggestive of TB with smear negative result.

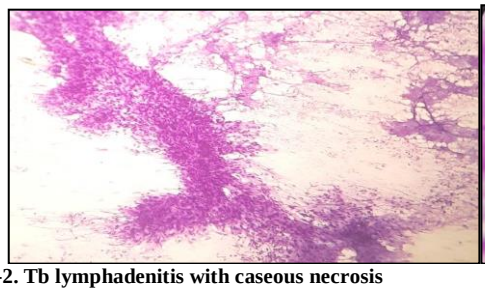

Fig-2. Tb lymphadenitis with caseous necrosis H & E stain 10x

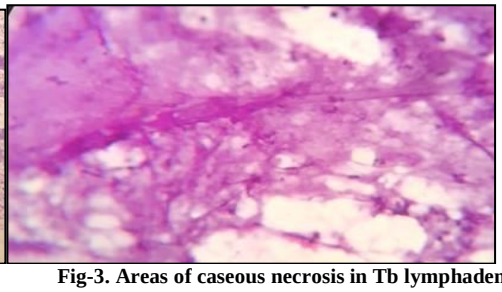

Fig-3. Areas of caseous necrosis in Tb lymphadenitis H & E stain 10x

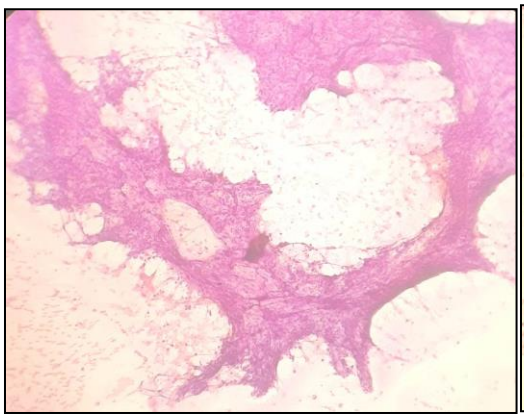

Fig-4. Well formed Granuloma without necrosis H & E stain 10x

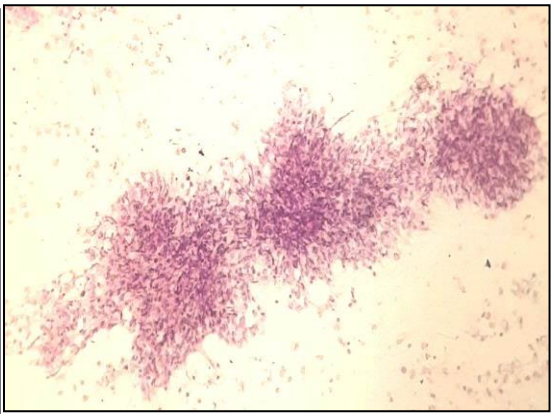

Fig-5.Tb abscess H & E stain 10x

The culture Drug susceptibility testing is complex, time consuming usually takes 2-8 weeks which makes delay in treatment decision[11]. While patients await diagnosis, they are likely to receive inappropriate or in effective treatment and consequently disease may progress. This results in an increased chance of morbidity from tuberculosis. They continue to transmit drug-resistant TB to others; especially family members and the resistance might developed. All these factors lead to delay in definitive diagnosis[12].

There was 12 cases that was negative for tuberculosis on cytology and reported as Granulomatous and chronic non specific lymphadenitis but positive on XPERT for Mycobacteria; this is one reason why we use of XPERT in any purulent aspirate irrespective of clinical suspicion as the epithelioid granulomas and caseation may not be visible in the presence of suppuration. To address this issue there was a need for a simple and rapid diagnostic tool and a new diagnostic test, CBNAAT was developed. It is a rapid, fully automated test and based on PCR technique that detects DNA directly from the clinical specimens along with Rifampicin resistance detection.

Table 8: Cytological pattern comparison with other studies

Sr. No.	Pattern	Cases	Present Study	Shilpa G et al13	Bhavani et al14	Ligthelm et al15	Devi et al16	Naesreen et al17
1.	Granulomatous	306	64.42%	39.4%	42.26%	66.7%	31.2%	17.9%
2.	Reactive	76	16%	36.5%	35.47%	20.8%	55.2%	12.7%
3.	Metastatic	17	3.57%	14.4 %	11.32%	8.3%	6.25%	19.7%
4.	Suppurative	61	12.84%	6.7%	9.8%	2.1%	-	-
5.	Lymphoma and Lymphoproliferative	04	0.84%	2.8%	1.13%	-	6%	49.7%

The present study is a hospital based retrospective study in the diagnosis of suspected tubercular lymphadenopathy by CBNAAT in comparison to FNA. In this new diagnostic era, FNAC plays invaluable role in diagnosis of diseases. The mean age was 21-30 yrs and M:F ratio was 1.03:1, The most common site of presentation in our study was cervical lymph node swellings (posterior triangle of neck). For cytological diagnosis of tubercular lymphadenitis, we need to demonstrate granulomas composed of epithelioid cells, Langhans giant cells with or without caseous necrosis along with demonstration of AFB positivity. In granulomatous lesions the present study showed 46.32% granulomatous with necrosis(Table-1), 18.10 % granulomatous without necrosis and 12.84 % only necrosis (Table-1,8) whereas study done by Bhavani et al7 showed 9.8% granulomatous without necrosis, 27.17% granulomatous with necrosis and 5.28% only necrosis. The various cytological pattern comparison with other studies as shown in Table.8

Table 9: Comparison of age and sex wise distribution along with CBNAAT positive cases of other studies (n=475).

Sr. No.	Study	Year of study	Place of study	Age (years) group	Female	Male	Percentage of CBNAAT Positivity
1	Rock RB et al., [18]	Jan1993-Dec 2003	Minnesota	15-24	46%	54%	43%

2	Tadesse M et al., [19]	May-Sept 2013	Jimma, Belgium	16-30	67%	76%	58%
3	Komanapalli SK et al., [5]	April 2017-March 2018	Visakhapatnam, Andra Pradesh	11-30	23.8%	76.2%	58%
4	K Arpitha et al.[20]	July 2019-June 2020	Kalaburgi, Karnataka	21-30	42.85%	57.14%	21.84%
5	Present study	Jan 2018-July2021	Gujarat	21-30	49.26%	50.73%	20.84%

In this study, young adults are more commonly affected whereas in other studies where younger age groups were predominantly affected with Tb [Table/Fig-11][3,8,9] and male preponderance is seen in the present study which is correlated with other studies done by Komanapalli SK et al., Rock RB et al., and Tadesse M et al., K Arpitha et al[3,8,9].This study also compared the distribution of type of FNA aspirate along with CBNAAT result which is not correlated with Tadesse M et al.[9], study where caseous aspirates (58%) had more CBNAAT positivity compared to present study which has 58.75% cases of purulent aspirates with CBNAAT positivity which is in correlation with Komanapalli SK et al.[3]. In the present study, 20.48% cases of FNA aspirates had CBNAAT Positivity. These is because of large sample size we taken compared to other studies and It is possible that in these cases representative sample might not be obtained as bacterial load may have been too low for the GeneXpert to detect the Deoxyribo-nucleic Acid (DNA) from Mycobacterium Tuberculosis (MTB)- complex. In the present study, 12 cases out of non-correlated cases were CBNAAT positive. The importance of CBNAAT lies in detecting the above mentioned 12 Tb patients which were cytologically negative for Tb, who were surely benefitted by the CBNAAT.Therefore, FNAC should be included as first line investigation and it should be compared with CBNAAT to get more accurate results and to decrease the chances of missing cases by CBNAAT alone or FNAC alone.

Conclusion

FNAC is rapid, cost effective highly accurate test in diagnosing tuberculous lymphadenopathy. CBNAAT alone was found less sensitive for blood stained samples than purulent samples. But the present study highlights that FNAC combining it with CBNAAT can add benefit of detection of FNAC missed cases because of purulent material and it is a rapid test available with high specificity.

CBNAAT and FNAC testing both are complementary for early diagnosis TB lymphadenopathy and early identification of drug resistant TB lymphadenopathy.

These help in timely diagnosis and treatment of missed TB cases by any single diagnostic test like FNAC and CBNAAT alone. Early diagnosis will help in starting effective treatment on early basis that prevent the further spread of tuberculosis cases in the community and amplify effective utilization of TB control program in our country.

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