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Original Research Article

Utility of CBNAAT, Cytology and Histology in diagnosis of suspected tubercular solid lymph node

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ABSTRACT

Background : Cytology, Ziehl Neelsen staining and Mantoux test are conventionally used to test and treat lymph node tuberculosis. Cartridge Based Nucleic Acid Amplification Test (CBNAAT) is being now recommended for diagnosis of all extrapulmonary tuberculosis including tubercular lymphadenitis.
Aims and Objectives : To asses efficacy of various diagnostic tools for diagnosis of lymph node tuberculosis in solid state (non-caseating stage).
Material and Methods : It was a prospective study conducted by including all consecutive patients with lymph node swelling suggestive of tuberculosis. Fine needle aspirate was subjected for cytology and CBNAAT. If both turned negative, excision biopsy was performed and subjected for histology. Results were tabulated.
Results : Sixty patients were included after excluding certain patients with caseous aspiration. Thirty-one (51.67%) had mycobacterium detected in CBNAAT. Remaining patients after histology had following diagnosis; Tuberculosis in eight, nonspecific reactive hyperplasia in 11, metastasis and lymphoma in four and Non Tubercular Mycobacteria and actinomycosis in one each patient.
Conclusion : CBNAAT is an effective tool for diagnosing lymph node tuberculosis even in early stages of pathology. Relying solely on cytology to diagnose tuberculosis should be discouraged.

1. Introduction

Globally Lymph node tuberculosis (LNTB) is identified as the most common Extrapulmonary Tuberculosis (EPTB).[1–3] As a paucibacillary disease, LNTB poses a challenge in diagnosis, especially microbiological confirmation.[4] Hence, diagnosis was largely based on clinical background and cytopathology/smear examination with the support of elevated ESR and/or positive Mantoux test, which were prone for errors. It is postulated that 10^4bacilli are requiredto be able to demonstrate in smear preparations,[5] and 100 cfu are required to be positive in culture.[6] Then came a boon, CBNAAT- Cartridge based nucleic acid amplification and testing (Gene Xpert MTB/RIF), which revolutionised the diagnosis of tuberculosis in terms of efficacy and speed of diagnosis of tuberculosis disease.[7]

In 1993, National tuberculosis Programme (NTP) is renamed and restructured due to multiple lacunas, one important reason being, over reliability on chest X rays for diagnosis which is prone to over or under diagnosis, owing to subjective variability in reading chest X-ray.[8] Revised National Tuberculosis Control Programme (RNTCP) stressed and moved on to microbiological diagnosis which is a more certain diagnostic tool.[9] Only available rapid diagnostic microbiological tool was Ziehl Neelsen (ZN) staining technique and is good for sputum from Pulmonary tuberculosis.[4] Whereas paucibacillary EPTB required culture system which require 7 to 42 days depending on culture system used.[10,11] With the shift from ZN stain to CBNAAT for microbiological diagnosis, there is also added advantage of detecting Rifampicin resistance in

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addition to rapid testing time.[12] Also, ability of CBNAAT to detect as low bacterial load as 100-130 bacilli per ml of sample compared to 10^4 per ml for ZN staining makes it ideal for paucibacillary tuberculosis.[5,13,14] CBNAAT is at par with culture which demands 100 bacilli per ml of sample.[6]

World health organization endorsed CBNAAT for diagnosis tuberculosis in pulmonary and EPTB in December 2010, followed by adoption in national programme in 2012.[15,16] By 2019, 1180 laboratories across India had this rapid diagnostic tool[17]. Currently CBNAAT is the first mode of investigation in key population group like Children, HIV positive patients, EPTB.[9] Also, an index guideline for diagnosis and management of EPTB recommends CBNAAT as a must investigation in addition to conventional diagnostic tools such as smear, culture and cytology on Fine needle Aspiration (FNA) specimens[1].

LNTB pathologically progresses in five stages; Hyperplasia, Periadinitis, Cold Abscess, Collar stud abscess, Sinus formation.[18] Probability of finding bacilli increases as stage advances.[19] Multiple studies have already validated the efficacy of CBNAAT in EPTB including LNTB, especially in abscess stage and beyond. In this study we aimed to study the yield of CBNAAT in first two stages of lymph node i.e., in unripe or solid stage.

2. Aims and Objectives

To assess the usefulness of CBNAAT, Histology and Cytology in diagnosis of suspected tubercular lymph node in adenitis (pre-abscess/caseation) stage.

3. Materials and Methods

It was a prospective type of observational study conducted at Shridevi Institute of Medical Sciences and Research Hospital, Tumkur, over a period of 18 months from April 2017 to November 2019, after obtaining institutional ethical committee approval. Palpable, firm lymph node in a patient who came to department of respiratory medicine with clinical features of tuberculosis or LNTB patients referred from other departments for initiation of Antitubercular Treatment (ATT) were included in our study. Those patients with Positive serology for HIV, Fluctuant node, on ATT and if aspiration of pus/cheesy material from lymph node were excluded from study. After explaining the procedure and complications, written informed consent was taken from all patients. FNA was done by passing 18- or 20-Gauge needle into lymph node under two finger guidance, with suction applied, needle was moved back and fro around 10-15 times. Procedure was repeated from same node or other node if material obtained was unsatisfactory. Material obtained was smeared for cytology and ZN staining and remaining aspirate was flushed with normal saline to empty contents in syringe and hub into Falcon's tube for CBNAAT. If CBNAAT or Acid Fast Bacilli (AFB) in ZN stain smear turns negative, excision biopsy of easily approachable node was performed and histopathology was sought irrespective of cytology result. Diagnosis of Tuberculosis is established with positive CBNAAT/ AFB or Epithelioid granuloma with or without necrosis in histopathology with clinical response to ATT. Results were tabulated, analysed using MS Excel 2019 and expressed in mean, median and percentages.

4. Results

A total of 82 patients underwent FNA and 22(22.83%) were excluded from study due to aspiration of pus/cheesy material. Remaining 60 patients were included in for study. Demographic details and lymph node examination findings of included population are depicted in table one. Most common site of disease is cervical region in 52(86.67%) followed by inguinal in two, axillary in one and remaining five had generalized lymphadenopathy. Out of 60 patients 31(51.67%) had got MTB detected in CBNAAT and remaining 29(48.33%) were subjected for excision biopsy. Demonstration of AFB by ZN stain was done in 9 (15%) cases and all of them were positive in CBNAAT. Two among 31 MTB detected samples in CBNAAT had Rifampicin resistance. Final diagnosis made after histopathology of excision biopsy is shown in table two. Results of Cytological features of FNA in relation to final diagnosis are depicted in table three. CBNAAT could not detect 20.51% (8/39) cases of Tuberculosis which were diagnosed by histopathology. CBNAAT detected Tuberculosis in 22(70.96%) cases which were missed by AFB smear and 16 cases missed by Cytology. Sensitivity, specificity, positive predictive value and negative predictive value of Cytology, ZN stain, Mantoux test, Elevated ESR and CBNAAT for diagnosis of Tuberculosis is shown in table four. Cytological finding correlated with final diagnosis (including Tuberculosis and other diagnoses) in 56.67 % (34/60) cases and final diagnosis was different from cytology in 43.33 % (26/60) cases.

5. Discussion

Our study population included majority of male gender and with mean age 28.42 years similar to other studies.[20,21] Most common site for lymphadenopathy was cervical region, which could be due to pathogenesis of lymph node TB by spread of tuberculosis through lympho-hematogenous route from lungs and, cervical nodes drains major parts of lungs.[22,23] On examination 55% of patients had mattedness and 60% had multiple lymph nodes. This could be explained by late presentation of patients to health care, by the time disease would have progressed to periadinitis stage and beyond. Similar findings were noted by Nidhi et al who noted 40.7% tubercular patients had multiple lymph node and Saurabh et al reported 32.8% cases

Table 1: Demographic profile, clinical history and lymph node examination.

Mean age	28.42 years
Gender	
Male	41(68.33%)
Female	19 (31.67%)
Mean total duration of illness	99.8 days
Symptoms of tuberculosis	
Present	45(75%)
Absent	15(25%)
Past history of Tuberculosis	
Present	16(26.67%)
Absent	44(73.33%)
Lymph node characteristics Number	
Solitary	19(31.67%)
Multiple	36(60%)
Generalised	5(8.33%)
Tenderness	
Present	9(15%)
Absent	51(45%)
Mattedness	
Present	33(55%)
Absent	27(45%)

Table 2: Cytology features of lymph node(rows) charted against final diagnosis obtained (columns).

	Tuberculosis	**Metastasis**	**Lymphoma**	**Reactive Hyperplasia**	**Actinomycosis**	**NTM**	**Total**
Granulomatous lymphadenitis	12	0	1	0	0	0	13
Necrotising Granulomata	9	0	1	0	0	0	10
Metastasis	0	3	0	0	0	0	3
Reactive lymphadenitis	15	0	1	9	0	1	26
Actinomycosis	0	0	0	0	1	0	1
Inconclusive	3	1	1	2	0	0	7
Total	39	4	4	11	1	1	60

Table 3: Performance of various diagnostic techniques against composite diagnostic methods for diagnosis of LNTB

	Sensitivity% (95%CI)	**Specificity% (95% CI)**	**Positive predictive value% (95% CI)**	**Negative predictive value% (95%CI)**
Cytology	53.85(37.18-69.91)	90.48(69.62-98.83)	91.30(91.30-97.59)	51.35(53.31-78.31)
ZN stain	23.08(11.13-39.33)	100(83.89-100)	100(-)	41.48(37.08-45.39)
Mantoux Test	46.15(30.09)	42.80(21.82-65.98)	60.00(47.59-71.25)	30.00(19.46-43.19)
Elevated ESR	76.92(60.67)	28.57(11.28-52.18)	66.67(59.21-73.37)	40.00(21.55-61.8)
CBNAAT	79.49(63.54-90.70)	100(83.89-100)	100(-)	72.41(58.6-82.96)

Table 4: Final diagnosis obtained

Final Diagnosis	**Number(%)**
Tuberculosis	39(65)
Metastasis	4 (6.67)
Lymphoma	4(6.67)
Non specific Reactive lymphadenopathy	11(18.33)
Actinomycosis	1(1.67)
NTM	1(1.67)
Total	60

with matted node.[20,24] In a study by Mengistu 16.5% had past history of tuberculosis,[25] whereas our study population composed 26.67% patients with past tubercular treatment history, may be due to high tuberculosis burden in our country.

Common predefined cytological features suggestive of tuberculosis are granulomatous inflammation, granulomatous inflammation with necrosis and only necrosis.[26] Cytology had modest sensitivity and specificity in our study. We noted 53.84% (21/39) of tubercular patients had granulomatous inflammation with or without necrosis. Two patients with granuloma were turned out to be lymphoma in histology. Shigeyuki describes multiple causes of granulomatous inflammation of lymph node which included both Hodgkin and Non-Hodgkin lymphoma.[27] Khurana et al. identifies granuloma in lymph node draining malignancy as a recognized phenomenon.[28]

Significant number of patients (26/60, 43.33%) had a cytological feature of reactive hyperplasia in our study. Out of 26, 15 (57.69%) turned out to be tuberculosis in either CBNAAT or histology. In a follow-up study by Ijaz conducted on nonspecific reactive hyperplasia of lymph nodes upon re-biopsies,[29] showed 17% had Tuberculosis, 11% had lymphoma, 6% developed acute lymphadenitis and 27% of patients had persistent benign nonspecific hyperplasia.[29] This emphasizes the need for early histology in cases of reactive hyperplasia.

AFB smear had low sensitivity and negative predictive value with high specificity and positive predictive value and this is noted in other studies too and is due to paucibacillary nature of LNTB.[4] Bacillary load increases as disease progress to necrotic stage and highest in purulent material. In a study by Hemalatha et al. AFB positivity rate among different cytological features were noted as follows; granulomatous reaction- 21%, necrotising granulomatous-55% and necrosis only-73.5%.[19] Since we excluded purulent or caseous samples from our study, naturally AFB smear positivity rate will be reduced. Mantoux test (MT) is also used as an adjunct in diagnosis of tuberculosis in routine clinical practice, but is limited by low sensitivity, specificity, high false positive and negative rate. We noted 53.85% patients with tuberculosis had false negative MT and implies its limited value in diagnosis of tuberculosis. Also, in a study published by Anju Jain,[30] only 54.6% of EPTB had MT positive. Similarly, elevation of ESR for diagnosing Tuberculosis is misleading.[30]

Performance of CBNAAT on suspected LNTB have been published with varying yields. Our study had sensitivity and specificity of 79.49% and 100% respectively which can be compared to a pooled results by two different meta-analysis of Guocan and Denkinger who found sensitivity and specificity of 80% & 96% and 81.2 & 99.1% respectively.[31,32] Many studies reported sensitivity of CBNAAT >95% on lymph node specimens in those meta-analysis. Our study could be having lesser yield due to the exclusion of purulent aspirations. A study by Megintsu et.al had a difference in CBNAAT positive rate among hemorrhagic, caseous and purulent aspirates, yielding only 20% (12/60) CBNAAT positivity in hemorrhagic aspirates and 68.4% (63/92) in caseous and purulent aspirates.[25] Our study had CBNAAT positive rate of 79.89% (31/39) despite inclusion of only non-caseous or non-purulent material for CBNAAT. This relative higher yield is may be due to microscopic necrosis which grossly doesn't look like pus or may be due to technical differences like using wide bore needle (we used 18- or 20-gauge needle instead of traditional 22 gauge needle) for aspiration with more number of needle strokes or repeat sampling. One of the limitations of our study is not conducting culture of mycobacterium on aspirated material or biopsy specimen.

6. Conclusion

CBNAAT is an effective tool for diagnosing lymph node tuberculosis even in adenitis or non-caseous stage, although yield is lesser compared to that in caseous stage. We recommend to perform excision biopsy of lymph node in feasible centers if CBNAAT or AFB smear is negative, irrespective of cytological features suggesting tuberculosis.

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None.

9. Conflict of Interest

None.

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