EFFICACY OF CBNAAT Vs. AFB IN CYTOLOGY DIAGNOSIS OF TB

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***Abstract*- Cytology, Ziehl Neelsen staining and Mantoux test are conventionally used to test and treat tuberculosis. Cartridge Based Nucleic Acid Amplification Test (CBNAAT) is being now recommended for diagnosis of all tuberculosis. Aims and Objectives: To asses efficacy of various diagnostic tools for diagnosis of tuberculosis. Material and Methods : It was a prospective study conducted by including all consecutive patients suggestive of tuberculosis. Fine needle aspirate was subjected for cytology and CBNAAT. Results were tabulated. Results: Two hundred patients were included along with patients having caseous aspiration. 103 (51.5%) had mycobacterium detected in CBNAAT. Remaining patients after histology had following diagnosis. Conclusion: CBNAAT is an effective tool for diagnosing tuberculosis even in early stages of pathology. Relying solely on cytology to diagnose tuberculosis should be discouraged.**

***Index Terms*-Tuberculosis, CBNAAT, Cytology.**

INTRODUCTION

Tuberculosis (TB) is an infectious disease that most often affects the lungs and is caused by a type of bacteria. It spreads through the air when infected people cough, sneeze or spit. Tuberculosis is preventable and curable. About a quarter of the global population is estimated to have been infected with TB bacteria.

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.[1] Revised National Tuberculosis Control Programme(RNTCP) stressed and moved on to microbiological diagnosis which is a more certain diagnostic tool. [2] Only available rapid diagnostic microbiological tool was Ziehl Neelsen (ZN) staining technique and is good for sputum from Pulmonary tuberculosis. [3] Whereas paucibacillary EPTB required culture system which require 7 to 42 days depending on culture system used. [4,5] With the shift from ZN stain to CBNAAT for microbiological diagnosis, there is also added advantage of detecting Rifampicin resistance in addition to rapid testing time. [6] Also, ability of CBNAAT to detect as low bacterial load as 100-130 bacilli per mlof sample compared to 10^4 per ml for ZN staining makesit ideal for paucibacillary tuberculosis. [7] 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. [8,9] By 2019, 1180 laboratories across India hadthis rapid diagnostic tool [10]. Currently CBNAAT is the firstmode 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 Fineneedle Aspiration (FNA) specimens[1].

This study is done to compare CBNAAT Vs. Cytology test in the diagnosis of Tuberculosis.

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.

Materials and Methods

It was a prospective type of observational study conducted at Government Medical College at Chandrapur, Maharastra over a period of 18 months from April 2021 to November 2022, after obtaining institutional ethical committee approval. Patients who came to department of Pathology for diagnosis of Tuberculosis with clinical features of tuberculosis or patients referred from other departments for initiation of Antitubercular Treatment (ATT), if aspiration of pus/cheesy material from lymph node were included in our study. Those patientswith Positive serology for HIV, Fluctuant node, on ATT andexcluded 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 for 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 emptycontents 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 nodewas performed and histopathology was sought irrespective of cytology result. Diagnosis of Tuberculosis is established with positive CBNAAT/ AFB Results were tabulated, analysed using SPSS Version 26 and expressed in mean, median and percentages

RESULTS

A total of 282 patients underwent FNA and 22.83% were excluded from study due to aspiration of pus/cheesy material. Remaining 200 patients were included in for study. Demographic details examination findings of included population are depicted in table one. Out of 200 patients 103(51.6%) had got MTB detected in CBNAAT and remaining 48.33% were subjected for excision biopsy. Demonstration of AFB by ZN stain was done in 15% cases and all of them were positive in CBNAAT. Two among 31 MTB detected samples in CBNAAT had Rifampicin resistance. Final diagnosis madeafter histopathology of excision biopsy is shown in table two. Results of Cytological features of FNA in relationto final diagnosis are depicted in table three. CBNAAT could not detect 20.51% (8/39) cases of Tuberculosis whichwere diagnosed by histopathology. CBNAAT detected Tuberculosis in (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.

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

Mean ageGender	28.42 years
Male	68.33%
Female	31.67%
Mean total duration of illness	99.8 days
Symptoms of tuberculosis	
Present	75%
Absent	25%
Past history of Tuberculosis	
Present	26.67%
Absent	73.33%
Lymph node characteristics Number	
Solitary	31.67%
Multiple	60%
Generalised	8.33%
Tenderness	
Present	15%
Absent	45%
Mattedness	
Present	55%
Absent	45%

Table 2: Performance of various diagnostic techniques against composite diagnostic methods for diagnosis of TB

	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
Histopathology	65(59-72.3)	99.8 (92-99.8)	69(56-69)	53(67-69)

Figure 1: Epithelioid cell cluster, Cytology

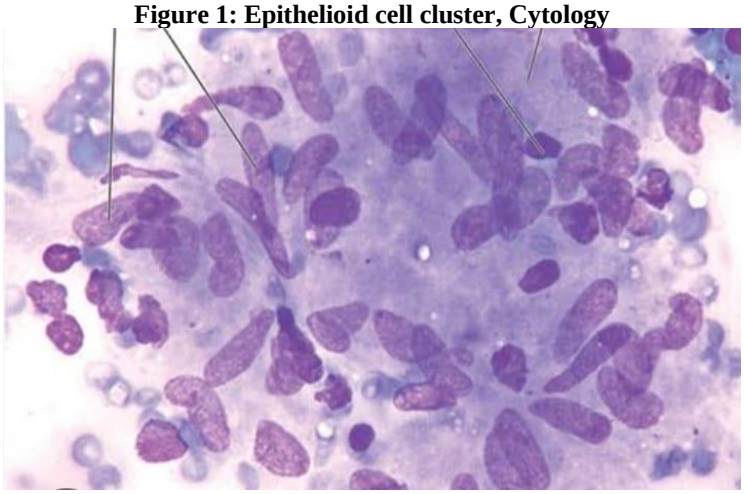
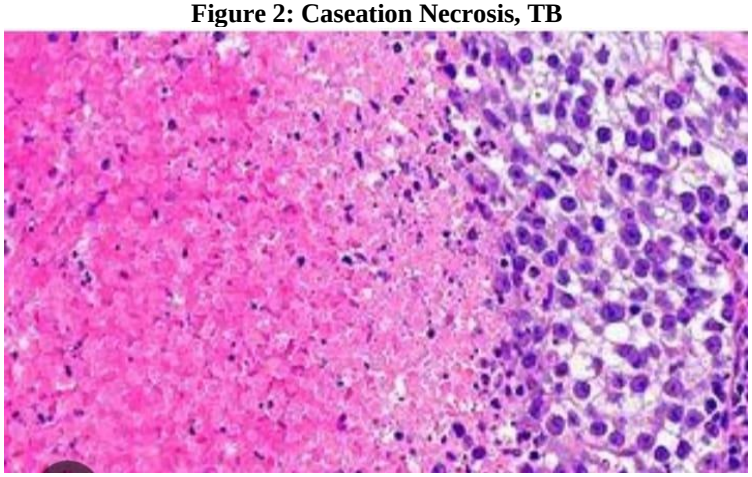


Figure 2: Caseation Necrosis, TB



DISCUSSION

Our study population included majority of male gender andwith 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 periadenitis 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 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 specificityin our study. We noted 53.84% (21/39) of tubercular patients had granulomatous inflammation with or without necrosis. Two patients with granuloma[11-15] were turnedout to be lymphoma in histology.

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. This emphasizes the need for early histologyin 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 purulentmaterial. In a study by Hemalatha et al. AFB positivity rateamong different cytological features were noted as follows;granulomatous reaction- 21%, necrotising granulomatous- 55% and necrosis only-73.5%. [19] Since we excludedpurulent or caseous samples from our study, naturally AFBsmear 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 whichcan 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.[16-20] Our study could be having lesser yield due tothe 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] Ourstudy had CBNAAT positive rate of 79.89% (31/39) despiteinclusion of only non-caseous or non-purulent material for CBNAAT. This relative higher yield is may be dueto microscopic necrosis which grossly doesn't look likepus 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.

Conclusion

CBNAAT is an effective tool for diagnosing tuberculosis. 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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Conflict of Interest

None.

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