Original Article

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Cytological Study of Tuberculosis in Pulmonary and Extrapulmonary Lesions in Correlation with Cartridge Based Nucleic Acid Amplification Test (CBNAAT)

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ABSTRACT

Introduction: Various methods can be used for the detection of tuberculosis of which microscopy,culture and identification of the organism's DNA are known. Newly introduced Nucleic acid amplification test (NAAT) if used in combination with other tests provide improved diagnostic yield.

Aims and Objectives: Todiagnose tuberculosis bacilli in various cytological samples and sputum samples. To evaluate the diagnostic efficacy of combined approach of conventional smear study and and CBNAAT.

Material and Methods: A Total of 50 cytology samples received in Pathology Department were studied over a period of 6 months.These included sputum(20), lymph node(22),body fluids(8).For these samples routine staining and ziehlneelsen are done and the remaining sample is sent for CBNAAT.Resultsare compared among AFB,CBNAAT for these samples. Sensitivity and specificity of microscopy and CBNAAT tests were calculated.

Results: Out of 50 cases 31 cases were positive for smear study and 29 cases were positive for CBNAAT.

Conclusion: Cytological examination of TB in association with CBNAAT is more confirmatory,sensitive and specific tool for the early diagnosis of MTB in variety of clinical samples.

Keywords: *Tuberculosis,Polymerase Chain Reaction Amplification,Pleural Fluid,Smear Microscopy.*

Introduction

Tuberculosis (TB) is caused by organisms of the Mycobacterium tuberculosis complex, which includes M. tuberculosis, the most common and important agent of human mycobacterial disease[1].It is an important health problem in low and middle income countries and remains a key challenge to public health[2]. Different types of body fluids and tissues can be used for the detection of Mycobacterium tuberculosis (MTB) from patients who are clinically suspected for tuberculosis.

Various methods can be used for the detection of tuberculosis of which microscopy, culture and identification of the organism's DNA are known. Newly introduced Nucleic acid amplification test (NAAT) if used in combination with other tests provide improved diagnostic yield. For Cartridge Based Nucleic Acid Amplification Test (CBNAAT) sensitivity in most of the studies 80% and specificity is 98-99%. For microscopy sensitivity in most of the studies 46-78% and specificity is100%[3]. Hence present study will be undertaken.

TB is classified as pulmonary, extrapulmonary, or both. Pleural involvement is common and results from a hypersensitivity response to mycobacterial antigens or contiguous spread of parenchymal inflammation[1]. Cytological specimens contain aggregates of epithelioid histiocytes, lymphocytes, and Langhans giant cells[4]. A definitive diagnosis of tuberculosis rests on identifying the organisms with the help of a special stain, Ziehl-Neelsen (ZN) or by microbiologic culture[4] or identification of the organism's Deoxyribo Nucleic Acid (DNA) in clinical samples[1].

Infection by M. tuberculosis commonly results in granulomatous inflammation. In countries where M. tuberculosis is prevalent, the yield of acid-fast bacteria among all clinically suspicious lung masses can be quite high[5,6].Organisms may be found in multinucleate giant cells or at the periphery of necrosis. Lymph nodal involvement may be present[7].

Fine Needle Aspiration Cytology (FNAC) is highly cost effective and accurate as a first line investigative technique with differential diagnoses including inflammatory conditions, granulomatous disorders and malignancy, stratifying cases requiring further investigations, surgical intervention or clinical follow up.

Most forms of extra pulmonary tuberculosis (EPTB) have remained diagnostic challenge due to paucibacillary

nature of cytological specimens. Multidrug resistance has emerged in this organism and, consequently, the pathologic spectrum of tubercular infection is expanding. NAAT remain the only self-contained cartridge based fully automated DNA testing platform that can accurately detect both TB and resistance to rifampicin in less than 2 hours[8].

Aims and objectives

- To diagnose tuberculous bacilli in various cytological samples and sputum samples.
- To evaluate the diagnostic efficacy of combined approach of conventional smear study and and CBNAAT.

Materials and methods

A Total of 50 cytology samples received in Pathology Department were studied over a period of 6 months. These included sputum(20),lymph node(22),body fluids(8).For these samples routine staining and ziehl neelsen are done and the remaning sample is sent for CBNAAT. Sputum samples received at our department are tested for Acid Fast Bacilli (AFB) For body fluids cell count, cell type, cell blocks for Haematoxylin and Eosin (H&E) and ZN are done and the results are correlated with CBNAAT.

For cartridge based nucleic acid amplification test (CBNAAT), testing will be done at Revised National Tuberculosis Control Programme (RNTCP) unit, Siddhartha Medical College, Vijayawada. CBNAAT uses hemi-nested real-time Polymerase Chain Reaction (RT-PCR) assay to amplify a specific sequence of the rpo B gene, which is then probed with molecular beacons for mutations within the rifampicin-resistance determining region, providing a result within two hours[7]. For cartridge based nucleic acid amplification test (CBNAAT), testing will be done at RNTCP unit, Siddhartha Medical College, Vijayawada.

Results are compared among AFB, CBNAAT for these samples. Sensitivity and specificity of microscopy and CBNAAT were calculated.

Results

Out of 50 cases 31 cases were positive for smear study and 29 cases were positive for CBNAAT**.**

In which FNAC of lymph node are 22,body fluids are 8,sputum samples are 20.Out of 22 FNAC samples 14 are reported as granulomatous inflammation and 4 cases are positive for AFB,10 cases were positive for CBNAAT.

Out of 20 sputum samples, 17cases were positive for AFB and all these cases were positive for CBNAAT. Out of 8 body fluid samples all of them were reported as inflammatory effusions ,2cases were positive for CBNAAT.

Table 1: Distribution and results of various samples in the present study.

	H&E STAIN	AFB POSITIVE	CBNAAT POSITIVE	RIFAMPICIN RESISTANCE
FNAC OF LYMPH NODE TOTAL-22	GRANULOMAS SEEN IN-14CASES	4 CASES	10 CASES	_
BODY FLUIDS TOTAL-8	INFLAMMATORY EFFUSION	_	2 CASES	_
SPUTUM TOTAL-20	_	17 CASES	17 CASES	1 CASE

Table 2: Comparison of sensitivity and specificity of present study with most of the studies

	SENSITIVITY	SPECIFICITY
FOR MICROSCOPY IN MOST OF STUDIES	46-78%	100%
IN OUR STUDY	63%	100%
FOR CBNAAT IN MOST OF STUDIES	80%	98-99%
IN OUR STUDY	87.5%	94.4%

Table 3: Using culture as the reference standard, the pooled sensitivity of Xpert MTB/RIF for various samples.

Sample	Sensitivity
Lymph node tissues	84.9%
Pleural fluid	43.7%
In CSF	79.5%
Other tissue specimens	81.2%
Gastric fluid	83.8%

Table 4: Recommendations to be followed in various test results.

CBNAAT	OTHER TESTS	RECOMMENDATION
POSITIVE	AFB NEGATIVE	START ATT WHILE AWAITING CULTURE[17,18]
NEGATIVE	AFB NEGATIVE	CULTURES ARE MOST USEFUL[19,20,21]
POSITIVE	CULTURE NEGATIVE	CORRELATED WITH CLINICAL AND TREATMENT HISTORY
NEGATIVE	TB LIKE SYMPTOMS	NON TUBERCULOUS MYCOBACTERIUM

Table 5: Comparision with other studies.

	Present study(50)	Malakar et al(60) [21]	Sing et al(57) [2]	Narang et al(60) [22]
CBNAAT POSITIVE	29(58%)	54(90%)	44(77%)	51(85%)
CBNAAT NEGATIVE	21(42%)	6(10%)	13(23%)	9(15%)
RIFAMPICIN RESISTANCE	1	0	0	0

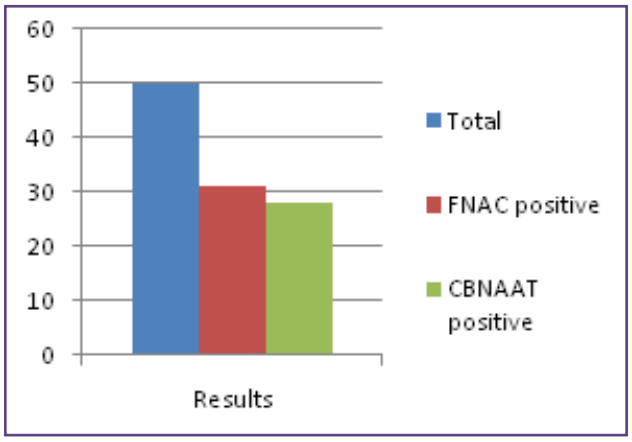

Fig. 1: Results for FNAC and CBNAAT samples-Out of 50 samples 31 are positive for FNAC and 29 are positive for CBNAAT.

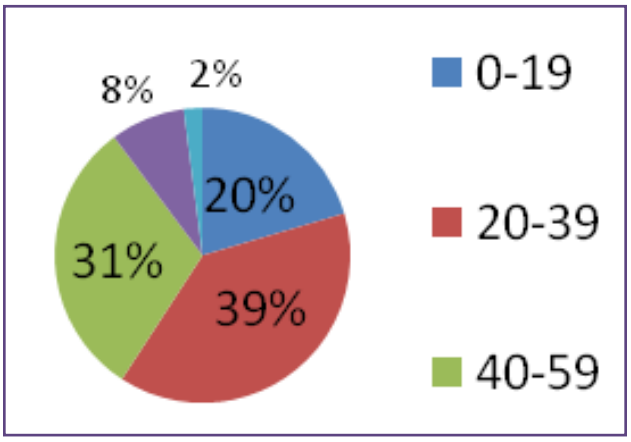

Fig. 2: Age distribution. 20-39 age group is predominantage group.

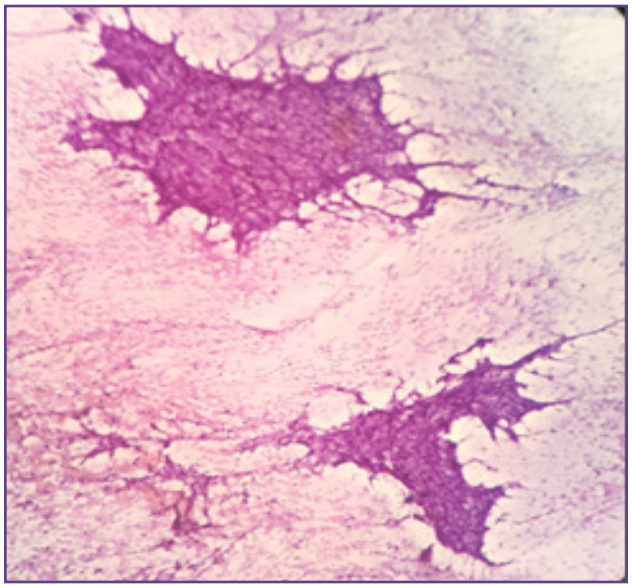

Fig. 3: H&E 4X showing epithelioid clusters.

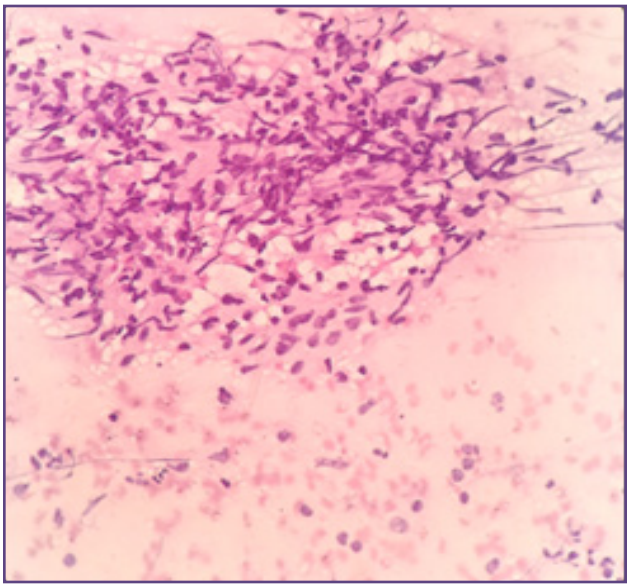

Fig 4: H&E 40X showing epithelioid clusters.

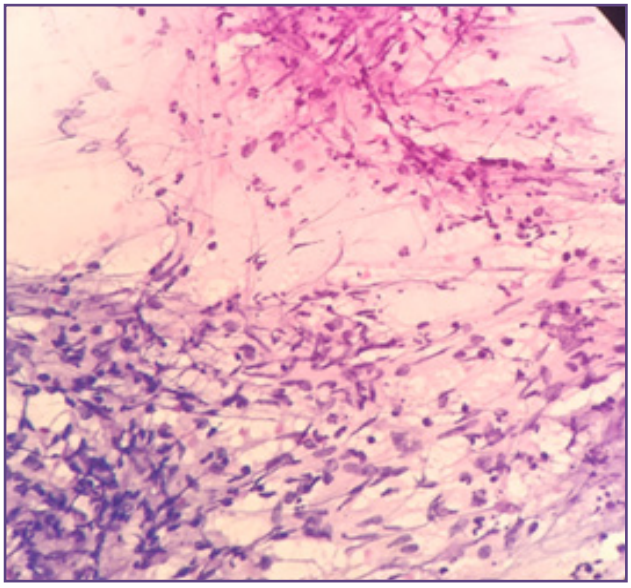

Fig. 5: H&E 40X showing epithelioid cell clustrers.

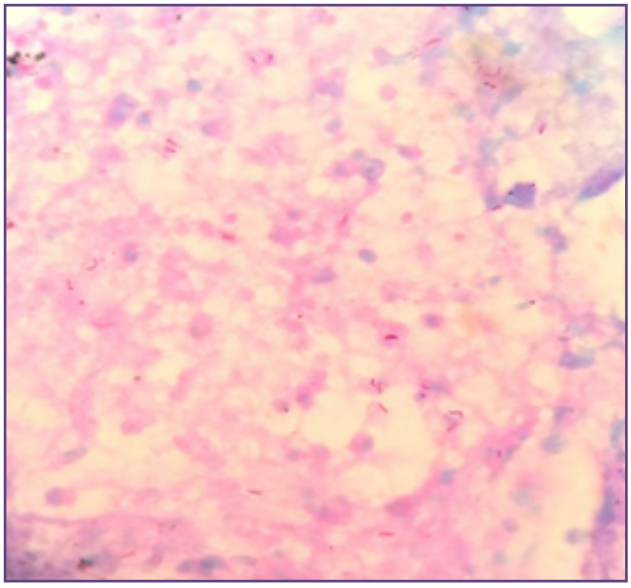

Fig. 6: AFB 100X showingacid-fastbacilli.

Discussion

The early and accurate identification of MTB facilitates control, prevention and treatment of this chronic disease. Several techniques are available for the diagnosis of TB such as smear examination, culturing, Enzyme linked immunosorbant Assay (ELISA) based and PCR-based detection methods. Among these culturing of the *M. tuberculosis* remains the gold standard method for diagnosing TB however it is time consuming and takes 5-6 week.

Mycobacteria have a unique waxy cell wall composed of unusual glycolipids and lipids including mycolic acid, which makes them acid-fast. Lymphadenitis is the most frequent presentation of extrapulmonary tuberculosis, usually occurring in the cervical region ("scrofula"), followed by mediastinal, axillary, mesenteric, hepatic portal and inguinal lymph nodes[9]. In tissue sections, the organisms are usually found extracellularly within the debris of the necrotic granulomas rather than in the surrounding viable cellular rim[10].

CBNAAT testing is recommended to perform the initial diagnosis of patients that are suspected to have TB, even in smear-negative patients. WHO recommends GeneXpert/ CBNAAT to be used as the initial diagnostic test in patients suspected extra-pulmonary tuberculosis (EPTB)[11].EPTB constitutes about 15–20% of TB cases and can constitute up to 50 of TB cases in HIV-infected individuals[12].

Compared with pulmonary disease, investigation for use in EPTB is far more complex because of the diversity of clinical sample types, difficulties in obtaining adequate tissue for analyses and in extraction of MTB DNA from samples, the challenge of providing a rigorous gold standard for comparison, and the range of potential ways of processing samples prior to analysis[13].GeneXpert assay has the potential to significantly improve and escalate the diagnosis of smear-negative pleural fluid specimens. A single negative NAA test result should not be used as a definitive result to exclude TB, especially when clinical suspicion of TB is moderate to high. Rather, a negative NAA test result should be used as additional information in making clinical decisions, to expedite testing for an alternative diagnosis, or to prevent unnecessary TB treatment.

False negative results

- Sensitivity of the PCR will be dropped in those improper taken specimens and sent samples as well as those inappropriate extracted specimens[14].
- Occasionally PCR inhibitors and other endogenous or exogenous host proteins (blood and even eukaryotic DNA in extrapulmonary specimens) are known to interfere with the sensitivity of PCR result in negative Xpert test result.
- A multi-step process is often required to eliminate PCR inhibitors and to obtain highly purified DNA.
- If the clinical picture is highly suggestive of TB,a second Xpert test should be ordered to increase sensitivity.
- The possible cause for CBNAAT negativity is solid nature of the cheesy material which usually have

very low bacillary load in nature compared to liquid caseous material which have high bacillary load[15].

False positive results

- These may occur due to contamination.
- False-positive results are likely to be linked to the detection by Xpert MTB/RIF of dead M. tuberculosis bacilli that would not be detected by culture, which is the reference standard.

Microscopy is the easiest and quickest diagnostic test but has limited sensitivity and cannot identify bacterial species. Requires 10,000 cfu /ml.

CBNAAT: Requires 131 cfu/ml of given sample[16]

Conclusion

Cytological examination of various samples is of paramount importance not only diagnosing cancer but also reveal various inflammatory conditions, infections with bacteria etc.

Hence the present study has been undertaken to assess cytological diagnosis by combined approach of conventional microscopy, AFB staining and more advanced CBNAAT procedure which can detect rifampicin resistance also.

By using this combined approach, we can detect more number of tuberculosis cases, hence it has national importance. Cytological examination of TB in association with CBNAAT is more confirmatory, sensitive and specific tool for the early diagnosis of MTB in variety of clinical samples.

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LIST OF ABBREVIATIONS

WHO	World Health Organisation
FNAC	Fine Needle Aspiration Cytology
CBNAAT	Cartridge Based Nucleic Acid Amplification Test
TB	Tuberculosis
AFB	Acid Fast Bacilli
RR	Rifampicin Resistance
MTB	Mycobacterium tuberculosis
RIF	Rifampicin
rpoB	Gene encoding for the ß-subunit of the DNA-dependent RNA polymerase of Mycobacterium tuberculosis.
Xpert MTB/RIF	Cartridge based nucleic acid amplification test for diagnosis of Mycobacterium tuberculosis and rifampicin resistance.
NAAT	Nucleic Acid Amplification Test
ZN	Ziehl-Neelsen
H&E	Haematoxylin and Eosin
ELISA	Enzyme linked immunosorbant Assay
PCR	Polymerase Chain Reaction
DNA	Deoxyribo Nucleic Acid
RNTCP	Revised National Tuberculosis Control Programme

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