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DIAGNOSTIC IMPORTANCE OF CBNAAT IN SMEAR-NEGATIVE PULMONARY & EXTRA-PULMONARY TUBERCULOSIS

Pulmonary Medicine

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ABSTRACT

Introduction: Despite of decreasing trend in TB, an alarming increase in childhood TB, MDR-TB,XRD-TB, and HIV-TB co-infection are the newer challenges, as they are difficult to diagnose and detect. CBNAAT has become a boon in early diagnosis, especially in difficult-to-diagnose cases, it simultaneously detects rifampicin resistance. This study aims for molecular confirmation of smear-negative pulmonary and extra-pulmonary TB by CBNAAT and simultaneous detection of rifampicin resistance in a very short time (2hrs). **Material & Method:** 115 patients >18 years, with persistent fever, cough>2 weeks, unexplained weight loss, hemoptysis, abnormal CXR s/o TB despite the antibiotic course with 2 sputum smear-negative and clinically diagnosed cases of extra-pulmonary TB were recruited, appropriate samples taken and were subjected to microscopy and CBNAAT. **Result:** In Smear-negative pulmonary and extra-pulmonary cases, 65.9% & 57.3% were diagnosed with help of CBNAAT, and rifampicin resistance was detected in 8.5% & 4.4% of cases respectively. In patients with past H/O TB, 50% of cases had rifampicin resistance. **Discussion:** For effective treatment of TB and prevention of the emergence of resistance, early diagnosis, bacteriological confirmation & drug sensitivity testing is of utmost importance. Smear has low sensitivity and cultures take more time, thus CBNAAT can provide an effective and quick technique to serve the purpose.

KEYWORDS

CBNAAT, smear-negative pulmonary & extra-pulmonary TB, rifampicin resistance.

Tuberculosis is a chronic infectious granulomatous disease caused by Mycobacterium tuberculosis**.** It primarily affects lung but can involve extra-pulmonary sites also like lymph-nodes, skin, mucosa, serosa and other visceral organs. Affection of extra-pulmonary sites can be secondary to spread from primary pulmonary foci or may occur de-novo due to direct implantation of mycobacterium tuberculosis. Tuberculosis is a great menace to mankind since ancient times. Approximately 10.6 million people were affected with TB in 2021, an increase of 4.5% from 10.1 million in 2020, the 30 countries with high TB burden accounted for 86% globally detected new TB cases. Out of 30 high burden countries, eight countries account for two thirds of the total, with India leading the count. The World Health Organization (WHO) considers TB as one of "the top 10 causes of death worldwide and the leading cause of death from a single infectious agent. Tuberculosis related deaths are rising in the world in an alarming trend. No wonder that this chronic infection is setting the major brakes in human resources and economy of a nation particularly the developing ones. The main reasons for this high mortality are because of lack of proper diagnosis in the apt time. This is particularly important in patients with HIV and TB co infection and extra pulmonary TB in special because the detection rates are low. There is an urgent need to implement newer diagnostic modalities for detection of TB. Till now, microscopy has an important role in the diagnosis of tuberculosis as it is simple, economical and less time consuming. Although culture of M. tuberculosis using solid or liquid medium is the gold standard in the diagnosis of TB, it requires specialized laboratories and highly skilled staff. Solid culture takes a longer duration (6-8 weeks).Though, liquid cultures detects M.TB earlier (10-14 days), it is easily prone for contamination. Drug susceptibility testing using these phenotypic (culture) methods can be done only after obtaining primary growth in the media. This further increases the time taken for detecting resistance (3-4 months) among anti-tuberculous drugs. This affects the patient treatment response and spread of resistant tuberculosis among the population. CBNAAT (Cartridge based nucleic acid amplification tests) has now become a boon in the early and prompt diagnosis of tuberculosis especially in extra-pulmonary TB cases. The CBNAAT besides providing faster results also detects resistance to rifampicin simultaneously. It is now being used as a first line of investigation in HIV –TB co-infection and in extra-pulmonary tuberculosis.

AIM:

Molecular confirmation of smear negative pulmonary and extra pulmonary tuberculosis cases by CBNAAT and determination of Rifampicin Resistance in very short time (2hours).

OBJECTIVES:

1. To assess the clinical profile of the patient of smear negative pulmonary and Extra-pulmonary tuberculosis .
2. To determine the percentage of molecular confirmation of the smear negative pulmonary TB cases by CBNAAT.
3. To determine the percentage of molecular confirmation of the smear negative Extra pulmonary TB cases by CBNAAT.
4. To determine the percentage of Rifampicin Resistance among smear negative Pulmonary and Extra-pulmonary cases.

MATERIALS AND METHODS

Place Of Study

Department of Respiratory Medicine, CAREER MEDICAL COLLEGE AND HOSPITAL.

Study Design

Prospective, observational study

Sample Size : 115

Study Period: May 2020 to September 2021.

Inclusion Criteria:

Adult patient age >18 years

1. With symptoms of persistent fever or Cough for >2 weeks , unexplained weight loss,hemoptysis and abnormal chest X- ray suggestive of TB despite antibiotic course with 2 sputum smear negative.
2. Clinically diagnosed Extra pulmonary TB involving lymph node,spine,pleura.

Exclusion Criteria:

1. Patients with sputum positive pulmonary TB.
2. Uncontrolled asthma, COPD, active hemoptysis, recent eye surgery and other conditions where fibro optic bronchoscopy or induction of sputum is contraindicated.
3. Lack of informed consent.

Study Technique:

Patients having inclusion criteria were enrolled in the study, informed consent was taken, and history was noted. Clinical examination was done with emphasis on Pulmonary and extra pulmonary manifestations of TB.

- Sputum smear examinations were done as per the RNTCP algorithm.
- Induced sputum was done by 3% hypertonic saline.
- Gastric lavage was collected in the morning after an overnight fast.
- Fiber optic bronchoscopy was done in indicated cases with study of BAL fluid , bronchial brush , biopsy and post bronchoscopy sputum.
- Culture of sputum/BAL fluid/gastric lavage/lymph node aspirate/pus was done for *Mycobacterium tuberculosis* from an RNTCP accredited laboratory.
- GeneXpert test of sputum/gastric lavage/BAL fluid/post bronchoscopy sputum/lymph node aspirate/pus of cold abscess/pus of empyema/pleural fluid were done.

Other relevant investigations were:

1) Peripheral lymph node FNAC/biopsy.
2) USG study of abdomen.
3) Pleural fluid analysis, Pleural biopsy.
4) MRI Brain, CSF study.
5) CTFNAC from extra pulmonary sites like paravertebral abscess / cold abscess/ other skeletal sites involved.

RESULTS AND ANALYSIS

Table 1: Age wise distribution of patients

Sl. No.	Age	Pulmonary samples n=47	Extra pulmonary samples n=68
1	18-40	27(54%)	41(60.2%)
2	41-60	14(29%)	20(29.4%)
3	>61	6(12.7%)	7(10.2%)
Total		47	68

The men age of presumptive pulmonary TB was 37+/- SD 15.19 years. About 54% belong to age group of 18-40years, 29% belong to 41-60 years and 6% in geriatric age group(>60 years).

The mean age of presumptive extra-pulmonary TB was 33+/- SD 12.36 years. About 60.2% belong to age group of 18-40 years, 29.4% belong to age group 41-60 years and10.2% belong to Geriatric age group.

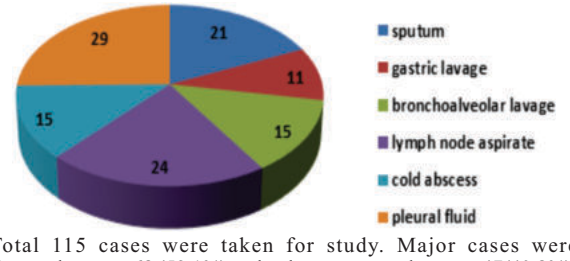
Table 2: Gender wise distribution of patients

Gender	Pulmonary(n=47)	Extra pulmonary (n=68)
Male	26(55.3%)	37(54.0%)
Female	21(44.6%)	31(45.5%)
Total	47	68

In smear negative presumptive pulmonary TB 55.3%(26/47) were male and 44.65 %(21/47) were female. In smear negative presumptive Extra pulmonary TB 54%(37/68) were male and 45.5%(31/68) were female.

Table 3: Types of samples

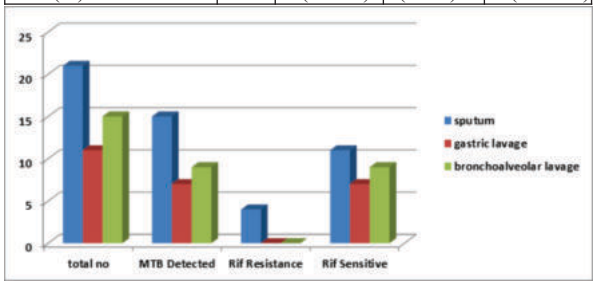
Sl. No.	Samples	Total n=115
1	Sputum	21(18.2%)
2	Gastric lavage	11(9.5%)
3	Bronchoalveolar lavage	15(13%)
4	Lymph node aspirate	24(20.8%)
5	Cold abscess	15(13.0%)
6	Pleural fluid	29(25.2%)



Total 115 cases were taken for study. Major cases were Extrapulmonary 68 (59.1%) and pulmonary samples were 47(40.8%). Among the samples 21(18.2%) were sputum, 11(9.5%) were Gastric lavage,15(13%) were BAL fluid, 24(20.8%)Lymph node aspirate,15(13%) cold abscess and 29(25.2%0 were pleural fluid.

Table 4: Pulmonary samples MTB detected by CBNAAT

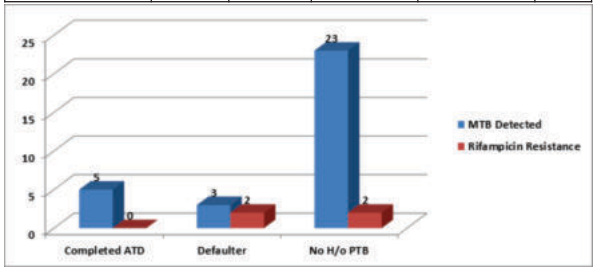
Types of sample	Number	MTB Detected by CBNAAT	Rifampicin Resistant	Rifampicin Sensitive
Sputum	21	15	4	11
Gastric lavage	11	7	0	7
Bronchoalveolar lavage	15	9	0	9
Total(%)	47	31(65.9%)	4(8.5%)	27(57.44%)



Total 47 cases of 2sample AFB smear negative presumptive pulmonary TB were taken. Bacteriological confirmation by CBNAAT was in 65.9% cases (31 out of 47). Rifampicin resistance was detected in 8.5% of cases (4 cases out of 47). CBNAAT is very important diagnostic tool for not only bacteriological confirmation but also Detection of Rifampicin Resistance in short time.

Table 8: Result of CBNAAT of pulmonary samples having AFB smear negative among patients with past history of PTB

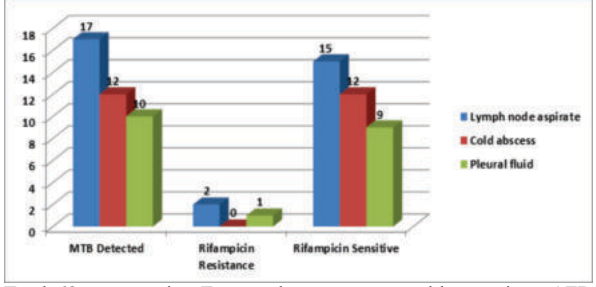
ATD History	Number	MTB Detected	Rifampicin Resistance	Rifampicin sensitive	P value
Completed ATD	9	5	0	5	P=.01
Defaulter	4	3	2(50%)	1	
No H/o PTB	34	23	2(5.88%)	21	
Total	47	31	4	27	



In this study Rifampicin Resistance was Detected in 50% cases(2out of 4) of PTB defaulter. Overall primary MDR was detected in 4.25% (2/47) of cases

Table 11: Extra pulmonary samples MTB Detected by CBNAAT

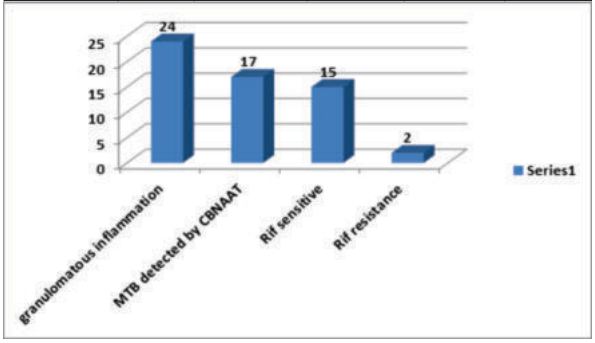
Types of sample	Number	MTB Detected by CBNAAT (n=39)	Rifampicin Resistant (n=3)	Rifampicin Sensitive (n=29)
Lymph node aspirate	24	17(70.8%)	2	15
Cold abscess	15	12(80%)	0	12
Pleural fluid	29	10(34.4%)	1	9
Total	68	39(57.35%)	3(4.4%)	36(52.9%)



Total 68 presumptive Extra pulmonary cases with negative AFB smear of sample were taken for study. Bacteriological confirmation By CBNAAT was in 57.3%(39 out of 68) cases and Rifampicin Resistance was Detected in 4.4%(3 out of 68) cases.MTB detection Rate is high in Lymph node aspirate(70.8%) and pus of cold abscess (80%) and low in Pleural fluid(34.4%).

Table 12: Result of Lymph node aspirate CBNAAT among tuberculous Lymphadenitis

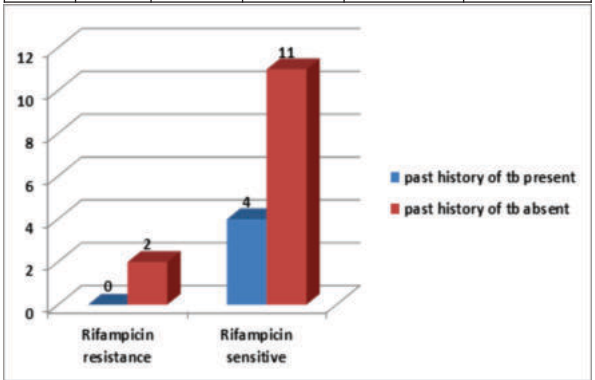
Category	Granulomatous inflammation	No Granulomatous inflammation	MTB detected by CBNAAT	Rifampicin Sensitive	Rifampicin Resistant
Lymph node FNAC	21	3	15	13	2
Lymph node Biopsy	3	0	2	2	0
Total(%)	24		17(70.8%)	15(62.5%)	2(8.33%)



Initially Total n=30 cases of presumptive tubercular lymphadenitis were taken for study. 6 were came out as other diagnosis(metastatic carcinoma, NHL) by FNAC or Biopsy, so excluded from study and total number of cases were 24 as granulomatous inflammation with caseous necrosis by FNAC or Biopsy. 17 out of 24 were MTB detected by CBNAAT ,15 were Rifampicin sensitive and 2 were Rifampicin resistant. In this study bacteriological confirmation by CBNAAT in lymph node is 70.8% and Rifampicin resistance is detected in 8.33% of population of lymph node TB in very short time for early initiation of treatment.

Table13: Result of Lymph node aspirate CBNAAT among patient with past history of tuberculosis

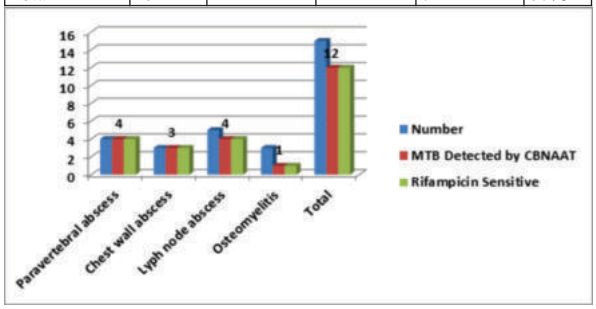
Past history of TB	Number	CBNAAT MTB detected	Rifampicin resistance	Rifampicin Sensitive	Percentage of Rifampicin Resistance
Present	7	4	0	4	0
Absent	17	13	2	11	11.7%
Total	24	17	2	15	



CBNAAT can detect Rifampicin Resistance in very short time. In this study 4 out of 7 patient of lymph node TB having past h/o TB were MTB detected by CBNAAT and all were Rifampicin Sensitive. 13 out of 17 without any h/o TB were MTB Detected by CBNAAT and 2 (11.7%) were Rifampicin Resistance.So primary MDR TB Lymphadenitis is also increasing among population and it can be detected by CBNAAT in very short time.

Table 14: Result of CBNAAT of pus of different site cold abscess

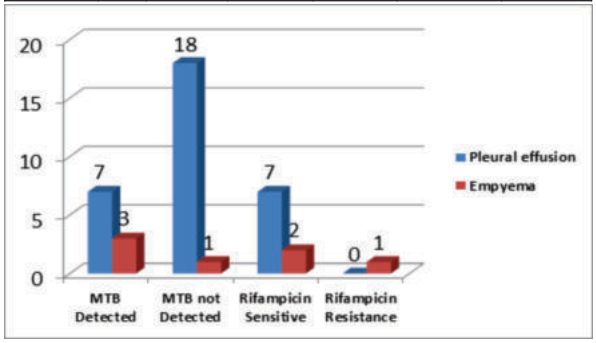
Type of samples	Number	MTB Detected by CBNAAT	Rifampicin Sensitive	Rifampicin Resistance	Percentage
Paravertebral abscess	4	4	4	0	
Chest wall abscess	3	3	3	0	
Lyph node abscess	5	4	4	0	
Osteomyelitis	3	1	1	0	
Total	15	12	12	0	80%



Out of 68 extra-pulmonary cases 15 were abscess at various site with radiological evidence of possible tubercular etiology in most of the cases. MTB were detected in all the sample of pus from paravertebral(n=4) and chest wall(n=3) abscess. 4 out of 5 from lymph node abscess pus and 1out of 3 osteomyelitis pus were MTB detected by CBNAAT. All the bacteriologically confirmed cases were Rifampicin sensitive.Where pus is the sample bacteriological confirmation by CBNAAT is high as in this study it is 80%.

Table 15: Result of CBNAAT of pleural effusion and empyema

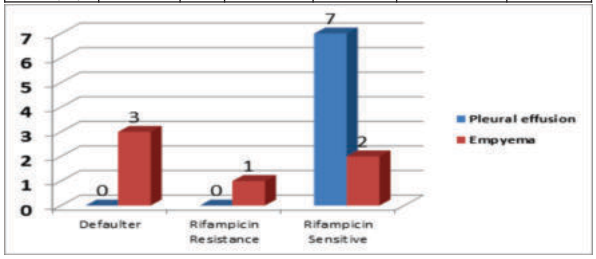
Category	Number	MTB Detected	MTB not Detected	Rifampicin Sensitive	Rifampicin Resistance	Percentage of MTB Detection
Pleural effusion	25	7	18	7	0	28%
Empyema	4	3	1	2	1	75%
Total	29	10	19	9	1	34.4%



25 out of 29 were tubercular pleural effusion with exudative,lymphocytic ADA >40 IU/L and respone to ATT were seen in all 25 cases. 7(28%) cases were MTB Detected by CBNAAT and all were Rifampicin sensitive. In 4 cases of tubercular empyema , bacteriological confirmation were in 3(80%) cases and 1 case was detected as Rifampicin resistance.In this study MTB detection rate is high in sample of pus(80%) and in case of exudative effusion with high ADA value is low (28%).

Table 16: Result of CBNAAT among patient with past h/o TB and Default in Empyema and pleural effusion

Category	Number	H/O TB	Defaulter	MTB Detected	Rifampicin Resistance	Rifampicin Sensitive
Pleural effusion	25	0	0	7	0	7
Empyema	4	4	3	3	1	2
Total(%)	29	4	3	10	1	9



3(75%)out 4 cases were defaulter. 1cases among Defaulter was detected as Rifampicin Resistance. In this study tubercular empyema was common among patient with Defaulter.

DISCUSSION

To achieve universal access to early accurate diagnosis of TB and enhancing case finding efficiency, identification of TB cases at the first point of care and linking them to the best available diagnostic test is of paramount importance. Early case detection is vital to interrupt the transmission of TB disease as highlighted in the 12th five year plan for TB control in India.

Early identification of people with a high probability of having active TB (presumptive TB) is the most important activity of the case finding strategy. Screening and diagnosing patient with appropriate tests and strategies will largely determine the response to appropriate treatment. There are certain limitations of sputum microscopy. According to Toman, *"...a specimen that is consistently found to be positive would have to contain at least 100 000 AFB per ml."* Sputum microscopy cannot differentiate M. tuberculosis from other mycobacteria. It fails to diagnose paucibacillary and extra pulmonary tuberculosis, the incidence of which is disproportionately higher in high HIV prevalent areas. The sensitivity of sputum microscopy ranges from 34 to 80%.

According to RNTCP diagnostic algorithm for pulmonary TB, all presumptive TB (especially for PTB symptoms) will undergo sputum smear examination (ZN/LEDFM). Two specimens will be collected (spot-early morning or spot-spot). If first smear is negative, Chest X-ray is suggestive of TB or not available and clinical suspicion is high of TB, the 2nd sample will be subjected to smear and CBNAAT simultaneously.

In this present study we have taken patients with two sputum smear negative with radiology suggestive of PTB subjected for CBNAAT for bacteriological confirmation.

Extra pulmonary tuberculosis (EPTB) refers to any microbiologically confirmed or clinically diagnosed case of TB involving organs other than lungs such as lymph nodes, pleura, bones and joints, meninges of brain, intestine, genitourinary tract, etc. A high level of suspicion of EPTB is important in patient with signs and symptoms.

In this present study we have taken cases of extra pulmonary TB with clinical, radiological, biochemical, cytological or histopathological evidence of TB for bacteriological confirmation by CBNAAT.

All efforts should be made to establish microbiological confirmation and drug sensitivity in cases of smear negative PTB and EPTB. In this aspect molecular method especially cartridge based nucleic acid amplification test (CBNAAT) has opened up new horizon in diagnosis TB both pulmonary and extrap-ulmonary.

In this study using CBNAAT it has been observed that, out of 47 cases of smear negative PTB bacteriological confirmation was done in 65.9% (31/47) cases by CBNAAT of which 71% (15/21) was sputum and induced sputum, 63%(7/11) was gastric lavage and 60%(9/15) was BAL fluid. In a study by Ranjana R Khorgade and Pramod R Bhise at Amravati Medical college, Maharastra ,115 smear negative presumptive PTB, sputum/BAL tested for Gene-Xpert.MTB Detected in 26.95%(31 in 115) cases and Rifampicin Resistance in 6% (9 in 115) cases.

In a study by S.Subbarao et.al at S.V Medical college, Tirupathi CBNAAT study of 303 sputum smear negative presumptive PTB cases 74 were MTB Detected making diagnostic yield of 24.42%.

Sensitivity and specificity of CBNAAT- results of different studies

Name of the study	Sputum positive PTB		Sputum negative PTB	
	sensitivity	Specificity	Sensitivity	Specificity
Dewan R et al	100%	100%	32.3%	100%
Theron et al	94.7%	95%	46.8%	94.4%
Geleta et al	95.2%	96.3%	48.6%	96.3%
Agarwal M et al	100%	90%	79.1%	93.1%
Sharma SK et al	99.2%	99.6%	77.7%	99.6%
Sowjanya DS et al	99.8%	100%	37.5%	100%

Different studies showed sensitivity of CBNAAT in sputum smear negative samples ranges from 32.3% to 79.1% with specificity 93-100%.

Besides the bacteriological confirmation the unique advantage of CBNAAT is that it helps to detect Rifampicin Resistance by detecting rpo B gene mutation within 2 hours and Rifampicin Resistance is a surrogate marker of MDR-TB. In the present study Rifampicin Resistance was detected in 8.5% (4 in 47) of smear negative PTB among which 4.25% (2/47) was primary MDR .

In our study of 15 cases of sputum smear negative presumptive PTB, CBNAAT of BAL fluid showed diagnostic yield of 60%(9in 15). Culture was positive in 10 cases including those 9 cases MTB Detected by CBNAAT. Taking culture as 'Gold standard sensitivity is 90%, specificity is 100% ,PPV of 100% and NPV 83.3% and this is nearly similar with the previous studies.

In this present study it has been observed that Out of 68 cases of Extra pulmonary TB (EPTB) bacteriological confirmation was done by CBNAAT in 57.35%(39/68) cases of which 70.8%(17/24) was lymph node aspirate,80%(12/15) was pus of cold abscess and 34.45(10/29) was pleural fluid. Among EPTB, 4.4%(3/68) cases detected as Rifampicin Resistance of which 2.9%(2/68) was primary MDR. Lymph node Tuberculosis is commonest form of extra pulmonary Tuberculosis accounting 58% of all extrapulmonary TB.

In our study a total of 30 cases with presumptive lymph node TB were taken initially. In 6 cases FNAC/biopsy showed Non Hodgkin's Lymphoma or metastatic carcinoma, so excluded. 24 cases FNAC/biopsy showed granulomatous inflammation with caseous necrosis are taken for CBNAAT study. M.tuberculosis was detected in 70.8% (17/24) cases.

Diagnosis of pleural tuberculosis using routinely available diagnostic method is challenging due to the paucibacillary nature of the disease. Histopathology and pleural tissue TB culture requires expertise and appropriate equipment and time consuming. Xpert MTB/Rif test has been widely evaluated in sputum specimens but data on its performance in pleural TB is scarce.

In our study of 29 cases of Tubercular Pleural effusion 4 were empyema. In 25 cases were exudative & had ADA >40 U/L. MTB was detected in 28% (7/25) cases of pleural effusion and all were Rifampicin sensitive. In 4 cases of empyema MTB was detected in 75% (3/4) cases and 1 of them was Rifampicin resistant with past history of ATD Default. Pleural biopsy was done in 18 cases. Granulomatous inflammation with caseous necrosis found in 77.7% (14/18) cases. Of these 4 cases with ADA value>70U/L, all were histopathologically confirmed and 10 out of 14 with ADA >40-70 U/L were histopathologically confirmed.

There is not much data about CBNAAT of pus of cold abscess. In our study we have total 15 samples of pus from different site (4 paravertebral, 5chest wall, 3lymph node, 3osteomyelitis). In pus sample 80% (12/15) cases MTB detected by CBNAAT and all were Rifampicin Sensitive.

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