STUDY ON DIAGNOSTIC MODALITIES IN SMEAR NEGATIVE PULMONARY TUBERCULOSIS WITH SPECIAL REFERENCE TO SPUTUM INDUCTION (SI CBNAAT), BRONCHOSCOPY (BAL CBNAAT AND BAL CULTURE)

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ABSTRACT

BACKGROUND

Tuberculosis, an important preventable and treatable cause of death is a major health problem worldwide. However, in patients with a compatible clinical picture, sputum smear don't reveal acid-fast bacilli in all patients. So, alternative methods of obtaining sputum specimen are frequently needed in these patients. Rapid diagnosis of tuberculosis and detection of rifampicin (RIF) resistance are essential for effective disease management. CBNAAT (cartridge based nucleic acid amplification test) (DS Sowjanya, et al)[1] is a novel integrated diagnostic device for diagnosis of tuberculosis and rapid detection of RIF resistance in clinical specimen.

The aim of the study is to study different diagnostic modalities in sputum smear negative patients with special reference to CBNAAT.

MATERIALS AND METHODS

This was an observational study done in the Department of Pulmonary Medicine, SCBMCH, Cuttack. 100 patients were selected for the study. In all patients, induced sputum was sent for smear acid-fast bacilli. Those patients whose induced sputum was negative were undergone bronchoscopy for BAL (bronchoalveolar lavage). The specimen was sent for smear acid-fast bacilli, sputum CBNAAT and culture for Mycobacterium tuberculosis.

RESULTS

Total 100 patients, 74 males and 26 females. SI sputum smear positive 29 out of 100. SI CBNAAT positive 23 (32%), BAL CBNAAT yield 38/71 (54%), BAL culture reference standard yield 42/71(59%). Sensitivity, specificity, PPV, NPV, SI CBNAAT 61.9%, 96.5%, 96.3%, 63.6%, respectively. BAL CBNAAT sensitivity, specificity, PPV and NPV 88.09%, 96.5%, 97.36%, 84.84%, respectively. Kappa (Anthony J Viera, et al)[2] score 0.808 (substantial agreement).

CONCLUSION

Sputum induction procedure is simple, relatively safe, cost effective and is widely available. Therefore, in a patient with a suspected sputum smear, negative pulmonary tuberculosis sputum induction should be the initial procedure of choice reserving bronchoscopy for the non-responders.

KEYWORDS

Bronchoscopy, Rifampicin, Mycobacteria, Lignocaine.

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BACKGROUND

TB still constitutes a major health problem worldwide with an 8.8% (Poojan, et al)[3] incidence and 1.3% mortality rate (Alladi Mohan, et al).[4] However, almost 50% produce a negative smear microscopy. For several decades, smear microscopy and conventional culture techniques have been the mainstay of diagnostic testing. While smear microscopy has poor sensitivity and issues related to quality control, conventional cultures have the limitation of long

turnaround time of 6-8 weeks. Liquid culture have a turnaround time of 21 days.

GeneXpert/CBNAAT/MTB/RIF is an automated, real time PCR that detects MTB and also test for rifampicin sensitivity using molecular beacons. Results for both are available in 2 hours. It is a cartridge based test without biosafety risk. This method detects complex DNA in sputum samples whether spontaneous or induced. Various methods have been used in smear negative cases to obtain material for other modalities of investigation to diagnose PTB.

Sputum induction procedure first used by Hensler et al[5] has been put to use in cases with inadequate sample, paediatrics, immunocompromised. Induced sputum smear positivity can go up to 63% (Nageswar Rao)[6] as against L. Saglam, et al[7] (47%) and Hartung, et al[8] (42%), Swetha, et al[9] (32%). Induced sputum subjected to Xpert assay as an initial replacement for smear microscopy in a meta-analysis shows a pooled sensitivity of 89% and pooled specificity of 99% and as an add on test following negative smear microscopy, pooled sensitivity and specificity were 67% and 99% (Poojan Srestha, et al).[3]

The Xpert test was first endorsed by WHO in 2010 (Poojan, et al).[3]

In developed countries, FOB (fibreoptic bronchoscopy) is considered a good option for cases that pose a diagnostic challenge, although smear exhibits a low sensitivity on FOB samples, i.e. 5.35% on aspirates and 10-30% on BAL (Pierre, et al).[10]

Since December 2010, WHO has recommended the GeneXpert MTB/RIF assay for sputum samples, but no specific recommendation is there for FOB samples. FOB sampling with Xpert outperforms smear with a sensitivity around 80% (Pierrre, et al).[10] BAL Xpert when compared to culture as a reference standard has a sensitivity, specificity, PPV and NPV as 91.86%, 71.42%, 97.53% and 41.66% (Kanwal, et al).[11]

BAL culture for MTB can have a yield from 60-90%.

RNTCP is currently using Xpert/MTB/RIF to diagnose paediatric TB and pulmonary TB in the immunocompromised as well as to detect rifampicin resistance.

AIM

There is lack of studies comparing the validity of different modalities of investigation in smear negative PTB suspects especially with CBNAAT, a novel diagnostic tool for rapid and specific detection of mycobacterial in pulmonary samples.

Hence, the current study was conducted to study the validity (sensitivity, specificity, PPV, NPV) of SI CBNAAT and BAL CBNAAT with BAL culture taken as the reference standard.

MATERIALS AND METHODS

This is a hospital-based prospective observational analytical study conducted in 100 patients who were clinicoradiological suspects of pulmonary tuberculosis with initial spontaneous sputum smear negative were studied during the period from April 2016 to March 2017 in the Department of Pulmonary Medicine, SCB Medical College, Cuttack, Odisha.

The inclusion criteria was all sexes above 14 years of age who were clinicoradiological suspects of PTB and had their initial sputum smear negative and had never taken ATT. Patients with HIV were excluded from the study. Consecutive sampling was done to include 100 eligible study subjects.

Sputum induction was done in a well-ventilated room with an ultrasonic nebuliser and nebulisation done with 10-20 mL of 3% hypertonic saline until patient coughed up at least 2 mL of sputum or a maximum of 15 minutes.

Samples were collected in sterile specican and sterile falcon tubes.

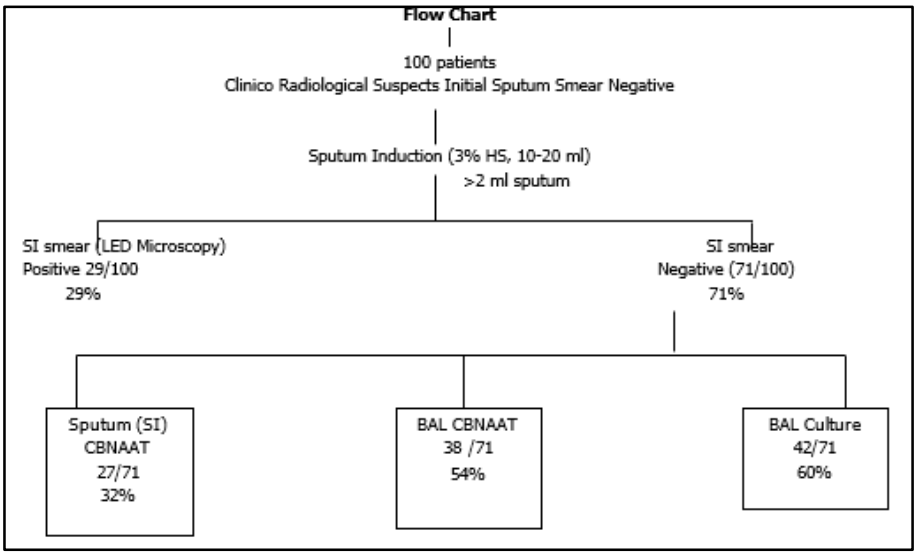
Sputum sample was subjected to LED microscopy and fluorescent staining. Procedure was repeated if necessary. Those found negative on smear supplied samples for CBNAAT in the adjacent state run IRL.

All the SI negative patients also underwent bronchoscopy. Bronchoscopy was done with Pentax FOB under local anaesthesia with 2% lignocaine and Xylocaine jelly and mouth spray. Bronchial washing and lavage was performed by instilling 20 mL aliquots of normal saline at room temperature upto 100-120 mL and collected into a sterile suction trap by aspiration.

Bronchial washing and BAL was subjected to culture and CBNAAT along with smear by concentration method in the IRL (Intermediate Reference Laboratory of the State (located adjacent to the department).

Statistical Analysis- The data were entered in Microsoft Excel 2007 and analysed using SPSS Version 21 (PAS W Statistics for Windows, Chikago: SPSS Inc.) the categorical values were expressed in terms of proportions.

Ethical Clearance - Certificate attached.



RESULTS

A total of 100 patients were recruited for the study including 74 males and 26 females. The mean age of the males was (43.35 ± years) and that of females was 33.07 ± years. The mean BMI recorded was 16.94 in males and 15.7 in females. Diabetes as a comorbidity was found in 12% (9) cases in males and 7% in females. Cavitary lesion was seen in 12 cases (16%) in males and 4 cases (15.4%) in females. Cough was the most common symptom noted in 74% cases. After induction of sputum with 3% HS SI sputum smear positive was seen in 29 cases out of 100 subjects. Out of 71 patients who were SI smear negative SI CBNAAT was positive in 23 (32%) patients. CBNAAT conducted on BAL fluid in the same 71 patients had an yield of 54% (38/71). BAL culture, which was accepted as the gold standard had an yield of 59% (42/71).

The sensitivity, specificity, PPV and NPV of CBNAAT conducted on SI fluid/specimen was found to be 61.9%, 96.5%, 96.3% and 63.6%, respectively. The sensitivity, specificity, PPV and NPV of CBNAAT on BAL fluid was 88.09%, 96.5%, 97.36%, 84.84%, respectively.

Item	Male (74 Nos.)	Female (26 Nos.)
Mean age in years	43.35 (± 16.7)	33.07 (±16.3)
Mean BMI	16.94 (± 2.8)	15.7 (± 2.08)
Mean weight in kg	49.35 (± 10.3)	59.4 (± 8.1)
Diabetes	9 (12%)	2 (7.6%)
Cavitary lesion	12 (16.2%)	4 (15.4%)
Table 1.		

(Characteristics of study subjects, N=100).

Item	Sensitivity	Specificity	PPV	NPV
Induction SI CBNAAT	61.9	96.5	96.3	63.6
Bronchoscopy BAL CBNAAT	88.09	96.5	97.36	84.84
Table 2				

FOB culture gold standard with reference to validity of two different modalities.

(Comparative validity of SI CBNAAT and BAL CBNAAT taking BAL culture as gold standard).

	BAL CBNAAT		
SI CBNAAT	**Item**	**Positive (+)**	**Negative (-)**
	Positive (+)	26	1
	Negative (-)	12	32
Table 3			

Agreement between SI CBNAAT vs. BAL CBNAAT as diagnostic modalities Kappa=0.808=80% substantial agreement.

DISCUSSION

Primary health centres successfully implanted sputum smear microscopy under RNTCP. Early diagnosis of PTB by smear microscopy is the approved method.

However, smear negative and culture positive state is observed in 22-61% (J. Balakrishna, et al).[12] Mycobacterial culture considered as a gold standard requires a good quality sputum and a turnaround time of 6-8 weeks.

Causes of SSNPTB (sputum smear negative PTB) include poor quality sputum and low bacterial load. Smear negativity is also seen in the immunosuppressed and paediatric population. Because mortality rate for smear negative and culture positive cases is around 14% and 50% need chemotherapy by 12 months, it is very important to diagnose SSN-PTB cases and to break the chain of transmission. The various methods used in SSNPTB cases include Sputum Induction (SI), gastric lavage, TTNA (transthoracic needle aspiration), bronchoscopy (bronchial aspirate, washing, BAL) and samples so collected to be subjected to smear, culture, HP study and molecular methods. Sputum induction has a smear positivity ranging from 32-64% in various studies as follows (Swetha et al,[9] (32%); Katayoun, et al[13] (40%); Hartung, et al[8] (42%); L. Saglam, et al[7] (47%) and Nageswar Rao, et al[6] (63.3%).

In our study, induced sputum smear positivity was 29%. The yield of smear positivity can be increased by multiple induced sputum samples.

GeneXpert/CBNAAT and AFB smear microscopy share almost same specificity, but sensitivity in GeneXpert/CBNAAT/MTB/RIF is much higher in respiratory samples (Monika Agrawal, et al).[14] Xpert study done by Surendra K. Sharma et al,[4] shows-

In smear positive cases, a Sen 95.7%, Sp 99.3%.

In smear negative cases, Sen 77.7%, Sp 99.3%.

WHO reported the sensitivity in detecting TB by CBNAAT/GeneXpert from 70-100% in culture positive and around 60% in those with smear negative and specificity ranging from 91-100%.

Exclusive studies on SI CBNAAT in smear negative PTB cases in adults are not very many.

In our study, we subjected SI samples in SSN-PTB cases to CBNAAT and in 71 cases obtained a Sen (61.9), Spec (96.5), PPV (96.3) and NPV (63.6) study by Monika Agarwal, et al[14]- Sn (86.8%), Sp (93.1%), PPV 78.5% and NPV 96% when compared to BAL culture.

Our study had an unique approach where BAL samples subjected to culture in IRL was the reference standard. When the same 71 cases (SSN-PTB) were subjected to bronchoscopy and BAL samples from the radiologically-guided segments were collected and subjected to CBNAAT the overall Sen, Spec, PPV, NPV were 88.09%, 96.5%, 97.36% and 84.84%, respectively.

Similar studies in the past have result as follows- (Monika Agrawal, et al).[14]

Pierrae, et al[10] - Sn 80, Sp 98.6, PPV 88.9, NPV 97.2.

HY Lee, et al[15] - Sn 81.6, Sp 100, PPV 100, NPV 97.1.

Kanwal, et al[11] - Sn 91.86, Sp 71.42, PPV 97.53, NPV 41.6.

SK Sharma, et al[4]- Sn 90, Sp 100, PPV 100, NPV 98.1.

Our result is comparable.

In our study, when a Kappa statistics (Anthony J Viera, et al)[2] is calculated between SI CBNAAT and BAL CBNAAT with BAL culture taken as the reference standard a score of0.80 (80%) was noted (Table 3), which indicates substantial agreement between the two procedures.

Hence, the two methods of diagnosis maybe used interchangeably as the situation favours.

CONCLUSION

So, it may be concluded that SI CBNAAT can be a powerful tool in diagnosis of SSN-PTB in tertiary and non-tertiary centres and BAL CBNAAT may be reserved to evaluate the non-responders.

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