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CBNAAT Co-Testing of Sputum and BAL Fluid with Sputum Microscopy: May it Halt the March of Tuberculosis !

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ABSTRACT

Introduction: Growing concern for Tuberculosis (TB) epidemic forces World Health Organization (WHO) and government of India (GOI) to incorporate newer rapid and highly specific diagnostic test like Cartridge Based Nucleic Acid Amplification Test (CBNAAT).

Aim: To find the usefulness of CBNAAT in increasing Acid Fast Bacilli (AFB) positive patient pool over and above the yield of traditional sputum microscopy.

Materials and Methods: The cross-sectional survey was conducted in the Department of Respiratory Medicine, Nilratan Sircar Medical College and Hospital (NRSMCH), Kolkata, India. The study involved 94 smear negative TB suspects referred from other health facilities as well as diagnosed by the department itself. After collecting baseline information like age, sex, previous history of TB and its treatment by interview and scrutinizing records using predesigned questionnaire, the patients were put on sputum CBNAAT and Broncho-Alveolar Lavage (BAL)-CBNAAT testing. Data were analysed by estimating mean, Standard Deviation (SD), proportion and using independent t-test, chi-square test.

Results: Overall, average age of participants was 44.7±15.3 (mean±SD) years. Male-female ratio was 1:2.8. Altogether 44.7% patients were detected sputum positive out of which 34.0% were detected only by sputum CBNAAT and another 10.7% detected when BAL-CBNAAT testing was used among the negatives yielded from sputum CBNAAT only. These differences were statistically significant.

Conclusion: Utility of CBNAAT over and above traditional diagnostic methods was reaffirmed. With added advantage of detecting MDR cases simple, sensitive, speedy and automated CBNAAT seems new mile stone in 'Stop TB' strategy and needs utilised to its highest potentiality through monitoring and supervision.

Keywords: Drug resistant tuberculosis, Lavage, Nucleic acid amplification, Sputum microscopy

INTRODUCTION

In May, 2012 India declared TB a notifiable disease [1]. TB can present with clinical features and some radiologic findings indistinguishable from those of Community-Acquired Pneumonia (CAP) [2]. Revised National Tuberculosis Control Programme (RNTCP) guidelines seemingly motivate physicians waiting 02 weeks before initiating diagnostic investigations for presumptive tuberculosis [3]. During this interval, clinicians usually prescribed courses of antibiotics for lower respiratory tract infection before pulmonary TB is correctly diagnosed [4]. It fosters development of antimicrobial resistance apart from Adverse Drug Reactions (ADRs), financial burden and emotional turbulence to patients and spreading of TB in the mean time. Initial sputum negatives were further put on antibiotic treatment for 10-14 days and provided with repeat sputum smear for AFB if symptoms persist. Finally, with radiological findings the patient is categorised as sputum negative TB [3]. Thus, the patients have got the scope for spreading disease for almost 3 weeks or more whatever may be the intensity of transmission.

Being simple, rapid yielding, Sputum Microscopy (SM) has been the main diagnostic tool for nearly a century, followed by sputum culture, the 'gold standard'. However, both tools have limitations like sensitivity (as low as 50%) of SM and 2-6 weeks duration to obtain results of culture [5]. Though cheap (costs USD 0.50) and highly specific, the low sensitivity of SM is further reduced in patients with extra-pulmonary TB, children and HIV/TB co-infected patients [6]. The smear negative TB also spreads the disease and only tool for diagnosing has been chest X-ray with low specificity [7].

Accurate and prompt diagnosis of all cases is required for control of TB and can only be achieved through affordable newer diagnostic tools. It may help reduce the direct costs of diagnostic burden on patients and their families and also help national TB control programs to start early treatment. For this purpose SM shouldn't be relied upon as a primary diagnostic tool (being so in resource limited settings) because of its high yield of false negatives [6]. The CBNAAT is one of these newer methods that simultaneously identifies Mycobacterium tuberculosis and detects rifampicin resistance as a surrogate of MDR, directly from clinical specimens. Since December 2010, WHO has recommended the CBNAAT as a bonafide test due to its high-quality performance as compared to SM, especially in cases of smear-negative cases [8]. It has high sensitivity and specificity and results can be obtained much quicker but at the expense of high cost (USD 25-30) [6]. Although SM exhibits low sensitivity on fiberoptic bronchoscopy samples with 5-35% on Bronchial Aspirates (BA) and 10-30% on BAL, CBNAAT of BAL has been established as a good diagnostic tool for the purpose of bacteriological confirmation of TB suspects who were otherwise sputum negative or could not produce adequate sputum for SM [7].

Studies have already established its utility in Indian perspective with more than 90% sensitivity and 90-100% specificity [9,10]. Indian guidelines on TB care are envisaged in RNTCP 'National strategic plan for TB control 2012-2017' [11]. RNTCP is currently using Xpert MTB/RIF to diagnose Pulmonary TB, Paediatric TB, Extrapulmonary TB and Rifampicin resistance and MDRTB in high risk populations like HIV positives as recommended by WHO under 2013 policy recommendations [12-14]. The present study aim to find the effectiveness of CBNAAT test in detecting the AFB positivity among the smear negative TB patients.

MATERIALS AND METHODS

A cross-sectional survey was carried out from October, 2016 to March, 2017 in the Department of Respiratory Medicine situated at Nilratan Sircar Medical College and Hospital (NRSMCH), Kolkata. The hospital has CBNAAT facility and acts as a referral unit for providing the opportunity of this test, specially to all the smear negative TB cases referred to as well as those who were self-reporting to the Department of Respiratory Medicine.

94 radiologically suspected sputum negative TB cases either referred from other health facilities or diagnosed in the Department of Respiratory Medicine, NRSMCH, Kolkata, were included in the present study. After obtaining informed consent, the patients were interviewed using a predesigned questionnaire for collecting baseline information like age, sex, history of TB and its treatment etc. Relevant records were also scrutinised. Then, each of them were subjected to sputum CBNAAT.

Those who were found still AFB negative were further put on BAL-CBNAAT testing. Finally, the patients were categorised either into CBNAAT positive or CBNAAT negative TB and the referred patients were sent back to their original health facilities and those diagnosed at Department of Respiratory Medicine, NRMCH were put on TB treatment as per the RNTCP guidelines. The study was conducted after obtaining the approval of Institutional Ethics Committee.

Collected data were compiled in Microsoft (MS) excel sheet and analysed using statistical package for Social Science (SPSS) version-22. Continuous variables were described by mean, SD and the categorical ones were by proportion. Continuous data were tested for normality distribution by Shapiro-Wilk's test. Tables and charts were used for displaying data. Interrelationship among the variables was determined by inferential statistical test like chi-square ($\chi 2$) test, Fisher's-exact test, Odds Ratio (OR) with its 95% Confidence Interval (CI). A p-value less than 0.05 was considered statistically significant at 95% confidence limit.

RESULTS

Data collected from 94 sputum negative patients were analysed. Continuous data were found to follow normal distribution as reflected by normality test.

Half of the patients belonged to 41-60 years age group followed by 32.9% in 21-40 years group. The females were significantly higher in 21-40 years group compared to >60 years group containing no women participants [Table/Fig-1].

Age category (year)	Gender			χ^2	p-value at df 1
	Male No. (%)	Female No. (%)	Total No. (%)		
Up to 20	5 (83.3)	1 (16.7)	6 (100.0)	@	0.375
21-40	16 (51.6)	15 (48.4)	31 (100.0)	7.63	0.005
41-60	38 (80.9)	9 (19.1)	47 (100.0)	2.27	0.131
>60	10 (100.0)	-	10 (100.0)	*	*
Total	69 (73.4)	25 (26.6)	94 (100.0)	---	----

[Table/Fig-1]: Distribution of participants as per age category and gender (N=94). *Reference group, df: Degree of freedom; @ Fisher-exact test (two tailed)

Overall, average age was estimated to be 44.7±15.3 (mean±SD) with a range of 15-87 years. The corresponding values across the gender were 47.3±16.0, 17-60 years versus 44.7±15.3 and 15-87 years in males and females, respectively. As per independent t-test the female participants were significantly younger than their counterpart (t=2.821 at df 92 with p-value of 0.006). Eight (8.5%) patients had previous history of TB.

Either sputum CBNAAT or BAL-CBNAAT examination over and above the SM was found to be significantly more effective in regard to clinical benefit for guiding in the management of TB patients [Table/Fig-2]. Both the newer tools together provided a total 42 positive cases (32 in sputum- CBNAAT + 10 in BAL-CBNAAT) out of 94 patients revealed to be negative in SM. It was a statistically significant yield over and above SM {χ^2=54.1 at df 1 with p-value of 0.000; OR=0.00 (0.00-0.07)}. However, the effectiveness of BAL-CBNAAT providing a yield of 10 positive cases out of 62 patients found negative in sputum-CBNAAT was also shown to be statistically significant {χ^2=10.88 at df 1 with p-value of 0.0009; OR=0.00 (0.00-0.47)} .

Laboratory method	Results			χ^2, df, p	OR (95% CI)
	Positive No. (%)	Negative No. (%)	Total No. (%)		
Sputum microscopy	0	94 (100)	94 (100)	*	1
Sputum CBNAAT	32 (34.0)	62(66.0)	94(100.0)	38.56,1,0.000	0.00 (0.00-0.11)
BAL-CBNAAT	10(16.1)	52(83.9)	62(100.0)	16.20,1,0.000	0.00 (0.00-0.3)

[Table/Fig-2]: Distribution of participants as per results of sputum CBNAAT and BAL-CBNAAT.

Attributes		AFB Negative	AFB Positive	Total	χ^2, df	p-value
Sputum CBNAAT						
Sex	Female	19	6	25	1.530,1	0.216
	Male	43	26	69		
Total		62	32	94	----------	----------
BAL-CBNAAT						
Sex	Female	21	2	23	1.494,1	0.222
	Male	31	8	39		
Total		52	10	62	---------	----------

[Table/Fig-3]: Distribution of patients as per gender and CBNAAT results (N=94).

Test results of sputum CBNAAT and BAL-CBNAAT were found not to differ significantly across the gender [Table/Fig-3]. Even, both the yields together failed to reveal any significant difference across the gender {χ^2=2.22 at df 1 with p-value of 0.137; OR=0.48 (0.16-1.39)}.

DISCUSSION

TB control guidelines developed by Centres for Disease Control and Prevention (CDC), United States, recommends CBNAAT for at least one respiratory specimen of patients having clinical features suggestive of pulmonary TB and for whom diagnostic endeavour is going on but yet to confirm [15]. Similarly, Korean guidelines for management and control of TB adopted strategy for Nucleic Acid Amplification (NAA) testing in combination with SM for AFB and culture at least once for the pulmonary TB suspects [16].

CBNAAT should not be thought as a substitute for culture and SM. However, it can act as an adjuvant of traditional tests and clinical data for confirming TB. It cannot be used for monitoring the therapeutic response as it can produce false-positive results in presence of non-viable TB bacteria; though identification of M. tuberculosis out of Non-Tubercular Mycobacterium (NTM) is possible by it [17]. Thus, CBNAAT is helpful diagnostic armamentarium towards AFB smear-positive patients for rapidly detection of pulmonary TB and getting it differentiated from NTM [18]. In the present study 34% yield was obtained out of CBNAAT test.

However, a study conducted by Avashia S et al., reported 47.2% gain in respect of sputum positivity among smear negative TB cases [7]. The results of the present study have concurrence with this where 34.0% yield for sputum CBNAAT, 10.7% for BAL-CBNAAT and together it was 44.7%. This 10.7% increase in the yield arising out of BAL-CBNAAT over and above the yield of sputum CBNAAT was also revealed to be statistically significant. Here, the yield of 10.7% sputum positive patients would be taken as a major gain from the epidemiological point of view so far as the transmission of TB concerns.

A Cochrane systematic review done in 2013 showed high accuracy of CBNAAT compared to culture. It showed about 88% sensitivity and 98% specificity for pulmonary TB in adults. Among smear-negative TB patients, Xpert had a sensitivity of 67% [19]. Ioannidis

P et al., reported that GeneXpert MTB/RIF assay has positive predictive values for pulmonary and extra-pulmonary samples 93.5% and 50%, whereas negative predictive values for those are 91.7% and 100%, respectively. In case of microscopically negative specimens, the figures are 79% and 95.6% [20].

From their research conducted in 2012, Moure R et al., concluded that out of 108 smear-negative extrapulmonary samples 58.3% were positive with the Xpert MTB/RIF assay (GX) for Mycobacterium tuberculosis [21]. Vadwai V et al., carried out a similar study in 2011 and observed the sensitivity of the Xpert assay as 64% for smear-negative TB cases [22].

In a study on 132 patients in a single South-Korean centre Lee HY et al., reported sensitivity and specificity values for Xpert MTB/RIF assay and smear microscopy in the level of 81.6% and 100.0% versus 13.2% and 98.8% respectively compared to the culture [23]. In their South-African single-centre study involving 154 suspected TB patients, Theron et al., analysed the BAL samples in which sensitivity and specificity values compared to the culture were 92.6% and 96.0% for the Xpert MTB/RIF assay, and 57.7% and 99.3% for SM, respectively [24]. Palud PL et al., observed 80.0% and 98.6% sensitivity and specificity for the Xpert MTB/RIF assay as compared to culture [25].

LIMITATION

The participants were less in number restricting the external validity of the study. Radiologically suspected smear negative TB patients were considered for present study and the sensitivity as well as specificity of the CBNAAT tool could not have been estimated in this setting by comparing its effectiveness with that of the 'gold standard' i.e., culture.

CONCLUSION

Present study reaffirmed the usefulness of the CBNAAT over and above the traditional smear microscopy for a significantly higher yield. It leads to early detection and treatment of TB for stopping the transmission of the disease in the community. BAL-CBNAAT shows additional advantage of confirmatory diagnosis of the disease even if sputum CBNAAT is negative. Simplicity, sensitivity, speed and automation of CBNAAT makes this technique a very attractive tool for diagnosis of Mycobacterium tuberculosis from smear negative cases of TB suspects. With the added advantage of detection of multi-drug resistant cases, it seems to be another mile stone in 'Stop TB' strategy and needs to be utilised to its highest potential level through monitoring and supportive supervision at every level of RNTCP. Grass-root level workers, program implementers and managers require necessary re-orientation and motivation for integrated actions for maximum achievement.

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