ORIGINAL RESEARCH

Outcome of same-day smear method compared with conventional sputum method for diagnosing pulmonary tuberculosis

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Received: 16 November, 2023 Accepted: 18 December, 2023

ABSTRACT

Objective: The World Health Organization (WHO) has recommended to consider the early morning sputum samples test instead of the conventional two days for the diagnosis of smear positive tuberculosis (TB). **Materials and Methods:** This hospital-based cross sectional, comparative study was conductedat Medical College Hospital, Kolkata during November 2018 to May 2020.Two sputum specimens were collected from enrolled presumptive TB patients. In addition, a second sample was collected by traditional approach. All the samples were examined by Ziehl-Neelsen staining. Chi square testwas used to compare statistical differences in the proportion smear positive between the two approaches. **Results:** Total 230 patients were evaluated in the present study. The majority of patients (47.4%) belonged to the age group of 41-60 years, followed by ≤40 years age group (41.3%) and ≥61years age group (11.3%).Among 230 presumptive TB patients, 73 patients were detected as sputum positive presumptive TB (PTB) patients. High bacillary load (3+) was documented in conventional morning sputum compared to first day sputum (14.1% vs. 13.8%) and same day second sputum (14.1% vs. 7.4%). Total 71 patients were detected and 2 patients (2.7%) were missed on conventional sputum testing whereas, on the same day sputum testing, 67 patientswere smear positive while sixpatientswere found to bemissed. **Conclusions:** The conventional sputum microscopy method is more sensitive than same day method.

Keywords: Bacillary load, presumptive TB, sensitive, Ziehl-Neelsen.

INTRODUCTION

The rapidly increasing prevalence of tuberculosis (TB), as well as associated risk factors among Indians have substantially contributed to the high burden of mortality and morbidity.According to the latest report, the incidence of TB in India is 2.6 million which accounts for 27% of global patients.[1]

Early diagnosis and treatment of active TB patients can prevent disease prognosis, morbidity, mortality, and transmission of tubercle bacilli. Smear microscopy or National Tuberculosis Elimination Programme (NTEP) accredited cartridge-based nucleic acid amplification test (CB-NAAT or TrueNat) or Line Probe Assay (LPA) are the most commonly used techniques for the diagnosis of pulmonary TB.[2-4]As per the patient finding method used in NTEP, a presumptive TB patient has to visit healthcare facilities multiple times to give a sputum sample on the first day (spot), followed by early morning sputum on the second day, and to collect the report on subsequent days. Therefore, this approach can prolong the diagnostic process of TB and it may lead to dropout of TB patients due to the inconvenience associated with multiple visits.[5,6] Several studies have revealed that patient dropout is a major drawback of the SM approach accounting for 4.3-13% of dropout rate. As a result, the patients with active TB may be undiagnosed or delayed diagnosis results in delayed initiation of treatment and therefore

increased mortality, morbidity, and transmission of infection.[7]

The World Health Organization (WHO) has recommended to consider the early morning sputum samples (spot-spot) test instead of the conventional two days sputum smear method to yield a greater number of positive results than spot samples. This strategy can effectively reduce the dropout rate by decreasing the number of visits to the diagnostic facility.[3]Early morning sputum samples are generally considered to yield a greater number of positive results than spot samples with high sensitivity and specificity. Only a few Indian studies have addressed the accuracy and agreement of the positivity rates of the same-day sputum smear microscopy when compared to the conventional method, and the results of those few studies are conflicting.[8-11]Hence in the present study, the yield of sputum smear positivity between conventional sputum smear microscopy and same day sputum smear microscopy has been compared to improveknowledge, attitude, and practice regarding TB diagnosis.The objective of the present study is to assess and compare the smear positivity rate between the same day sputum sample (spot-spot) microscopy with the conventional sputum smear (spot-early morning) microscopy for diagnosis of microbiologically confirmed Pulmonary TB; (i) the number (proportion) of presumptive TB patients (ii) the number (proportion) of sputum smear positive TB patients diagnosed.

METHODS AND MATERIALS

Study design

This hospital-based cross sectional, comparative study was conducted among patients with TB attending outdoor and admitted in the indoor of respiratorymedicinedepartment, at Medical College Hospital, Kolkata during November 2018 to May 2020.

Inclusion and exclusion criteria

Patients (>14 years) of either sex with productive cough lasting for two or more weeks were included in the study. Patients<14 years of age with dry cough, and those visiting for the follow up for sputum smear examination for *acid fast bacilli* (AFB) were excluded from study. After obtaining approval from the Ethics Committee of the institution (MC/KOL/IEC/NON-SPON/195/12-2018; approved on 22.12.2018), a written informed consent was obtained from each patient prior to study enrolment.

Data collection

The data collected from the patients with a predesigned and pretested schedule included clinical examination, sputum smear microscopy for AFB and HIV antibody testing by enzyme-linked immunosorbent assay (ELISA).

Collection of sputum specimens

The patients were instructed about giving three first spot, second spot (one h after first spot) and next day early morning sputum samples. Patients who failed to give any one of the three samples were termed as ‘drop out’ and were not included in the study. The mucopurulent sputum samples of adequate volume collected after taking a deep breath were examined in the NTEP Designated Microscopy Centre (DMC). Smears were prepared on the properly labeled glass slides from the purulent portions of the sputum samples and were stained by Auramine O stain. The slides were then air-dried, heat-fixed, and stained with fluorochrome stain.Slides, without heating were decolorized with 0.5% acid-alcohol solution for about 2 mins followed by counter-staining with 0.5% potassium permanganate solution for a minute. Rinsed, air-dry stained slides were examined under Fluorescence Microscope. The low power objective 20X was used for focusing while 40X objective was used for reading. The slides were observed for bright yellow AFB against the dark background; grading of results was done as per NTEP guidelines.[12]

End points

Sputum samples were considered positive if AFB were seen on smear from any of the three sputum samples. The same day method consisted of the results of first and second spot sample, whereas the conventional method consisted of the results of first spot and early morning samples. The yield of same day method was compared with the yield of conventional method.

STATISTICAL ANALYSIS

Data was analyzed using Statistical Package for the Social Sciences (SPSS) version 23.0. Descriptive statistics was used to describe categorical variables (frequency and percentages). Chi square test was used to assess association between categorical variables. In patient of a cell value being zero, Fisher’s exact test was used for statistical association. P value ≤0.05 was considered statistically significant.

RESULTS

A total of 250 presumptive TB patients were enrolled, of which 20 patients who had not given any one of the three sputum samples were considered as drop out and were excluded from the study.Total 230 patients had given all the three sputum samples.

In this study, majority of patients (47.4%) belonged to the age group of 41-60 years, followed by ≤40 years age group (41.3%) and ≥61years age group (11.3%). About 171 (74.3%) patients were men. Most of the patients were from urban area (62.2%).Cough and feverwas the most common presenting symptom in 52.6% of patients followed by weight loss (41.6%) [Table 1].

Among 230 presumptive TB patients, 73 patients were detected as sputum positive presumptive TB

(PTB) patients . Of these 73 PTB patients, first sputum sample was (spot 1) detected positive in 65 patients, same day second sputum sample (spot 2) was detected positive in 54 patients and second day early morning sputum sample was detected positive in 71 patients. High bacillary load (3+) was documented in conventional morning sputum (14.1%) compared to first day sputum (13.8%) and same day second sputum (7.4%) samples (Table 2).

About 71 patients (97.8%) were detected and 2 patients (2.7%) were missed on conventional sputum testing whereas, on the same day sputum testing, 67 patients (91.8%) and 6 patients (8.2%) were detected and missed, respectively (Table 3).

Among the 73-sputum smear positive PTB patients, 30 (41.1%) were smoker, 31 (42.5%) were diabetic, and six (8.2%) were HIV positive (Table 4). Most common radiological finding among sputum smear positive PTB patients was consolidation (64.4%) followed by cavitary lesion (57.5%) and pleural effusion (4.0%).

Table 1. Demographic characteristics

Parameters	Number of patients (N = 230)
Age groups (years)	
≤40	95 (41.3)
41-60	109 (47.4)
≥61	26 (11.3)
Gender	
Male	171 (74.3)
Female	59 (25.7)
Religion	
Hinduism	160 (69.6)
Islam	70 (30.4)
Residence	
Rural	87 (37.8)
Urban	143 (62.2)
Associated presenting symptoms	
Fever	100 (52.6)
Anorexia	100 (52.6)
Weight loss	79 (41.6)
Chest pain	72 (37.9)
Shortness of breath	39 (20.5)
Outcome of first sputum sample	
1+	46 (20.0)
2+	10 (4.3)
3+	9 (3.9)
Outcome of second sputum sample	
1+	47 (20.4)
2+	3 (1.3)
3+	4 (1.7)
Outcome of conventional second sputum sample	
1+	50 (21.7)
2+	11 (4.8)
3+	10 (4.3)
Data presented as n (%).	

Table 2: Distribution of bacillary load according to the type of sputum sample among the participants who tested positive in any one sample

Parameters	First sputum sample (n = 65)	Same day second sputum sample (n = 54)	Conventional second (morning sputum sample) (n = 71)	P value
Bacillary load				
1+	46 (70.8)	47 (87.0)	50 (70.4)	
2+	10 (15.4)	3 (5.6)	11 (15.5)	0.218
3+	9 (13.8)	4 (7.4)	10 (14.1)	
Data presented as n (%).				

Table 3. Distribution of study participants according to patiet detection by same day sputum testing and overall diagnosis of tuberculosis patients

Parameters	Day sputum testing	Conventional sputum testing
Patiet detected	67 (91.8)	71 (97.8)
Patiet missed	6 (8.2)	2 (2.7)
Data shown as n (%).		

Table 4. Distribution of study participants according to presence of tuberculosis and smoking status, diabetic status, and HIV status of the participants

Parameters	Presence of tuberculosis		P value
	Present (n=73)	Absent (n = 157)	
Smoking status			
Non-smoker	43 (58.9)	113 (72.0)	0.048
Smoker	30 (41.1)	44 (28.0)	
Diabetes			
Present	31 (42.5)	40 (25.5)	0.009
Absent	42 (57.5)	117 (74.5)	
HIV			
Present	6 (8.2)	8 (5.1)	0.356
Absent	67 (91.8)	149 (94.9)	
Data presented as n (%).			

Table 5. Distribution of study participants according to presence of tuberculosis and radiological findings among the participants

Categories	Grade of bacterial load in sputum examination			
	1+ (n = 49)	2+ (n = 10)	3+ (n = 14)	Overall positive (n = 73)
Consolidation	31 (63.3)	7 (70.0)	9 (64.3)	47 (64.4)
Cavitary lesion	18 (36.7)	11 (110.0)	13 (92.9)	42 (57.5)
Pleural effusion	2 (4.08)	0 (0.0)	1 (14.3)	3 (4.0)
Data presented as n (%).				

DISCUSSION

In TB-endemic countries like India, early diagnosis and prompt initiation of treatment for PTB is a major challenge for the healthcare system. Sputum smear microscopy is rapida widely used inexpensive, relatively simplemethod especially low-income and middleincome countries for diagnosing pulmonary tuberculosis. Diagnosis is primarily done by identifying of AFB in sputum smear under light microscope using Ziehl-Neelsen (ZN) stain. It is a very useful point-of-care (POC) test. However, sputum smear microscopy method has high specificity but low sensitivity. To improve the diagnostic accuracy of the smear microscopy, fluorescent microscopy is introduced which is a very promising method.[13]Laifangbam S. etal. revealed that fluorescent microscopy has a higher diagnostic yield compared to that of ZN staining technique.[14] The fluorescent microscopy generated readings was three times faster than that of ZN technique with high sensitivity (84.5%) and specificity (100%).[15]Previously, three serial sputum specimens (spot-morning-spot) were examined for the microscopic diagnosis of sputum smear positive PTB according to RNTCP policies. Mase et al. demonstrated that the incremental yield of the third specimen (where first two specimens were negative) was only 3.1%. According to their study, majority (85.8%) of sputum smear positive PTB patients was detected with the first sputum specimen and 11.9% with the second sputum sample.[16] In another study conducted by Bonnet et al. demonstrated that examining only two sputum smears could reduce the workload of laboratories, particularly in high burden countries.[17] WHO in 2007 recommended that two sputum smear examination (spot-early morning)in a quality assured laboratory was adequate.[18]

RNTCP adopted the policy in April, 2009.[19] In 2011, WHO suggested implementation of same day sputum smear examination strategy (spot-spot) instead of conventional sputum examination (spot-early morning) to reduce the diagnostic 'dropout' rates. In this study 230 patients had preferred same day sputum microscopy while 20 patients failed to bring the second day specimen. In the previous study by Firdaus et.al. and Myneedu et al. 91.1% and 93.0% of patientspreferred same day sputum collection respectively.[20,21]However, previous study by Nayak et.al. observed counter-intuitive results. They noted results noted a higher drop-out rate with same-day approach as compared to conventional approach (7% vs. 5%).[11]

Davis et al. showed that examination of two smears taken in same day has the same specificity (98%) and almost same sensitivity (63.0%) compared to the conventional two days method of smear examination.[22]An expert group of WHO evaluated the diagnostic accuracy of same-day microscopy and found that the conventional approach of 'spot-morning' sputum smears hadsamilarsensitivity and specificity as those of 'same-day microscopy'.[23] The dropout rate of the patients in their study was 2% in patiet of same day sputum collection, compared to 5.8% in patiet of conventional two day sputum collection. A cross sectional study conducted by Shafiyabi et al. in Karnataka, India showed that the patiet detection rate was 39.6% & 40.4% with the same day smear examination (spot-spot) and conventional two day (spot-morning) method respectively.[24]Chandra TJ et al. from Andhra Pradesh, India in their study showed similar result and concluded that bothsame day smear microscopy and conventional method of smear microscopy were equally effective in diagnosing pulmonary tuberculosis.[25]

On the other hand, a cross sectional study conducted by Nayak et al. revealed that patiet detection rate in same day approach was 14%, whereas that in conventional two-day approach was 17%. Total 16.9% of patients were missed by same day sputum microscopy method compared to only 0.5% by conventional method.[11]Ravindra AG et al. from 1day smear method and conventional method (18.8% and 19.5%).[9]

Out of 189 patients with TB, eight patients were missed by the same day method and only onepatient by the conventional method diagnosed. Both the studies concluded that same day sputum microscopy was less sensitive compared to the conventional method. A study conducted by Myneedu et al. revealed higher smear positivity rate by the conventional method compared to same day sputum microscopy (18.5% vs, 13.0%). They concluded that the sensitivity of same day microscopy was loweras compared to conventional method (40.1% vs. 58.3%). Further it was revealed that the patients were more comfortable with the same day sputum collection method (spot-spot) than conventional method.[21]

In the present study, 73 patients among 230 presumptive TB patients were sputum positive. The rate of sputum positivity was 31.7%. Among three samples, high bacillary load (grade 2+ and 3+) was found higher in the morning sputum specimens than first and second spot specimens. Hence, it can be concluded that early morning sputum sample had the highest yield compared to the spot samples. The same inference was drawn by Cuevas LE et al. in their study.[26] Among 73 sputum smear positive patients, 67 patients (29.1%) were detected by the same day sputum microscopy method, whereas 71 patients (30.8%) were detected by conventional approach. Among 73 sputum positive patients, six (8.21%) patients were missed by the same day approach as compared to only 2 (2.7%) missed by the conventional method [P value <0.01]. Deka DJ et al. also concluded that the same day method was less sensitive than conventional method.[27]The results of present study was also in agreement with the study done by Nayak et al. which concluded that conventional method of sputum smear microscopy was better than the same day approach (smear positivity rate: 17% and 14% respectively) by conventional two day approach.[11] Now considering the results of the present study, it can be concluded that for the diagnosis of sputum positive PTB patients, the same day approach of collecting two sputum samples (spot-spot) is less sensitive than the conventional (spot-early morning) approach. Thus conventional sputum smear microscopy method is more sensitive than same day method. This statement is contrary to WHO statement, which concluded that the same day microscopy has an accuracy similar to the conventional method.

The discrepancy between studies that found a significant difference and those that observed no difference between the same-day and 2-day approaches may be due to differences in the intensity of grading of the smear results. If most of the smear-positive results were high positives (3+ or 2+), it is possible that the spot specimen itself could detect a patient as sputum smear positive, with little additional yield from the early morning specimen. The study done by Cuevas LE et al. reported no 'scanty positive' TB patients, and cited this as a possible reason for the lack of difference between the two approaches.[26] In contrast to above aforementioned results the present study revealed that over half of the sputum smear-positive TB patients were low positives (1+) and a more sensitive early morning specimen might thus have had added value and driven the results.

LIMITATIONS

The sample size of the present study was small hence, the derived conclusion may not be significant. On the other hand, sensitivity and specificity of each method could not be calculated, as we had not taken any gold standard investigation for the diagnosis of sputum smear positive PTB.

CONCLUSION

In diagnosing sputum positive pulmonary tuberculosis cases, conventional sputum microscopy method is more sensitive than same day method.

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