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Original article

The adequacy of two sputum smear examinations for diagnosis of Pulmonary Tuberculosis as per RNTCP- A Retrospective study in Pondicherry

R.S Bharatwaj*[1], T.Mangaiyarkarasi[2], D.Jeyakumari[3], P.Rajaram[4]

[1]Associate Professor, Department Of Community Medicine,Sri Lakshminarayana Institute Of Medical Sciences, Pondicherry, India

[2]Assistant Professor,[3]Professor, Department Of Microbiology, Sri Lakshminarayana Institute Of Medical Sciences, Pondicherry, India

[4]Epidemiologist, Department Of Community Medicine,Sri Lakshminarayana Institute Of Medical Sciences, Pondicherry, India.

ABSTRACT

Introduction: The failure to promptly identify and treat infectious cases stands out as a critical obstacle in the global control of tuberculosis. The RNTCP (Revised National Tuberculosis Control Program) guidelines specify that the numbers of sputum samples required are 2 with one of them being an early morning sample. **Methodology**: All the samples collected for A.F.B staining from suspect T.B patients from Jan 2009 onwards till Oct 2011 in Sri Lakshmi Narayana Institute Of Medical Sciences, a medical teaching hospital were retrospectively analyzed to compare two sputum samples to three samples in diagnostic yield. **Results**: Of all the samples given 391 (54.9%) suspect patients had given only one sputum sample. There were less number of female suspects (33.75%) in the total number of people examined. Looking at the diagnostic yield, three samples instead of two added only one extra case to the detection program among the 320 patients who gave two or more samples. **Conclusion**: Two smear examinations instead of three is a viable and efficient change in the armamentarium of the revised national tuberculosis control program in our country.

KEYWORDS: RNTCP, Sputum, Microscopy, Diagnostic Yield

INTRODUCTION

Tuberculosis (TB) persists as a major cause of human morbidity and mortality, affecting almost 9.4 million people and causing 1.8 million deaths yearly worldwide. India accounts for nearly one third of the global burden of tuberculosis. The major objectives of the tuberculosis control program are early detection and treatment of the infectious case of pulmonary tuberculosis.[1] Examining Mycobacterium tuberculosis bacilli, using light microscopy with Ziehl–Neelsen stain, is still considered the most specific, cost-effective, quick and reliable test for the diagnosis of

pulmonary TB. In revised national tuberculosis control program (RNTCP), microscopic examination of sputum for AFB plays an important role in the initial diagnosis of tuberculosis. The microscopic examination requires 10^4 bacilli per milliliter of sputum in order to be detected on smear. It has also been established that sputum smear microscopy is less sensitive in HIV –TB co infection where sputum smear tends to be negative.[2,3] Increasing the number of samples to be tested increases the smear positivity rate marginally when compared to three samples being tested in RNTCP.[4,5]. The failure to promptly identify and treat infectious cases stands out as a critical obstacle in the global control of tuberculosis. In addition to developing new technologies to support case finding, existing technologies, approaches and recommendations need to be examined carefully to determine if they are being utilized optimally and to identify areas in which improvements in efficacy and efficiency can be made.

The major reason for failure to control T.B includes incomplete case finding [6]. In resource poor countries where options are limited sputum microscopy is considered to be the method of choice for diagnosis of active disease. The reliability, low cost and ease of direct microscopic examination have made it the number one case finding tool. Smears for acid fast bacilli obtained from patients with pulmonary tuberculosis may be positive on one day and negative on the next necessitating a minimum of three samples to be collected to maximize the chances of detecting mycobacteria on sputum examination [7] The RNTCP guidelines specify that the number of sputum samples required is 2 with one of them being an early morning sample. Two samples are collected and one positive among them is enough to diagnose the patient as having smear positive T.B.[8]. This could increase the case detection by increasing the quality of examination of the first two smears. We wanted to see the impact of such a change in diagnostic modality at our institute.

Objectives

1. To find out the proportion of people who have given all three sputum samples for diagnosis of T.B as per RNTCP in a tertiary care teaching hospital

2. To compare the diagnostic yield among the people who have given up to two sputum samples and all the three samples.

METHODOLOGY

After obtaining approval from the institutional review board, all the samples collected for A.F.B staining from suspect T.B patients from Jan 2009 onwards till Oct 2011 in Sri Lakshmi Narayana Institute Of Medical Sciences, a medical teaching hospital as per RNTCP diagnostic guidelines were retrospectively analyzed . The institute has two trained technicians who were trained by the RNTCP state program managers to ensure quality sputum microscopy. The data was compiled and analyzed by SPSS 15 data analysis software, using tests for, significance of difference in proportions.

RESULTS

There were a total of 711 patients who were referred for sputum microscopy to the microbiological laboratory for acid fast bacilli staining as per the RNTCP norms for 3 sputum smears. Among these the number of females was 240 (33.75%) and the number of males was 471 (66.2%). Thus there were lesser numbers of females in the total number of people examined for AFB during the study duration .A total of 99 were identified to be positive for AFB that is a positivity of 13.92%in 711 patients. Among males 19.32% (91 out of 471) of the suspects were positive while among the females only 3.33% (8 out of 240) of the suspects were AFB+. This difference between males and females in chance of a suspect being positive was highly statistically significant.($p<0/001$)[Table 1]. The proportion of males who had given two or more samples was 47.6% and the proportion of females who had given two or more samples was 40%. Similarly the proportion of males who had given only one sample was 52.4% while the proportion of females who had given only one sample was 60% [Table 2].

Table 1: Overall distribution of the sputum samples and their results based on gender

Gender	Total	Sputum result		P VALUE
Males	471	AFB +	91	**<0.001**
		AFB -	380	
Females	240	AFB +	8	
		AFB -	232	

Table 2: Overall distribution of the patients based on gender and number of samples given

Number of samples given	Males	Females	Total
Only one sample	247(52.4%)	144(60%)	391
Two samples	146(30.9%)	70(29.1%)	216
All the three samples	78(16.5%)	26(10.8%)	104
Total	**471**	**240**	**711**

Of the total of 711 patients who were referred for investigation, for sputum AFB, as per the RNTCP guidelines, 391(54.9%) of them had given only one sample. The AFB positivity among these 391 patients who had given only one sample of sputum was 41(10.4%). There were 216 patients who had given only two sputum samples of which the sputum positivity was 39(18.05%). Among the total of 711 suspect patients referred only 104 (14.6%) had given all the three sputum samples of which 19(18.26%) were AFB positive. In between genders, among those who had given only one sample as well as those who had given only two samples the chance of being acid fast bacillus positive was significantly higher among the males [Table 3]. The high significance shows that if the suspect case was a male then, the probability that he would be AFB positive is much higher than if the suspect case were a female when only one or two samples were given but with all the three samples given this significance in chance of being AFB positive was rendered insignificant. This implies that for a female suspect the chance of being identified as AFB positive was higher if all the three smears were given.

Table 3: Tabulation of sputum sample results with the numbers of samples given, gender wise

Number of samples given		AFB +	AFB -	TOTAL	P VALUE
One sample	Males	39	208	247	**<0.01**
	Females	2	142	144	
	Total	**41**	**350**	**391**	
Two samples	Males	36	110	146	**<0.01**
	Females	3	67	70	
		39	**177**	**216**	
Three samples	Males	16	62	78	>0.05
	Females	3	23	26	
		19	**85**	**104**	

Among those who had given only two sputum samples 79.48% (31) of the patients with AFB positivity were identified in the first sample itself. Interestingly all the cases identified only in the second smear were of male patients. Similarly among those who had given all the three sputum samples 18 out of the 19 positives were identified in the first two samples itself.

The only extra case that was identified purely by a third smear was of a female. In the under 14 age category not even one suspect had sputum positive for AFB. Maximum numbers of cases were from the economically productive age category of 15 to 60 years [Table 4]. Comparing for the yield, the numbers of cases identified by one or at the most two samples itself was 98(13.78%) out of a total of 711 patients, while the extra yield only due to a third sample was 1(0.96%) out of a 104 patients. The difference when tested for a significance of difference in proportions was statistically very highly significant ($p< 0.001$) which means that the extra yield by the extra third sample is statistically very significantly low and hence is negligible. Thus in the overall yield a third sample did not contribute significantly to the diagnostic yield.

	Only one sample given				**Two samples given**				**All three samples given**			
	Males		Females		Males		Females		Males		Females	
	AFB+	AFB-	AFB+	AFB-	AFB+	AFB-	AFB+	AFB-	AFB+	AFB-	AFB+	AFB-
<14 yrs	0	13	0	4	0	7	0	3	0	3	0	4
15-60 yrs	34	142	2	116	26+**4***	63	1	50	13	37	1+**1**†	15
>60 yrs	5	53	0	22	2+**4***	40	2	14	3	22	1	4
Total	39	208	2	142	28+**8***	110	3	67	16	62	2+**1**†	23
	247		**144**		**146**		**70**		**78**		**26**	

Table 4: Distribution of AFB positivity among gender and age categories among the samples given

*Samples which were negative in the first and positive in the second smear

†Sample that was negative in both first and second smear but positive in the third smear

Among those patients having given all three sputum smears only 1 in 104 samples was positive only in the third sample (1/104=0.96%). Similarly among those who had given only two samples the chance of a smear being positive only in the second sample was 8 in 216 samples (8/216=3.7%).

Using these we can make a prediction of the number of cases we may have missed out as a result of collection of less than three samples from the patients. Making the calculations first among the 391 patients who had given only one sample the chance of a second sample being positive is in 15 samples(3.7/100*391) and the chance of a third sample being positive is 4 samples (1/104*391). Now among the 216 who had given only two samples the chance of a third sample alone being positive for AFB would be in 3 cases (1/104*216). Thus totaling up a total of 22(15+4+3) cases are probably missed due to inadequate sample collection.

Hence 99(81.18%) of the projected 121 cases were identified in spite of more than 50% of the patients having given only one sample. Also looking at the total picture of 711 samples the projected numbers that would have been positive only in the third smear is 8 i.e. 1.1% of the suspects.

DISCUSSION:

The RNTCP guidelines have instructed only two sputum smears to be collected instead of the previous three samples. In our study we found that the majority of the suspects are giving only one sputum smear. The likelihood of giving at-least

two samples is more if the suspect case is a male as compared to a female.

The likelihood of a female suspect being identified only in the second smear was nil in our study population. A huge difference in the AFB positivity with female suspects being far less likely to be sputum positive than their male counterparts in spite of having cough for more than three weeks could be due to the greater prevalence of other common respiratory complaints in females and their lesser exposure due to lesser mobility and hence exposure in the society.

The maximum numbers of detected cases were from the economically productive age category of 15 to 60 years. Looking at the diagnostic yield, three samples instead of two added only one extra case to the detection program among the 320 patients who gave two or more samples.

This trend of the majority of patients giving only one sample was observed in other studies also [9]. The two sample RNTCP approach is for field care. However in a tertiary care teaching hospital to enhance the diagnostic yield, sputum enrichment techniques can be adopted for higher efficiency of diagnosis.

Concentration with 5% sodium hypochlorite solution is a viable option. Considering its low cost, decontaminating and liquefaction properties with better sensitivity, this method is safe and can be of vital importance in sputum diagnosis of T.B and could be a method used as has been proven in a past study [10] Also the collection of two samples with the collection of the second sample next morning could be modified to two samples collected at the same time as has been shown to be of equal sensitivity and specificity in a past study. [11] This could save the out patients the trouble of coming back again with an early morning sample as well as the lab could declare the results on the same day to speedup the management process.

In a developing country like ours with economically poor patients and their unstable and challenging social conditions, giving his/her time and having motivation for coming back to give a second sample can hardly be expected. As well the lesser physician-patient time due to patient overload, leaves very little avenue for counseling the patient. So it would be appropriate to collect both the samples at the same sitting at-least in teaching college hospitals that contribute to more than 20% of sputum positive cases diagnosed in India as per 2011 report [12] This could enhance case detection and further our steps towards the goals of RNTCP.

Conflicts Of Interest: None

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*Corresponding author: Dr. R.S Bharatwaj

E mail – resure2@yahoo.com