Light Emitting Diode(LED) Fluorescence Microscopy(FM) for Same Day Diagnosis of Pulmonary Tuberculosis(PBT)

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ABSTRACT

Background: *The conventional approach for the diagnosis of pulmonary tuberculosis (PTB) involves collection of sputum sample for several days for which the patient makes multiple visits to health care center and does not take patients inconvenience into account. The sputum smears are stained by Ziehl-Neelsen (ZN) method and examined under light microscopy (LM) which is relatively insensitive diagnostic technique.*

Aims and Objectives:

1) *Evaluate the role of LM-ZN and light emitting diode (LED) fluorescent microscopy (FM) on spot samples collected 1 hour apart on first day and early morning sample collected on second day, in diagnosis of PTB.*
2) *Determine whether spot-specimen LED-FM is not inferior to conventional two specimens LED-FM.*
3) *Evaluate the diagnostic yield of overnight sputum with LM-ZN and LED-FM.*
4) *Compare the diagnostic yield between LED-FM and LM-ZN.*
5) *Determine the "loss to follow-up during diagnostic period".*

Methods: *All the presumptive PTB cases attending Department of Pulmonary Medicine, Navodaya Medical College Hospital and Research Institute, Raichur, including both in-patients and out-patients from 1st October 2011 to 30th April 2012 after informed consent were enrolled for the study. Total 3 sputum samples were collected from each patient (two spot sputum samples one hour apart and next day early morning sputum sample). Patients who failed to submit less than 3 sputum sample, were labelled as "lost to follow-up during diagnostic period". Four slides are prepared from each sputum specimen of which two slides from each sputum specimen are examined by LM following ZN staining and the remaining two slides are examined by FM following Auramine-O (AO) staining as per Revised National Tuberculosis Control Programme (RNTCP) guidelines.*

Results: *A total of 552 patients participated in our study, 492 patients (89.13%) submitted all the 3 sputum samples, whereas 60 patients (10.86%) were lost to follow-up during diagnostic period. Totally 80 cases (14.49%) were diagnosed as sputum smear positive (SSP) PTB. Among 492 cases who submitted all the 3 sputum samples, 67 cases (12.13%) were diagnosed as SSP-PTB and among the 60 cases lost to follow-up during diagnostic period, 13 (2.35%) cases were diagnosed as SSP-PTB. The 95% confidence interval (CI) for 1 hour apart sample is 9.88%-30.12% whereas the CI for the early morning sample is 94.45%-100%.*

Conclusions: *AO LED-FM is superior to ZN-LM in detection of SSP-PTB. Two smears prepared from a single sputum specimen had no potential role to improve the diagnostic efficiency of PTB among presumptive PTB cases. The loss to follow-up during diagnostic period in our study was 10.86%. Even though the yield is best with early morning sample, in view of high loss to follow-up during diagnostic period for early morning sputum sample, two spot sputum samples collected one hour apart and using AO-FM can be considered for diagnosis of PTB in presumptive cases for better global TB control.*

Key Words: *Tuberculosis, ZN Staining, Fluores Staining.*

INTRODUCTION

Since Robert Koch's discovery of Mycobacterium tuberculosis (MTB) in 1882, microscopic detection of the bacilli in clinical specimens has remained the mainstay of tuberculosis (TB) diagnosis in developing countries[1]. In PTB, sputum is the specimen of choice[2]. Despite recent advances in rapid diagnostic techniques, sputum smears microscopy remains the most widely used test in low-income countries and is likely the only means by which universal access to diagnosis and treatment can be achieved. The standard approach to smear microscopy under RNTCP involves sputum collection on two consecutive days and examination of sputum smears by LM-ZN staining technique or FM using fluorescent staining.

The sensitivity of LM-ZN staining is low, ranging from 20-60%[3,4]. Studies have reported that the additional yield of early morning sputum sample is around 10% when compared to spot sputum sample[5,6,7,8]. Studies have reported that the yield of same day smear examination results are as good as the conventional approach[9,10]. Two sputum smear approach does not take into consideration patients inconvenience for multiple visits to health center. As a result some patients fail to return and provide a second sputum specimen or receive the results thereby contributing to "loss to follow-up during diagnostic period"[11] which is as high as 50%, globally[12,13,14,15,16,17,18]. A recent meta-analysis found an average pre-treatment loss to follow up rate of 18% in African countries and 13% in Asian countries[19]. These patients are at high risk for spreading infection to others and have very high case-fatality rates.[17] In India, 4 studies found rates of pre-treatment loss to follow up ranging from 5-

22%[20,21,22,23] The reasons for loss to follow-up during diagnostic period could be patient-related like stigma leading to abandonment, ostracization and discrimination within the community and at the workplace, substance abuse, poverty, ignorance or health system-related like failure to contact patients with positive results, unfriendly staff, long waiting times at health centers. Hence urgent action is needed to reduce this loss to follow up.

The World Health Organization (WHO) has recommended that the maximum number of ZN smears examined by a microscopist in a day should not exceed 20, if attempted, visual fatigue will lead to deterioration of reading quality. FM is recommended where more than 50 smears are examined per day[24]. The sensitivity of LED-FM is 10% more than LM-ZN staining and can be done in 1/4th of the time needed for ZN-LM method[25].

We hypothesize that, with microscopic examination of two smears prepared from two spot samples collected 1 hour apart on same day, the sensitivity and specificity of case detection remains the same with reduced loss to follow-up during diagnostic period when compared to the conventional strategy.

AIMS AND OBJECTIVES

1. Evaluate the role of LM-ZN and LED–FM on spot samples collected 1 hour apart on first day and early morning sample collected on second day, in diagnosis of PTB.
2. Determine whether spot-specimen LED-FM is not inferior to conventional two-specimen LED-FM.
3. Evaluate the diagnostic yield of overnight sputum specimen with LM-ZN and LED-FM.
4. Compare the diagnostic yield between LED-FM and LM-ZN.
5. Determine the "loss to follow-up during diagnostic period".

MATERIAL AND METHODS

Inclusion criteria:

1. Presumptive PTB [26] patients as per RNTCP guidelines who submitted one or two or three sputum samples.

Exclusion criteria:

1. Patients who have taken anti-tubercular therapy more than 1 month.
2. Patients not consenting for the study.

Study design:

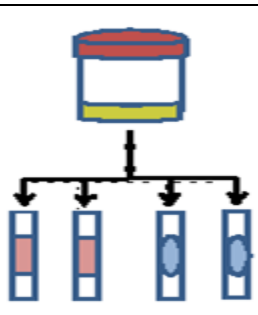
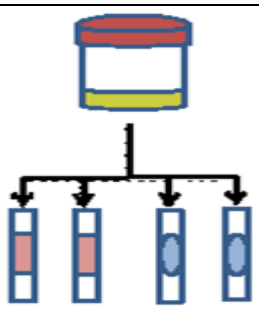
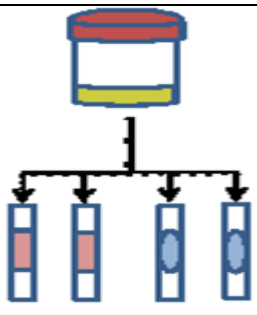
- Institutional based prospective, pilot study.

MATERIAL

Patients attending Department Of Pulmonary Medicine, Navodaya Medical College Hospital and Research Centre, Raichur, including both in-patients and out-patients from 1st October 2011 to 30th April 2012 after informed consent were enrolled in the study. Specimen used is sputum. Zeiss LED-FM microscope with AO staining and LM with ZN staining technique as per RNTCP guidelines were followed[27,28].

METHOD

Following Ethical Committee clearance, totally 3 sputum samples were collected from each patient (two spot sputum samples one hour apart and next day early morning sputum sample). Patients, who failed to submit less than 3 sputum sample, were labelled as "loss to follow-up during diagnostic period". Four slides are prepared from each sputum specimen collected, of which two slides from each sputum specimen are examined by LM following ZN staining and the remaining two slides are examined by FM following AO staining as per RNTCP guidelines[27,28].

<table>
<tr><th colspan="8">Day 1</th><th colspan="4">Day 2</th></tr>
<tr><th colspan="4">First Sample (X)</th><th colspan="4">Second Sample (Y)</th><th colspan="4">Third Sample (Z)</th></tr>
<tr><th colspan="4">Spot Sample</th><th colspan="4">1 Hour Apart Sample</th><th colspan="4">Early Morning Sample</th></tr>
<tr><td colspan="4">  </td><td colspan="4">  </td><td colspan="4">  </td></tr>
<tr><td>A</td><td>A1</td><td>A2</td><td>A3</td><td>H</td><td>H1</td><td>H2</td><td>H3</td><td>B</td><td>B1</td><td>B2</td><td>B3</td></tr>
<tr><td colspan="2">LM</td><td colspan="2">FM</td><td colspan="2">LM</td><td colspan="2">FM</td><td colspan="2">LM</td><td colspan="2">FM</td></tr>
</table>

LM	Light microscopy using ZN staining.
FM	Fluorescent microscopy *using* AO staining
X	Spot sputum sample
Y	1 Hour apart sample
Z	Early morning sample
A	Spot sample-ZN stained 1st slide
A1	Spot sample-ZN stained 2nd slide
A2	Spot sample-AO stained 1st slide
A3	Spot sample-AO stained 2nd slide
H	1 hour apart sample-ZN stained 1st slide
H1	1 hour apart sample-ZN stained 2nd slide
H2	1 hour apart sample-AO stained 1st slide
H3	1 hour apart sample-AO stained 2nd slide
B	Early morning sample-ZN stained 1st slide
B1	Early morning sample-ZN stained 2nd slide
B2	Early morning sample-AO stained 1st slide
B3	Early morning sample-AO stained 2nd slide

Fig. 1: Schematic representations of sputum smear microscopy strategy in our study

STATISTICAL ANALYSIS

Descriptive statistics such as frequency and percentage are used. As Mycobacterial cultures were not used we have considered B2 or B3 as gold standard (as all 67 cases were smear positive) done calculated sensitivity, specificity, positive predict value (PPV), negative predict value (NPV) and diagnostic accuracy. Comparison of diagnostic yield between different slides was done by using chi-square test or Fisher's exact test for small sample and reported 95% CI for "loss to follow-up during diagnostic period". p-value was calculated by applying statistical test chi-square test for comparison between slides. p-value of < 0.05 is considered as significant.

RESULTS

Total 552 patients were enrolled in our study. Of which, 360 patients (65.66%) were males and 192 patients (34.78%) were females. The majority of the patients (46.92%) were in the age group of 41-60 years. Among 552 patients, 492 patients (89.13%) submitted all the 3 sputum samples, whereas 60 patients (10.86%) were lost to follow-up during diagnostic period. Total 80 cases (14.49%) were diagnosed as SSP-PTB among the 552 patients. Among 492 cases who submitted all the 3 sputum samples, 67 cases (12.13%) were diagnosed as SSP-PTB and among 60 cases of lost to follow-up during diagnostic period, 13 (2.35%) cases were diagnosed as SSP-PTB. Among 60 cases of loss to follow-up during diagnostic period, 38 (63.33%) were males, 22 (36.67%) were females. Only 48 of them submitted Y sample (48/60;80%) having loss of follow up rate of 20% and only 1 case submitted Z sample (1/60; 1.6%) having loss to follow up rate of 94.45%. The 95% CI of loss to follow-up during diagnostic period for Y sample is 9.88%-30.12%, whereas for the Z sample 95% CI is 94.45%-100%. The combinations of the sputum sample X+Y was submitted by 48 cases, X+Z by 1, Y+Z by none.

Table 1: Percentage distribution of sputum samples submitted and loss to follow-up during diagnostic period cases on both day

Day	**First Day**								**Second Day**			
Sample	**Spot sample (X)**				**1 Hour apart sample (Y)**				**Early morning sample (Z)**			
Staining	ZN	ZN	FM	FM	ZN	ZN	FM	FM	ZN	ZN	FM	FM
Slide	A	A1	A2	A3	H	H1	H2	H3	B	B1	B2	B3
Samples submitted	60	60	60	60	48	48	48	48	1	1	1	1
%	100	100	100	100	80	80	80	80	1.7	1.7	1.7	1.7
Loss to follow-up during diagnostic period	0	0	0	0	20	20	20	20	98.3	98.3	98.3	98.3
95% CI of loss to follow up during diagnostic follow up					9.88%	9.88%	9.88%	9.88%	94.45%	94.45%	94.45%	94.45%
					30.12%	30.12%	30.12%	30.12%	100%	100%	100%	100%

Total SSP-PTB cases diagnosed from X sample excluding lost to follow-up during diagnostic period cases was 62 (62/492). In our study the total number of SSP-PTB cases diagnosed are 67 excluding lost to follow-up during diagnostic period cases. All the four sputum smear slides (A, A1, A2, A3) were positive in 48 patients (48/67; 71.64%), all the four slides were negative in 2 patients (2/67; 2.98%). Both the ZN stained slides (A, A1) were positive in 48 cases (48/67; 71.64%). Both the AO stained slides (A2, A3) were positive in 62 (62/67; 92.53%) cases. Total SSP-PTB cases diagnosed from Y sample excluding lost to follow-up during diagnostic period cases were 66 (66/492). In our study the total number of SSP-PTB cases diagnosed are 67 excluding lost to follow-up during diagnostic period cases. All the four sputum smear slides (H, H1, H2, H3) were positive in 47 patients (47/67; 70.14%), all the four slides were negative in 1 patient (1/67; 1.49%). Both the ZN stained slides (H, H1) were positive in 47 cases (47/67; 70.14%). The AO stained slides H2 was positive in 65 cases (65/67; 97.01%), whereas H3 was positive in 66 cases (66/67; 98.50%). Total SSP-PTB cases diagnosed from Z sample excluding lost to follow-up during diagnostic period cases were 67 (67/492). In our study the total number of SSP-PTB cases diagnosed are 67 excluding lost to follow-up during diagnostic period cases. All the four sputum smear slides (B, B1, B2, B3) were positive in 48 patients (48/67; 71.64%), all the four slides were negative in none (0/67; 0%). Both the ZN stained slides (B, B1) were positive in 48 cases (48/67; 71.64%). Both the AO stained slides (B2, B3) were positive in all the 67(67/67; 100%) cases. Total 80 cases (14.49%) were diagnosed as SSP-PTB among the 552 patients enrolled in our study. This included sputum from 53 (9.60%) patients diagnosed as SSP-PTB by both ZN and AO staining methods and sputum from an additional 27 (4.89%) patients were positive by AO staining only. Among 492 cases who submitted all the 3 sputum samples, total SSP-PTB cases detected were 67 (13.61%), 48 cases (9.75%) were diagnosed as SSP-PTB by both ZN and AO staining methods and sputum from an additional 19 (3.86%) patients were positive by AO staining only. Among the 60 (10.86%) cases of lost to follow-up during diagnostic period, 13 (21.66%) cases were diagnosed as SSP-PTB, 5 cases (8.33%) were diagnosed as SSP-PTB by both ZN and AO staining methods and sputum from an additional 8 (13.33%) patients was positive by AO staining only.

Table 2: Comparison of diagnostic yield between sputum smears

S. No	Slides Compared I vs. II	I (%)	II (%)	p-Value	Remark
1.	A vs. A1	71.6	71.6	p=1.0	Not significant
2.	A vs. A2	71.6	92.5	p=0.001	Highly significant
3.	A vs. A3	71.6	97	p<0.0001	Highly significant
4.	A1 vs. A2	71.6	92.5	p=0.001	Highly significant
5.	A1 vs. A3	71.6	97	p<0.0001	Highly significant
6.	A2 vs. A3	92.5	97	p=0.44	Not significant
7.	H vs. H1	70.1	70.1	p=1.0	Not significant
8.	H vs. H2	70.1	97	p<0.0001	Highly significant
9.	H vs. H3	70.1	98.5	p<0.0001	Highly significant
10.	H1 vs. H2	70.1	97	p<0.0001	Highly significant
11.	H1 vs. H3	70.1	98.5	p<0.0001	Highly significant
12.	H2 vs. H3	97	98.5	p=0.56	Not significant
13.	B vs. B1	71.6	71.6	p=1.0	Not significant
14.	B vs. B2	71.6	100	p<0.0001	Highly significant
15.	B vs. B3	71.6	100	p<0.0001	Highly significant
16.	B1 vs. B2	71.6	100	p<0.0001	Highly significant
17.	B1 vs. B3	71.6	100	p<0.0001	Highly significant
18.	B2 vs. B3	100	100	p=1.0	Not significant
19.	A vs. H	71.6	70.1	p=0.849	Not significant
20.	A vs. H1	71.6	70.1	p=0.849	Not significant
21.	A vs. H2	71.6	97	p<0.00001	Highly significant

22.	A vs. H3	71.6	98.5	p<0.00001	Highly significant
23.	A1 vs. H	71.6	70.1	p=0.849	Not significant
24.	A1 vs. H1	71.6	70.1	p=0.849	Not significant
25.	A1 vs. H2	71.6	97	p<0.00001	Highly significant
26.	A1 vs. H3	71.6	98.5	p<0.00001	Highly significant
27.	A2 vs. H	92.5	70.1	p=0.001	Significant
28.	A2 vs. H1	92.5	70.1	p=0.001	Significant
29.	A2 vs. H2	92.5	97	p=0.44	Not significant
30	A2 vs. H3	92.5	98.5	p=0.21	Not significant
31.	A3 vs. H	97	70.1	p<0.0001	Highly significant
32.	A3 vs. H1	97	70.1	p<0.0001	Highly significant
33.	A3 vs. H2	97	97	p=1.0	Not significant
34.	A3 vs. H3	97	98.5	p=1.0	Not significant
35.	A vs. B	71.6	71.6	p=1.0	Not significant
36.	A vs. B1	71.6	71.6	p=1.0	Not significant
37.	A vs. B2	71.6	100	p<0.0001	Highly significant
38.	A vs. B3	71.6	100	p<0.0001	Highly significant
39.	A1 vs. B	71.6	71.6	p=1.0	Not significant
40.	A1 vs. B1	71.6	71.6	p=1.0	Not significant
41.	A1 vs. B2	71.6	100	p<0.0001	Highly significant
42.	A1 vs. B3	71.6	100	p<0.0001	Highly significant
43.	A2 vs. B	92.5	71.6	p=0.001	Significant
44.	A2 vs. B1	92.5	71.6	p=0.001	Significant
45.	A2 vs. B2	92.5	100	p=0.06	Not significant
46.	A2 vs. B3	92.5	100	p=0.06	Not significant

47.	A3 vs. B	97	71.6	p<0.0001	Highly significant
48.	A3 vs. B1	97	71.6	p<0.0001	Highly significant
49.	A3 vs. B2	97	100	p=0.49	Not significant
50.	A3 vs. B3	97	100	p=0.49	Not significant
51.	B vs. H	71.6	70.1	p=0.849	Not significant
52.	B vs. H1	71.6	70.1	p=0.849	Not significant
53.	B vs. H2	71.6	97	p<0.0001	Highly significant
54.	B vs. H3	71.6	98.5	p<0.0001	Highly significant
55.	B1 vs. H	71.6	70.1	p=0.849	Not significant
56.	B1 vs. H1	71.6	70.1	p=0.849	Not significant
57.	B1 vs. H2	71.6	97	p<0.0001	Highly significant
58.	B1 vs. H3	71.6	98.5	p<0.0001	Highly significant
59.	B2 vs. H	100	70.1	p<0.0001	Highly significant
60.	B2 vs. H1	100	70.1	p<0.0001	Highly significant
61.	B2 vs. H2	100	97	p=0.496	Not significant
62.	B2 vs. H3	100	98.5	p=1.0	Not significant
63.	B3 vs. H	100	70.1	p<0.0001	Highly significant
64.	B3 vs. H1	100	70.1	p<0.0001	Highly significant
65.	B3 vs. H2	100	97	p=0.496	Not significant
66.	B3 vs. h3	100	98.5	p=1	Not significant

Further analysing the diagnostic accuracy by considering B2 and B3 as gold standard group (as all 67 cases were positive) are results are as follows

Table 3: Diagnostic accuracy

Slide	Sensitivity	Specificity	PPV	NPV	Diagnostic Accuracy
A	100	0	71.6	0	71.6
A1	100	0	71.6	0	71.6
A2	100	0	92.54	0	92.5
A3	100	0	97.01	0	97.01
H	100	0	70.1	0	70.1
H1	100	0	70.1	0	70.1
H2	100	0	97.01	0	97.01
H3	100	0	98.5	0	98.5
B	100	0	71.6	0	71.6
B1	100	0	71.6	0	71.6

Regarding our hypothesis, the sensitivity and specificity of case detection by microscopic examination of two smears prepared from two spot samples collected 1 hour apart on same day when compared to the conventional strategy, is statistically reduced but in view of SSP-PTB cases among lost to follow-up during diagnostic period, case detection by sputum microscopic examination of two smears prepared from two spot samples collected 1 hour apart is feasible.

DISCUSSION

Sputum smear examination for AFB is the key diagnostic tool used for diagnosis of PTB in RNTCP, as it is easy to perform at the peripheral laboratories, economic, has low inter and intra observer variation, simple, requires minimum training and can be used for diagnosis, monitoring and defining cure. If good diagnostic practices are followed, it is expected that at least 50% of the new PTB patients diagnosed, will be SSP[29].

Sputum smear examination in addition to being relatively insensitive for PTB diagnosis, it cannot distinguish viable and dead bacilli, cannot identify the species of Mycobacterium, this approach generally requires multiple visits to a health center and does not take patients inconvenience into account. For a variety of reasons, up to 50% of patients fail to return to provide a second specimen or receive results[17,30].

RNTCP previously recommended examination of 3 sputum smears for diagnosis of PTB. This was not practicable especially in difficult areas. It further added to the cost of diagnosis and causes inconvenience to patients. In order to study the diagnostic yield of examining only two smears and the additional yield by the third smear, a retrospective study of the data from the RNTCP area of the IRS Institute was carried out for the years 1998 and 1999. In 1998, in all, 719 SSP patients were diagnosed out of 3738 new chest symptomatics examined (19.2%). In 1999, there were 1044 SSP patients from 4189 new chest symptomatics examined (24.9%). However, sputum positivity of two or more sputum smears did not affect diagnostic yield. Further, of the three sputum smears examined (spot, early morning, spot), the early morning specimen had the best result. It was concluded that under field conditions, two sputum smears (one of which is early morning) is as effective as three smears for screening of chest symptomatics. Reduction in the number of smears to two is expected to reduce cost without compromising quality. However, before changing national programme policy, more studies in different situations was recommended[31].

A systematic review of 37 eligible studies that quantified the incremental diagnostic yield of serial sputum specimens was performed by Mase et al and published. The results clearly demonstrated that the vast majority of TB cases (on average 85.8%) were detected with the first sputum specimen. With the second sputum specimen, the average incremental yield was 11.9%, while the incremental yield of the third specimen, when the first two specimens were negative, was 3.1%[32]. Study conducted in Kenya by Bonnet et al. demonstrated that decreasing the number of smears examined for the detection of new PTB cases lead to a reduction of patient's visits to a clinic and the laboratory workload. Examining only two smears could therefore alleviate the workload of laboratories, particularly in countries with a high microscopy workload - by one third [33]. Under RNTCP, diagnosis of SSP-PTB new guidelines, effective from 1st April 2009, PTB presumptive cases at designated microscopy centers (DMC) are subjected for two sputum examinations,

with one of them being a morning sputum specimen[34]. We hypothesize that, with microscopic examination of two smears prepared from two spot samples collected 1 hour apart on same day, the sensitivity and specificity of case detection remains the same with reduced diagnostic dropout rates when compared to the conventional strategy. Recent studies have evaluated same-day smear microscopy performed using two specimens collected 1 hour apart and found the strategy to be as sensitive as smear microscopy performed using 2-day specimen collection.[35,36] However, to reduce the high direct and indirect patient costs and inconvenience associated with multiple health facility visits and loss to follow up for diagnosis, our findings suggest that collection of a two sputum samples one hour apart may be sufficient. The concept of examining multiple smears from a single sputum specimen is an age old one that has largely been forgotten. In 1949, Freiman and colleagues reported that examination of a second smear from the same specimen resulted in a 12% increase in the proportion of SSP specimens[37]. In 1969, Rao reported increased sensitivity when multiple smears were prepared from culture-positive specimens[38]. In 1993, Wilkinson and Sturm reported that performing one direct and one concentrated smear on a single specimen had increased sensitivity compared with direct or concentrated smears made from different specimens[39]. In our study, examination of the second slide from the same sample (A v/s A1, A2 v/s A3, H v/s H1, H2 v/s H3, B v/s B1, B2 v/s B3) has no statistically significant improvement in the diagnostic yield (Table 2).

Our study suggests that the sensitivity is increased with LED-FM on comparing with ZN-LM (Table 2). A systematic review of studies mostly from high income and low HIV prevalence settings reported similar findings: sensitivity was increased by 6% with LED-FM compared with LM[40]. The other advantages of LED-FM are that it has simpler technique, examined at a lower magnification; slides are read more quickly and efficiently than ZN-LM; and lower electric power requirements, longer lifespan relative to conventional fluorescence microscopes[41,42]. For a variety of reasons, up to 50% of patients fail to return to provide a second specimen or receive results [12,13,14,15,16,17,18]. In our study 60 patients (10.86%) lost to follow-up during diagnostic period. Among these, 13 (21.66%) cases were diagnosed as SSP-PTB, 5 cases (8.33%) were diagnosed as SSP-PTB by both ZN-LM+AO-FM methods and sputum from an additional 8 (13.33%) patients was positive by AO-FM method only. All the 60 cases lost to follow-up during diagnostic period, submitted the X sample (60/60; 100%) having loss to follow up rate of 0%, whereas 48 cases submitted Y sample (48/60; 80%) having loss to follow up rate of 20% and only 1 case submitted Z sample (1/60; 1.6%) having loss to follow up rate of 94.45%. The 95% CI for Y sample is 9.88%-30.12% whereas the CI for the Z sample is 94.45%-100%. Hence collecting two samples one hour apart on the same day would reduce the loss to follow-up during diagnostic period cases without reducing the diagnostic yield (Table 1). The combinations of the sputum sample X+Y was submitted by 48 cases, X+Z by 1, Y+Z by none. It was understood from table 3, that the PPV of H3 is 98.5% and diagnostic accuracy is 98.5%, which in turn, gives better diagnostic accuracy than other slides when B2 and B3 are considered as gold standard. The conventional dogma that sputum collection should occur over multiple days and include an overnight sample to increase the sensitivity of smear microscopy holds good. A systematic review reported an average 12% absolute increase in the proportion of SSP with examination of morning versus spot specimens based on only four studies[43]. However, more recent studies have shown no difference in the incremental yield of smear microscopy with spot versus morning specimens[44,45,46]. Perhaps due to differences in study populations, smear-positivity thresholds, and increased attention to sputum collection procedures. Although collection of an additional specimen may be warranted if the initial specimen is salivary, sputum collection on multiple days may not translate into increased SSP case detection after lost to follow-up during diagnostic period cases considered[47].

LIMITATIONS

Mycobacterial cultures were not used as gold standard for diagnosis of PTB due to financial constraints. Sample size is small and is a single center institutional study. The reasons for loss to follow-up during diagnostic period cases could not be elicited.

CONCLUSION

To summarise AO LED-FM is superior to ZN-LM in detection of SSP-PTB. Two smears prepared from a single sputum specimen had no potential role to improve the efficiency of evaluation for patients suspected of PTB. The loss to follow-up during diagnostic period in our study was 10.86%. Even though the yield is best with early morning sample, in view of high loss to follow-up during diagnostic period rate for early morning sputum sample, two spot sputum samples collected one hour apart and using AO LED-FM can be considered in diagnosis of PTB in presumptive cases for better global TB control.

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