
The Yield of First Spot Double Slide Smears for the Diagnosis of Pulmonary Tuberculosis

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Abstract: Background: Direct sputum smear microscopy is the cornerstone for the diagnosis of pulmonary tuberculosis (PTB) in resource-poor countries. However, the requirement for repeated visits to submit specimens and receive results is associated with considerable diagnostic delay, work load, patients drop-out and high expenses for patients. Although the World Health Organization (WHO) has recently changed its policy from spot morning spot (SMS) to spot morning (SM), the SM method still involves two days visits for a patient. Objective: This study evaluated the yield of first spot double slides smears for the diagnosis of PTB in high TB setting. Methods: A total of 362 patients who visited the out-patient department (OPD) of Dilla referral hospital and who were suspected of PTB were involved in the study. In addition SMS sputum samples were collected; double slides smears were prepared, stained by the Ziehl–Neelsen (ZN) method and 100 fields were examined under oil immersion objective for acid fast bacilli (AFB). Results: Of 362, 54(14.92%) were smear-positives. Out of the 54 smear positive subjects, 53 (98.15%) were positives by the first spot specimen. Additionally, 1 of 54 (1.85%) were positives by the morning specimen. Using the 2-day protocol (SMS) among 362 patients, 54(14.92%) were smear positives by double slides and 53(14.64%) by single slide smears. Whereas using the 1-day protocol (first spot); among 362 patients, 53(14.64%) were smear positives both in double slides and single slide smears. Conclusion: The double slides smears from the first spot sputum samples appeared to be as effective as SMS strategy for the diagnosis of PTB though additional studies are required under various settings.

Keywords: Microscopy, AFB, Sputum, PTB

1. Background

Pulmonary tuberculosis (PTB) is a chronic infectious disease that remains an important public health problem throughout the world [1]. Hence, early diagnosis and treatment are essential to prevent the transmission of TB in the community. Direct sputum smear microscopy is the cornerstone of the diagnosis of PTB in resource-poor countries though it is less sensitivity. It is relatively cheap, rapid and simple to perform and identifies the most infectious cases amongst PTB patients [2, 3]. However, the requirement for repeated visits to submit three specimens and receive results are associated with considerable diagnostic delay, technicians work load, low quality of laboratory services, patients' drop-out and unnecessary expenses for patients [4, 5]. Study showed that sputum smear microscopy using the three smear strategy also consumed the annual laboratory budget than any other investigation [6].

Studies showed controversial results regarding the number of sputum smears require to reduce laboratory work load, patients' time and cost. Recent study on the yield of SMS has shown that the majority of patients with smear-positive PTB are identified by the SM sputum specimens [3]. Zlwu et al. (2000) Saleem et al. (2007) suggested three sputum smear examinations for the effective diagnosis of PTB in high TB endemic areas [7, 8]. On the other hand, two sputum specimens (SM) were suggested as sufficient for the effective diagnosis of PTB [3, 6, 9-12]. A systematic review on the yield of serial sputum specimen examinations in the diagnosis of PTB suggested that the average incremental yield and/or the increase in sensitivity of examining a third specimen ranged between 2% and 5%. Hence, reducing the number of specimens examined from three to two (particularly two specimens collected on the same day) could benefit TB control programs, and potentially increase case detection for several reasons [13]. A retrospective study on records of patients with culture-proven PTB showed that 97%

of AFB was detected from the first specimen and only 3% was additionally obtained from the second smear [14]. A study conducted on one-day method for the diagnosis of PTB in rural Ethiopia, indicated that the same day approach would reduce the number of visits required for diagnosis, save resources for the health system and the patient, and ultimately improve case detection in poorer countries [15]. Furthermore, a study on an alternative 1-day smear microscopy protocol for the diagnosis of PTB showed that collection of a morning sample on the second day provided no significant benefit over collection of a second spot sample on the first day [16].

Even though WHO has recently changed its policy from SMS to SM [1], still SMS is done in Ethiopia. Moreover, in Ethiopia, mostly technicians are responsible for all routine laboratory activities of the health facility and are often short of time. Moreover, patients walk a long distance to reach health facilities in rural areas of Ethiopia and spend two-three days outside their home. Hence, the present study was performed to assess the feasibility of diagnosing PTB in a single day, using double slides smear using the first spot sputum sample.

2. Methods

2.1. Study Area and Population

A cross-sectional study on evaluation of the yield of the first spot double slides smear microscopy for the diagnosis of PTB using ZN staining method was conducted at Dilla University referral Hospital. It is found in Dilla City administration which is located 360 kilometer far away from the capital city, Addis Ababa, in the south of Ethiopia. It is the public hospital which is an affiliate of Dilla University providing training for health sciences student in a range of disciplines. Additionally, the hospital provides higher level of clinical care for nearly a million of catchment area populations. The hospital was purposely selected for the study because of it is only referral hospital for the diagnosis and treatment of TB. All patients (age ≥ 15 years) who were clinically suspected of PTB and sent to Dilla referral hospital laboratory for AFB and able to provide sputum samples were included in the study.

2.2. Sputum Sample Collection and Examination

Three sputum samples (SMS) were collected from the study participants using a coded disposable plastic sputum cup with a lid following the national guidelines for the diagnosis of PTB in Ethiopia. Each individual was instructed on how to expectorate a sputum sample and expectoration instructions given to patients was as per normal practice at the hospital. The quality of specimen was recorded on the data collection sheet as muco-purulent, purulent, blood stained (bloody) or partial saliva. Then, double slides smears were prepared from first day spot, early morning and the second day sputum samples. The smears were stained by the ZN method and observed under oil immersion for AFB

following the WHO guide line [17]. In order to avoid bias all the single and double smears and also their reporting or grading system was done the same an experienced technician. Additionally, randomly selected 10% negative and all positive smear results were reexamined another trained technician, who was unaware of the study protocol. The socio-demographic characteristics of the participants (name, age, sex) as well as sputum smear results were recorded on the data collection sheet. In accordance with the standard operating procedure for Bacteriology and reporting system of Dilla referral hospital, all smears with ≥ 1 AFB/100 high power fields (HPF) were considered positives [18]. At least 100 microscopic fields were examined to declare a slide negative. In case of positive smear, the bacterial load was classified (1 to 9 AFB per 100 fields, 10 to 99 AFB per 100 fields; 1 to 10 AFB per field after examining at least 50 fields and more than 10 AFB per field after examining at least 20 fields) using standard operating procedure for Bacteriology and reporting system of Dilla referral hospital and WHO guidelines [18, 19].

2.3. Data Analysis

The data was entered into Epi Data version 3.1 and analyzed using STATA version 8. The detection yield of the first spot double slides smear and the incremental yields of the morning and 2nd spot were calculated. Patients' characteristics were compared using the chi-square test. A p -value of < 0.05 was accepted as indicating statistical significance.

2.4. Ethical Consideration

The study protocol was reviewed and approved by Dilla University ethical clearance committee. Each individual was instructed on how to expectorate a sputum sample and expectoration instructions given to patients was as per normal practice at the hospital.

3. Results

Table 1. Socio-demographic characteristics of the study participants.

Gender	Total (%)	Positive (%)	Negative (%)
Male	170(46.96)	24(6.63)	146(40.33)
Female	192(53.04)	30(8.29)	162(44.75)
Age group			
15-24	70(19.34)	8(2.21)	62(17.13)
25-34	98(27.07)	14(3.87)	84(23.20)
35-44	88(24.31)	18(4.97)	70(19.34)
45-54	54(14.92)	8(2.21)	46(12.71)
55-64	32(8.84)	6(1.66)	26(7.18)
65 and above	20(5.52)	0(0.00)	20(5.52)

A total of 392 patients (age ranged from 15 -79 years, mean age, 36.47 years) submitted sputum samples for AFB smear microscopy examination. Out of these, 30 (7.65%) participants provided only the first spot sputum sample for smear microscopy and they excluded from the finally data analysis. Of the total number recruited, 60 patients (15.31%) were found smear-positives and 332 (84.69%) were found to

be negative by smear microscopy. Table 1 shows the socio-demographic characteristics of 362 patients who submitted three sputum specimens and included in the final analysis. Out of the 362 patients, 54(14.92%) were found positives for AFB.

Table 2 shows the results of double slides smears in six different combinations and table 3 shows the yield of positive smears identified on first spot, early morning and second spot specimens of 362 suspects. Of the 54 positive smears, 53 (98.15%) were positives by the first spot specimens. An

additional 1.85% (1 of 54) was positive by the early morning specimen, but not by the first spot. On the other hand, 3.7% (2 of 54) was negative by the second spot specimen, but not by the previous two samples. Overall, using 2-day protocol (SMS) among 362 patients, 54(14.92%) were smear positives by the double slides smears, while 53(14.64%) were positives by the single smear. Using the 1-day protocol (first spot); 53(14.64%) patients were positives by double slide and single slide smears.

Table 2. Results of double slides smear of 362 suspects that have different results in six combinations.

Total	PpPpPp %	PpPpNp %	PpPpNn %	NpPpPp %	NnNpNn %	NnNnNn %
362	50(13.81)	1(0.28)	2(0.55)	0(0.00)	1(0.28)	308(85.08)

Key: P-Positive for 1st slide, p- positive for 2nd slide, N-Negative for 1st slide, n- negative for 2nd slide

Table 3a. Incremental yield of positive, first spot, early morning and second spot specimen of 362 suspects for double slides smear.

Total suspects with 3 sputum samples	At least 3 positives n(%)	At least 2 positive n (%)	At least one positive n (%)	Positive by 1 st spot smear PXX n (%)	Positive by early morning smear NPX n (%)	Positive by 2 nd spot smear NNP N (%)
362	50(13.81)	53(14.64)	54(14.92)	53(98.15)	1(1.85)	0(0.00)

Table 3b. Incremental yield of positive, first spot, early morning spot and second spot specimen of 362 suspects for single slide smear.

Total suspects with three sputum samples	At least three positive n (%)	At least two positive n (%)	At least one positive n (%)	Positive by 1 st spot smear PXX n (%)	Positive by early morning smear NPX n (%)	Positive by 2 nd spot smear NNP n (%)
362	50(13.81)	53(14.64)	53(14.64)	53(98.15)	0(0.00)	0(0.00)

Key: P-Positive, N-Negative for 1st slide, n- number of patients, X-negative or positive

Generally, 1086 sputum specimens were examined. Out of 38 muco-purulent specimens, 28 (73.68%) were positives, whereas out of 406 purulent specimens, 128(31.53%) were positives. None was positive out of 28 bloody specimens. Out of 614 partly saliva specimens, 6 (0.98%) were positives. There was a significant difference between appearance of sputum samples and AFB positivity ($p < 0.001$). But there was no difference in appearance between the first spot, early morning and second spot sputum samples from the same individual.

Of the slides reported as positives, 26.54%, 45.68%, 26.54% and 1.23% were found to be 3+, 2+, 1+ and scanty in grading, respectively (Table 4). The bacterial load grading was differed according to the appearance of the sputum ($p < 0.05$). However, the bacterial load grading did not vary between the first spot, the early morning or the second spot sample ($P = 0.952$).

Table 4. Bacterial load grading according to sputum appearance.

Grading system	Sputum appearance			
	Muco-purulent	Purulent	Partly saliva	Total
Scanty	0(0.00)	2(1.56)	0(0.00)	2(1.23)
1+	4(14.29)	35(27.34)	4(66.67)	43(26.54)
2+	2(7.14)	70(54.69)	2(33.33)	74(45.68)
3+	22(78.57)	21(16.41)	0(0.00)	43(26.54)
Sub total	28(100.00)	128(100.00)	6(100.00)	162(100.00)

4. Discussion

Direct sputum smear microscopy is considered as the cornerstone for the diagnosis of PTB in resource-poor countries. It is relatively cheap, simple and identifies the most infectious cases. However, the requirement for repeated visits to submit specimens and receive results is associated with considerable diagnostic delay, work load and drop-out of patients from the diagnostic process [4,5,6]. This study was designed to assess the yield of first spot double slides smears for the diagnosis of PTB in high TB setting. The results revealed that the yields of the double slides smears from the first spot and the single slide smear from SMS samples had no significant difference.

Studies carried out on one day protocol [5,15,20] for the diagnosis of PTB showed that the yield of two sputum samples in the first day and sputum collection on two days protocol had not significant difference. In present study, 53 of 54 (98.15%) smear positive subjects were detected by the first spot specimens, while an additional 1.85% (1 of 54) was found positive by the early morning specimen. A recent systematic review quantified the diagnostic yield of each of three sputum specimens showed that the average incremental yield and/or the increase in sensitivity of examining additional sputum samples ranged between 2% and 5% [13]. Studies conducted on 2-days sputum collection method for the diagnosis of PTB also showed that the average

incremental yield and/or the increase in sensitivity of examining a third specimen was < 5% [3, 6, 9, 10, 11, 12] which indicated that reducing the number of specimens examined from three specimens to two specimens could benefit TB control programme by reducing laboratory work load and patients' time and expense.

Recently, the WHO has changed its policy from SMS to SM sputum smears for the diagnosis of PTB [1]. However, SM method does not reduce patients' visit and drop out, since it requires two days specimen collection. The present study showed that the double slides smears (1-day protocol) for the diagnosis of PTB is as effective as the two-day protocol currently recommended by the Ethiopian national guideline for the diagnosis of PTB [21]. Thus, the one-day protocol would miss about 1.85% of the patients identified by the three-specimen strategy, as suggested by a previous systematic review [13]. This loss may not significantly affect the diagnostic yield of PTB and can be improved by proper instruction of patients on how to expectorate a sputum sample, by increased the number of slides and increased quality of microscopy [22]. Moreover, reducing the number of sputum specimens and clinic visits would reduce the diagnostic delay, workload of laboratory staff and patient's costs. On the other hand, a reduction in the number of slides could increase the proportion of false negativity especially in patients with low bacterial load [6]. The morning sputum sample is considered to contain respiratory secretions that have accumulated overnight and hence, most likely to be positive [5]. However, the findings of the present study indicated that there was no difference between the first spot, morning or second spot sputum samples collected from the study participants in terms of appearance or smear grade.

A study conducted on the number of sputum smears require for case finding in PTB in southern region of Ethiopia reported higher number of smear-positive patients drop out during the diagnostic process than the gain achieved by requesting a third specimen [6]. A study on the value of three sputum smears showed that about 24% of PTB suspects failed to return after submitting the first spot specimen and 4.5% of these suspects were smear positives [7]. In addition, study carried out on front loading sputum microscopy services showed that $\geq 10\%$ of patients failed to return for the second day of diagnosis [4]. Similarly, in the present study, among the 30 suspects who failed to return after submitting the first spot specimen, 6(10%) were found to be smear-positives based on the results of the first day samples. However, as the Ethiopian national guideline of TB control recommends three sputum smear microscopic examination before dispatching smear examination results. Those patients who were found positives, but defaulted on the second day would remain unaware of their smear status and were not referred for treatment. This indicated an additional limitation of the two-day protocol for the diagnosis of PTB, while the one-day protocol could reduce drop-out rates of patients from the process of diagnosis.

The present study has its own limitations. One of the limitations of the present study was that smear-negative and

smear-positive cases were not confirmed by culture as well as by molecular methods, because of budget constraint. It would also be important to collect information on the effect of HIV infection on the one-day protocol for the diagnosis of PTB. However, data on HIV status of the patients was not collected as this issue was not indicated in the study protocol and approved by Dilla University ethical clearance committee.

5. Conclusion

The results of this study showed that double slides smears from the first spot sputum (one-day protocol) would miss about 1.85% of the patients identified by the SMS strategy. The loss is likely to be compensated by increased quality of sample collection and microscopic examination.

Since data were not showed the smear status on HIV/ADIS patients and even smear negative result didn't confirm on culture. Nevertheless, this study tried to provide base line information about double smear PTB diagnosis using first spot. Thus, the double slides smears from the first spot sputum samples appeared to be as effective as SMS strategy for the diagnosis of PTB though additional studies are required under various settings.

Author Contributions

Basha A. designed the study, participated in data collection, analysis and manuscript

Basha A. is the guarantor of the paper

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