A Study on Adequacy of Sputum Samples to Detect Pulmonary Tuberculosis

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ABSTRACT:
INTRODUCTION: Tuberculosis is one of the commonest infectious diseases in Nepal. The current guidelines specify that pulmonary tuberculosis suspects are required to submit three sputum samples the first and the third being spot specimens taken at the centre, and the second one an early morning sputum. The aim of this study was to find out adequacy of two versus three sputum specimens to detect pulmonary tuberculosis.

METHOD: This analytic descriptive study was conducted among patients suspected to have pulmonary tuberculosis over a period of six months. Three samples of sputum smear (Spot S1-Morning M-Spot S2) were collected from a suspect from over two consecutive days. Smear microscopy with Zeihl-Neelsen (ZN) staining was performed. Numbers of AFB were calculated and tabulated as per hundred immersion fields and analyzed.

RESULT: In this study 1614 cases were included; 162 patients were smear-positive. In 122 cases acid fast bacilli were detected in the first specimen; while the second specimen detected 40 more cases and none were detected from the third specimen. The proportion of positives were different for S1, M and S2 (Cochran’s Q =54.12, p<0.001). Mc Nemar test shows that morning and S2 test revealed similar distribution of positives and negatives (p=0.72), however, morning and S1 samples showed significant difference of proportion of positives (p<0.001). Similar results were found for S1 and S2 (p<0.001) also.

CONCLUSION: Under field conditions, 2 sputum smears are as effective as 3 smears for diagnosing smear-positive tuberculosis. If the first 2 samples were negative, the possibility of missing a third positive sample was very low.

KEY WORDS: Anti-tubercular agents; pulmonary tuberculosis; sputum microbiology; predictive value for Mycobacterium tuberculosis. The technical guidelines of WHO specify that pulmonary tuberculosis (PTB) suspects are required to submit three sputum samples the first and the third being spot specimens taken at the centre, and the second one an early morning sputum. A diagnostic strategy based on 3 serial smears maximizes sensitivity, but it has substantial drawbacks. First, it implies a vastly increased number of false-positive patients who will be put on treatment. Second, doing 3 sputum smear examinations for each patient suspected of having TB may overload these laboratories.

INTRODUCTION
Tuberculosis (TB) remains one of the commonest infectious diseases in Nepal as almost half of the over 20 million population is infected with TB. Of these, up to 90 000 people have active TB and there are 44 000 new cases of the disease every year.

Early diagnosis and treatment are essential to prevent transmission of disease in community. Smear microscopy with Zeihl-Neelsen (ZN) staining is currently the most feasible microbiological method for diagnosis of pulmonary TB in developing countries due to its rapidity, low cost, and high positive predictive value for Mycobacterium tuberculosis. The technical guidelines of WHO specify that pulmonary tuberculosis (PTB) suspects are required to submit three sputum samples the first and the third being spot specimens taken at the centre, and the second one an early morning sputum. A diagnostic strategy based on 3 serial smears maximizes sensitivity, but it has substantial drawbacks. First, it implies a vastly increased number of false-positive patients who will be put on treatment. Second, doing 3 sputum smear examinations for each patient suspected of having TB may overload these laboratories.

Various studies in different countries like India and other countries in Africa they have shown that PTB cases can be diagnosed by doing two smears examination rather than the present recommended three, saving time as well as cost. Although many studies have been conducted in other countries, such studies in Nepalese population is scarce. In a study from the DOTS clinic of Kanchanpur district concluded that under routine
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conditions, two sputum smears (one of which is early in the morning) can be recommended in place of three smears for screening the chest symptomatics. Study from National Tuberculosis Center, Thimi, observed that the first two smears examination was sufficient to detect 95% of the sputum positive patients with overall positivity in the group were 14.78%.

The adequacy of two sputum samples for the diagnosis of PTB has to be readdressed considering methods, significance and costs. The objective of this study was to find the adequacy of two versus three sputum specimens for the diagnosis of pulmonary tuberculosis in developing country like Nepal where there are limited health personnel and significant cost consideration. The reduction in the workload may give more time to an over burdened laboratory for improving quality of sputum microscopy. So this study was conducted.

METHOD

After approval from the institutional review board and informed consent from the study participants, a prospective analytic descriptive hospital based study was conducted at a regional hospital in the western region of Nepal over a period of six months.

All outpatients with suspected tuberculosis defined as a patient with cough for more than three weeks with or without haemoptysis, evening fever, weight loss, night sweats or anorexia were included for sputum examination. Diagnosed, follow-up or referred case of known pulmonary tuberculosis and children below five years of age were excluded from the study.

Operational definitions

A PTB suspect: any patient attending the outpatient department with cough for more than 3 weeks with or without haemoptysis /evening fever/weight loss/anorexia.

A smear positive PTB patient is a patient who has at least one Positive sputum sample for acid fast bacilli.

Criteria for the diagnosis of pulmonary tuberculosis:

Smear-positive case was defined as at least two positive smears, or one positive smear and radiographic abnormalities compatible with pulmonary tuberculosis, or one positive smear and one positive culture.

Smear-negative case was defined as at least three negative smears and one or more positive cultures, or at least two series of negative smears from samples taken at least 2 weeks apart, with persisting radiographic abnormalities compatible with active tuberculosis, not improved with treatment using broad-spectrum antibiotics for at least 1 week.

The following grading method for smears stained by Ziehl-Neelsen (immersion lens 100)26 was used.

No AFB /100 immersion field =0
1-9 AFB /100 immersion field =0
10-99 AFB /100 immersion field = +
1-10 AFB/field = ++
More than 10 AFB/field = +++

STATISTICAL ANALYSIS

Data was analysed using IBM SPSS Statistics 16 (IBM, New York, USA). Descriptive data was summarised using standard techniques and reported as percentages and means with standard deviation. The Cochran’s Q test was used to determine the differences on a dichotomous dependent variable between three related groups. McNemar’s test was used on paired nominal data. A p-value less than 0.05 were considered significant.

RESULT

Over the study period of six months, a total of 1614 patients suspected to have pulmonary tuberculosis were included in the study. Incremental yield of positive, first, second and third specimens of 1614 suspects are presented in table 1.

Table 1: Incremental yield of positive, first, second and third specimens

<table>
<thead>
<tr>
<th>Total suspects with three sputum smears</th>
<th>At least two positive n (%)</th>
<th>At least one positive n (%)</th>
<th>Positive identified on first smear PNN n (%)*</th>
<th>Positive identified on Second Smear NPN n (%)*</th>
<th>Positive identified on Third smear NNP n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1614</td>
<td>162(10.03%)</td>
<td>0(0%)</td>
<td>122(7.56%)</td>
<td>40(2.4%)</td>
<td>0(0%)</td>
</tr>
</tbody>
</table>

P-positive, N-negative
Three sputum smear results of 1614 suspects in eight possible combinations are presented in Table 2.

Table 2: Number of positive samples and grading of smears

<table>
<thead>
<tr>
<th>Grade</th>
<th>S1 Frequency</th>
<th>S1 Percent</th>
<th>M Frequency</th>
<th>M Percent</th>
<th>S2 Frequency</th>
<th>S2 Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>1+</td>
<td>70</td>
<td>4.3</td>
<td>62</td>
<td>3.8</td>
<td>70</td>
<td>4.3</td>
</tr>
<tr>
<td>2+</td>
<td>25</td>
<td>1.5</td>
<td>52</td>
<td>3.2</td>
<td>48</td>
<td>3.0</td>
</tr>
<tr>
<td>3+</td>
<td>27</td>
<td>1.7</td>
<td>44</td>
<td>2.7</td>
<td>38</td>
<td>2.4</td>
</tr>
<tr>
<td>4+</td>
<td>0</td>
<td>2.5</td>
<td>1</td>
<td>0.1</td>
<td>1</td>
<td>0.1</td>
</tr>
<tr>
<td>Total positive</td>
<td>122</td>
<td>7.56</td>
<td>159</td>
<td>9.9</td>
<td>157</td>
<td>9.7</td>
</tr>
</tbody>
</table>

Three sputum smear results of 1614 suspects in eight possible combinations are presented in Table 3.

Table 3: Results of three sputum smears in eight possible combinations

<table>
<thead>
<tr>
<th>Total Suspects</th>
<th>PPP n</th>
<th>PPN n</th>
<th>PNP n</th>
<th>PNN n</th>
<th>NPP n</th>
<th>NPN n</th>
<th>NNP n</th>
<th>NNN n</th>
</tr>
</thead>
<tbody>
<tr>
<td>1614</td>
<td>114</td>
<td>5</td>
<td>3</td>
<td>0</td>
<td>40</td>
<td>0</td>
<td>0</td>
<td>1452</td>
</tr>
<tr>
<td>%</td>
<td>7.06</td>
<td>0.3</td>
<td>0.18</td>
<td>0</td>
<td>2.478</td>
<td>0</td>
<td>0</td>
<td>89.96</td>
</tr>
</tbody>
</table>

The proportion of positives are different for S1, M and S2 (Cochran’s Q =54.12, p<0.001). However, McNemar test shows that morning and S2 test reveals similar distribution of positives and negatives(p=0.72), morning and S1 showed significant difference of proportion of positives(p<0.001). Similar results were found for S1 and S2(p<0.001).

DISCUSSION

In this study 1614 cases were included. Among them, 162 patients were smear positive in any two samples, while other 1452 patients were found negative in all three sputum samples. Among the positive cases 122 patients had first specimens being positive. A further 40 patients were positive on the second specimen but not the first. There were no patients who had positive on the third specimen but not on the previous two.

It is a well-accepted fact that the smear positivity yield improves as the number of sputum specimens per PTB suspect increases. National guidelines of tuberculosis control recommend that microscopic examination of three sputum specimens is necessary for the evaluation of persons suspected of having pulmonary tuberculosis. The optimum number of sputum specimens required to establish diagnosis of tuberculosis has been documented in several studies.

In our study 162(10.03%) patients had at least one positive smear result confirmed by second smear examination. Positivity in any two samples was in 10.03%. Among smear positive, 7.56% AFB were detected in the first specimen while the second specimen detected further 40(9.9%) patients and third smear detected 9.7%. The third specimen did not contribute to find a new positive case. The proportion of positives are different for S1, M and S2 (Cochran’s Q =54.12, p<0.001). However, McNemar test shows that morning and S2 test reveals similar distribution of positives and negatives(p=0.72), morning and S1 showed significant difference of proportion of positives(p<0.001). Similar results were found for S1 and S2 (p<0.001).

A similar study conducted in Kasturba Medical College, Manipal University showed among 1762 suspected patients, and positivity in any 2 samples was found to be 17.7% while 19% were found to be positive in a single smear. A statistically insignificant association was found between the grading and positivity of the sputum samples using McNemar test. A positive third sample was found in 309 patients. If the first 2 samples were negative, the possibility of missing a third positive sample was 0.4%. Another similar studies in Nairobi showed that 53% AFB were detected from the first specimen while the second specimen detected 40% and third spot specimen contributed the remaining 8% cases. A study conducted in Zambia, found 77.1% AFB on first smear, a further 15% on the second smear and 7.9% additional cases were identified on the third smear. A reanalysis of data from a study involving 42 laboratories in four high burden countries showed that incremental yield from a third smear ranged 0.7-7.21%. The classic studies on incremental yield by
Nelson, found an incremental yield of around 5% on third sputum examination. A rigorously conducted systematic review of 41 studies found that, on average, the second smear detected about 13% and the third smear detected 4% of all smear positive cases.

In this study first smear detected 122 patients, second smear detected further 40 and the third detected none of smear positive cases. It also important to discuss what happens to patients who have one positive slide out of three. Those with positive smear are infectious and are responsible for the spread of disease. This makes the clinicians alert, these patients should have a further two specimens collected and if at least one is positive, the patient would be declared smear positive. In present study 10.03% of suspected PTB had two or three positive smears with combination of PPN, NPN, and PPP, whereas none of the suspects had a single positive smear with a combination if PNN, NNP and NPN.

Sputum microscopy is likely to be positive when there are at least 10,000 organisms per milliliter of sputum. In this study, first spot sputum specimen among the positive, most of 70 cases (4.3%) were found to be 1+ on sputum smear microscopy, indicating low number of bacilli while (25)1.5% had 2+, (27) 1.7% had 3+ and (0) 0% had 4+ indicating high number of bacilli. Sputum, which forms the basis of bacteriological diagnosis, is a variable source material. Type of specimen, its quality, quantity, bacterial content and viability of organism considerably influence the sensitivity and the specificity of the methods; and these in turn would vary under different diagnostic situations. The low grading (1+) in one smear positive 70(4.3%) cases may be partially explained by the fact that they have either minimal disease or without extensive cavitations. These patients cannot expectorate sputum properly resulting in insufficient sputum sample or saliva and contributed to isolated positives.

The need for systematic confirmation of an AFB positive result to diagnose a case of tuberculosis has been challenged. Critics argued that this practice, result in fewer cases being put on treatment, considering the numbers that do not return after a first positive smear. Three smears are helpful for case finding in those suspects where first or second sputum smear is negative. In our study 162(10.03) % of suspects fulfilled the case definition, having at least one positive smear result confirmed by another smear examination.

Considerable debate exist on reconsidering of examination of three sputum smears because more than two sputum samples were not rewarding, resulting in extra burden in areas where human and financial resources are significantly limited. These studies are in agreement with present study as incremental yield of third sputum smear is 0% However third specimen cannot be ignored in a country like Nepal where tuberculosis is one of the major health challenges. Three smears are preferable, in our setting cause no greater inconvenience to the patient than two because they are done at two consecutive days (spot, early morning, spot).

Out of the study population of 1614 patients, positivity in any two sputum samples was found in 162 suspects. In 122 patients AFB were detected in the first specimen while the second specimen detected further 40 patients that were not positive on the first. There were no patients who had positive results on the third specimen but not on the previous two. So the third specimen contributed none to the detection of positive cases.

In conclusion, under field conditions of western Nepal, two sputum smears were as effective as three smears for diagnosing smear-positive tuberculosis. If the first two samples were negative, the possibility of missing a third positive sample was very low.

However, there were few limitations to apply in clinical Practice like
1) The study was done in single hospital which may not reflect condition of the whole nation. 2) The duration of study was short.
3) Sputum culture was not taken as a gold standard in our study.
4) Moreover, laboratory error might have occurred as the study was done in single hospital lab.

It is clear that further experimental research is suggested for its use in clinical practice in future. In particular, randomized controlled trials should be conducted involving multiple hospitals in the country.

REFERENCES
A Study on Adequacy of Sputum Samples to Detect Pulmonary Tuberculosis

15