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Original Research Article

Factors affecting sputum and culture conversion in pulmonary tuberculosis patients on directly observed treatment, short-course

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ABSTRACT

Background: Sputum smear positive patients are infectious for a variable period after starting of treatment. Patients receiving anti-tuberculosis treatment by DOTS become noninfectious and times taken to become non-infectious are assessed by sputum smear conversion (SSC) by smear microscopy and culture conversion by growth on Lowenstein-Jensen medium. The aim of present study was to determine the time taken for SSC and culture conversion and factors delaying it.
Methods: A prospective cross-sectional study was undertaken in a tertiary hospital over a period of one year from January 2015 to December 2015 by Department of Respiratory medicine. 150 patients diagnosed as pulmonary tuberculosis by sputum smear microscopy and on DOTS were included and followed at regular intervals of 4th, 8th, 12th, 16th and 20th week or until they were sputum and culture negative which was earlier. At each follow-up sputum, smear and culture were done as per standard guidelines.
Results: 150 patients were enrolled which included 63 (42%) males and 87 (58%) females with mean age of 36.41 years and all were followed up. Fever was the most common clinical symptom (98.67%). 146 patients (97.33%) underwent sputum and culture conversion. The median time taken for sputum conversion for cases in the study was by the end of 4th week [day 28] and culture conversion was by end of 5th week [day 35]. 4 cases were positive for sputum and culture which were seropositive for HIV.
Conclusions: Smear-positive patients are infectious to close contacts and to the community. Hence initiation of infection control measures should be applied until the patient is non-infectious. The results of our study reveal that patients of smear positive pulmonary tuberculosis continue to expel the bacilli for a considerable period of time after initiation of DOTS regimen. There is a strong need to reinforce infection control measures until the patients are judged noninfectious.

Keywords: DOTS, Pulmonary tuberculosis, Sputum smear conversion

INTRODUCTION

Tuberculosis is one of the diseases as mentioned in WHO report that records around 1.5 million deaths worldwide with a majority of young people and adults. The incidence of TB has increased significantly after emergence of HIV pandemic throughout the world. India is said to be one among the 22 high burden countries as reported by world health organization, with annual recorded cases of 1.2 million as per 2015 global annual WHO report.[1] There is a resurgence of TB after HIV with more mortality, morbidity, and drug resistance. To combat this overwhelming increase, India adopted the WHO recommended "TB control strategy".[2] Effective implementations of diagnostic facilities, control measures, and treatment modalities are part of this

strategy. One of the key components in this strategy is diagnosis through a quality assured network of TB direct microscopy centers. Implementation of Directly observed therapy short course (DOTS) is one of the important factors which has been adopted in RNTCP to control and treat newly diagnosed cases of tuberculosis.

As a measure to monitor the treatment response, sputum acid-fast bacillus smears (AFB) and cultures of *Mycobacterium tuberculosis* are regularly checked during follow-up.

Sputum smear positive (SSP) tuberculosis patients are significant source of infection and should be initiated DOTS regimen with two months of intensive and four months of continuation phase. Sputum sterilization achieved by effective DOTS, determined by sputum smear conversion [SSC] is a cardinal index of treatment success. 80 - 90% of patients achieve sputum smear conversion by end of 2nd or 3rd month of treatment.[3]

However multiple factors influence the outcome of SSC which may delay or prolong the SSC and culture conversion which may be DM, HIV, initial bacillary load at time of diagnosis, duration of symptoms, hepatic disorder, old age, unilateral or bilateral involvement of lung with or without cavitary lesions and multi-drug resistant tuberculosis [MDR-TB].[4,5]

Documentation of sputum and culture conversion is recommended as necessary criterion for stopping anti-TB treatment as per new treatment guidelines.[6] Infection control measures are required to control the spread of pulmonary tuberculosis until noninfectiousness is demonstrated by sputum and culture conversion. SSC is less sensitive than culture conversion, but can be reported earlier. But lack of facilities and prolonged time taking are limitations in assessing the culture conversion.[7]

Data available on factors influencing the outcome of SSC and culture conversion in India are few and limited. The present study was undertaken to determine the time taken to smear and culture conversion in Category I DOTS patients receiving uninterrupted therapy and to determine factors that prolong smear and/or culture conversion.

Smear conversion is defined as new smear-positive PTB cases who became smear negative after a period of anti-TB treatment and are therefore no longer infectious (confirmed by at least two consecutive negative sputum acid-fast bacilli (AFB).

METHODS

It was a prospective cross-sectional study which was conducted for a period of one year from January 2015 to December 2015 at Santhiram Medical College and Hospital a tertiary care hospital, Andhra Pradesh. The study was approved by the Hospital research committee and Institutional ethical committee. The study was done by Department of Respiratory medicine in association with Department of Microbiology.

Patient profile

All the patients attending the Department of Respiratory medicine and diagnosed as pulmonary tuberculosis by sputum smear microscopy were enrolled in the study. Two consecutive sputum specimens were collected from the patients [Spot and early morning] and performed Ziehl – neelsen [Zn] staining and smear positive specimens by microscopy were graded as per RNTCP guidelines.[8] The positive specimen was decontaminated and homogenized by N-acetyl-L- homocysteine sodium hydroxide method and sediment was inoculated on Lowenstein-Jensen medium [LJ].

Culture specimens were observed daily for one week, biweekly thereafter up to twelve weeks. The absence of growth until 12 weeks was declared as negative for culture. Any growth on LJ medium was confirmed by acid fastness and species identification by colony characters, rate of growth, heat labile catalase test and Niacin test.[9] All the positive cases were started on Direct observed therapy short course [DOTS] and followed up at regular intervals of 4th, 8th, 12th, 16th and maximum 20th week of treatment or until patients were smear and culture negative whichever was earlier. At each follow-up two early morning sputum specimens on consecutive days were collected and performed Zn staining and Culture on LJ medium.

Informed consent was obtained from all the patients enrolled in the study. The clinical, demographic and social profiles of patients (age, gender, comorbidities etc) were interviewed at time of enrolment and entered in a separate predesigned questionnaire form. Involvement of lung unilateral or bilateral with or without cavities was confirmed by chest physician by X-ray and any kind of hepatic disorder was evaluated by performing Liver function profile [LFT].

Exclusion criteria

Cases not adhering to the DOTS regimen, cases of extra pulmonary tuberculosis, patients who didn't attend the full follow-up, cases of age less than 12 years were excluded from the study.

Statistical analysis

Smear conversion was the time in weeks from initiation of treatment to first of the two serial negative smears for AFB. Culture conversion was the time in weeks from initiation of treatment to first of the two serial negative mycobacterial cultures. Median time in weeks to smear and culture conversion was calculated and used for further analysis. Data was entered into Microsoft Excel. Factors that influence and prolong sputum and culture

conversion were analyzed and a P value <0.05 was considered significant.

RESULTS

In present study, a total of 150 patients were enrolled which included 63 (42%) males and 87 (58%) females. Patients were aged between 19 to 67 years.

Table 1: Socio, clinical, demographic profile of cases in study.

Profile	No	%
Gender		
Male	63	42
Female	87	58
Age		
<20 years	2	1.33
>21 -30 years	38	25.33
>31 -40 years	59	39.33
>41 -50 years	37	24.67
>51 years	14	9.33
Smoking		
Yes	78	52
No	72	48
Alcohol		
Yes	75	50
No	75	50
BCG		
vaccinated	138	92
Non-vaccinated	12	8
HIV		
Reactive	12	8
Non -reactive	138	92
DM	42	28
Non-Diabetic	108	72
Hepatic disorder		
Present	59	39.33
Absent	91	60.67
Lung involvement		
Unilateral	51	34
Bilateral	99	66
Cavity		
Present	78	52
Absent	72	48
Clinical symptoms		
Fever	148	98.67
Cough with expectoration	124	82.67
Haemoptysis	96	64.00
Loss of appetite	112	74.67
Grading of smear by microscopy		
3+	37	24.67
2+,1+, Scanty	113	75.33

The mean age for both men and women in the study was 36.41 years and for men 36.57 years, women 36.41 years.

The most common age group affected in the study was between 31 -40 years. The social, demographic and clinical profile with comorbidities are shown in Table 1.The most common clinical symptom of presentation was fever (98.67%) followed in order by a cough with expectoration (82.67%), loss of appetite (74.67%) and hemoptysis (64%). 12 cases (8%) in the study were HIV co-infected, 42 cases (28%) were diabetic controlled and 59 cases (39.33%) were suffering from a hepatic disorder (Eg: Cirrhosis, fatty liver, Hepatitis etc) (Table 1).

All the specimens collected at the time of diagnosis were performed Ziehl- neelsen staining and culture on Lowenstein –Jensen medium. All the specimens' positive by microscopy grew *Mycobacterium tuberculosis*. The average time taken for the growth was 22 days and earlier growth was observed in specimens with 3+ grading when compared with 2+, 1+ and scanty. The average time taken for culture positivity for scanty,1+,2+ and 3+ was 26, 24,23, and 18 days.

All the patients recruited in the study were able to complete the study by strict follow-up measures taken. The median time taken for sputum conversion for cases in the study was by the end of 4th week [day 28] and culture conversion was by end of 5th week [day 35]. Smear conversion was observed earlier than culture conversion in the total study. In the entire study group, 4 cases (2.67%) remained positive for smear and culture until 20th week.

In our study by the end of 4th week, sputum smear conversion (SSC) was seen in 54 cases (36%) and culture conversion in 28 cases (18.67%) followed by 8th week with additional 38 cases a gain by 25.33% by sputum conversion and culture conversion with 38 cases and gain by 25.33%.

By the end of 12th week in our study 116 cases (77.33%) were sputum negative and 92 cases (61.33%) were culture negative and additional 21 cases (14%) turned sputum negative by end of 16th week and 32 cases (21.33%) were culture negative by end of 16th week. By the end of 20th week, 4 cases (2.67%) remained both sputum and culture positive.

All the cases which remained positive were HIV seropositive (Table 2). Out of 54 cases which turned out sputum negative by end of 4th week, 15 were scanty, 23 and 16 were 1+ and 2+. In additional 38 cases, which became sputum negative by 8th week, 10, 16, 12 were scanty, 1+ and 2+. None of the cases with 3+ sputum grading were negative by end of intensive phase.

By end of 12th week, additional 16 cases of 2+, 8 cases with 3+ turned out sputum negative. Of total 137 sputum negative cases by end of 16th week, 26,39, 45 and 27 were scanty,1+,2+ and 3+. By the end of 20th week of follow-up 146 cases, additional one case of scanty, 1+ and 2+ became negative but 7 cases of 3+ became

negative (Table 3). In the total study none of the cases with 3+ grading turned culture negative by end of 12th week, 14 cases, and 34 cases turned culture negative by 16th and 20th week. Out of total 46 cases graded 2+, 28 turned culture negative by 12th week, additional 27 by end of 16th and last one by end of 20th week.

Table 2: Sputum conversion rates by microscopy.

Time period	No of positives	No of negatives	% of positives	% of conversion
End of 4th week	96/150	54	64	36.00
End of 8th week	58/150	92	38.67	61.33
End of 12th week	34/150	116	22.67	77.33
End of 16th week	13/150	137	8.67	91.33
End of 20th week	4/150	146	2.67	97.33

Table 3: Sputum culture conversion rates.

Time period	No of positives	No of negatives	% of positives	% of conversion
End of 4th week	122/150	28	81.33	18.67
End of 8th week	84/150	66	56.00	44.00
End of 12th week	58/150	92	38.67	61.33
End of 16th week	26/150	124	17.33	82.67
End of 20th week	4/150	146	2.67	97.33

All the 4 cases which remained sputum and culture positive until 20th week are HIV seropositive with bilateral involvement of lung with cavitary lesions. Two of them were having cirrhosis of liver.

DISCUSSION

Regular follow-up of pulmonary tuberculosis cases on DOTs treatment, by sputum smears and cultures, are thought to be one of the major and reliable predictors of treatment response. Studies have shown that nonconversion of positive smears at the end of the two months of treatment is one of the strongest predictors for treatment failure.[10-12] However this factor alone in consideration is not effective because of low positive predictive value.[13]

Smear positivity alone does not permit us to know whether they are viable or not. Hence culture conversion can be a reliable indicator to predict noninfectiousness of a patient. But lack of facilities, long time for growth by conventional method makes it as a limitation. The duration of infectiousness after the initiation of effective treatment is still a subject of discussion. Many of the studies on sputum conversion are limited to developed countries and in India are limited.

In present study, we highlighted the time taken for smear conversion and culture conversion as a measure of noninfectiousness and factors that delay in patients on DOTS treatment. The follow up was done regularly at end of 4th, 8th, 12th, 16th and 20th week because, patients treated under CAT I would be judged as "treatment failure" if they were smear-positive at the end of 5th month of treatment.

In present study out of 150 cases, 97.33% (146/150) had a favorable treatment outcome as they became smear and culture negative by the end of 20th week. This is more than the target cure rate of 85% under the Millennium Development goals. [14] The median time taken for smear conversion was 28th day and culture conversion by 35th day with a gap of 1 week between both. In present study also smear conversion preceded culture conversion as seen in many studies. Findings of our study were in contrast to findings of Long et al where median time for sputum conversion was 43 days and only 34.4% of patients were culture negative at time of sputum conversion.[15] Findings of our study were on par with Telzak et al who reported smear conversion by end of 23 days and culture conversion by 26 days.[16] some studies reported culture conversion prior to smear conversion which may be due to excretion of dead tuberculous bacilli in sputum, which was not observed in our study.[17,18]

Out of 150 patients, four remained sputum and culture positive until 20th week indicating the possibility of MDR-TB and were shifted to Cat-II, while 146 patients completed CAT-I treatment. Patients who were smear and culture negative by the end of 20th week were considered successfully cured.[19] Smear conversion and culture conversion started increasing gradually from 3rd and 4th week of starting of DOTS regimen.

By the end of 16th week, 91.33% of cases turned sputum negative; this is comparable to national average of 90%.

This is similar to the findings of Long et al and Pachas et al who reported in their studies the 93% of SSC by the end of 16th week. [20, 21] In prersent study four patients remained sputum and smear positive by the end of 20th week of treatment and was shifted to CAT-II regimen. The possible reason for this may be drug resistance of *My.tuberculosis* which was not undertaken in our study which is a limitation. Based on the findings of this study, and depending on resources available, Drug susceptibility testing (DST) should be carried out on all such patients who continue to remain smear-positive at the end of the 2nd month or 3rd month and should not be delayed until the end of 5th month.

This would prevent spread of MDR-TB in case the continued smear positivity is due to drug-resistant strains. This is especially important in a country like India with high rates of primary drug resistance.[22,23] Statistical significance for analysis of risk factors causing delayed sputum and culture conversion was observed in patients with initial high bacillary load (3+), hepatic disorder, HIV positivity and bilateral lung involvement with cavitary lesions. DM was not a significant risk factor in delayed sputum and culture conversion. These findings are on par with findings in the studies of Gopi PG et al, Holtz TH et al and Guler M et al where high sputum bacillary load and cavitary lesions are significant risk factors in delayed conversion of sputum smear and culture conversion.[24-26]

Cavities in the lung contain millions of TB bacilli and bilateral involvement is one more associated condition which causes high bacillary load and leads to development of drug-resistant strains. HIV positivity is one more risk factor observed in many studies; in our study 10 cases were positive and completed the study. Four of the cases were sputum and culture positive by end of 20th week. All the four had bilateral cavitary lesions and two of them had cirrhosis of liver which is an additional risk factor which delays conversion. Patients with TB who are HIV co-infected are at increased risk of developing MDR strains. Rest of the six cases had delayed sputum and culture conversion which may be cavitary lesions and HIV coinfection. Hence persons diagnosed with HIV in TB infection should be performed DST to screen MDR cases to modify the regimen if necessary based upon DST.[27]

CONCLUSION

Smear-positive patients are infectious to close contacts and to the community. Hence initiation of infection control measures should be applied until the patient is noninfectious. The results of our study reveal that patients of smear positive pulmonary tuberculosis continue to expel the bacilli for a considerable period of time after initiation of DOTS regimen. Most of the patients undergo sputum conversion by the end of 3rd month and culture conversion by the end of 5th week. Patients who have cavitary disease, high pre-treatment smear grade, a past history of tuberculosis or MDR-TB are more likely to remain smear and culture positive for longer periods of time. Hence there is a need to reinforce DST at the starting of DOTS to prevent the development of MDR strains and failures. There is a strong need to reinforce strong infection control measures until the patients are judged noninfectious.

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