Original Article

A DOUBLE-BLIND STUDY OF ORAL SALBUTAMOL SUPPLEMENT AND REPEAT SPUTUM SMEAR MICROSCOPY IN ENHANCING DIAGNOSIS OF SMEAR-NEGATIVE PULMONARY TUBERCULOSIS IN SOUTH INDIA

R. Balasubramanian, R. Rajeswari, A.C. Naik, M. Duraipandian, K. Karunakaran and P.R. Narayanan

(Received on 5.5.2004; Accepted on 20.7.2004)

Summary:
Background: As routine culture facilities are not available in TB control programme in low income countries like India, there is an urgent need to improve the sensitivity of sputum microscopy, especially in diagnosis of smear negative pulmonary TB.
Methodology: In a double blind placebo controlled study, the role of repeat sputum microscopy after antibiotics and oral salbutamol supplement in improving the diagnosis of smear negative TB suspects was investigated in an urban TB clinic. We undertook culture examinations for all study patients to find out proportions of TB cases in this series.
Results: Of 206 enrolled, (101 salbutamol (S), 105 placebo (P) groups) 26 were positive by repeat sputum smear examination; similar in two groups (S 16, P 10, p = 0.25). In all, 40 (S 23, P 17) including 26 smear- positives, were culture -positive for *M. tuberculosis*.
Conclusions: Two thirds of initially smear negative but culture positive TB patients were smear positive on repeat sputum examination. Thus, repeat sputum smear microscopy for TB suspects improved the diagnosis, nevertheless oral salbutamol therapy was not beneficial. In resource poor settings, repeat sputum smear microscopy after a trial of antibiotics, could significantly improve the diagnosis of smear-negative PTB patients. ***[Indian J Tuberc 2004; 51:191-198]***

Key words: Diagnosis of smear –negative TB, Repeat sputum microscopy after salbutamol, TB control programme, Diagnostic algorithm

INTRODUCTION

Direct microscopic examination of sputum for acid - fast bacilli remains a cornerstone for the diagnosis of tuberculosis in both low income and industrialized countries. Even though the identification of acid - fast bacilli in a sputum specimen is highly specific for the diagnosis, it is estimated that in most developing and technically advanced countries, at least half the diagnosed cases of pulmonary tuberculosis are likely to be smear negative[1]. These patients constitute a substantial case load. Accuracy of the diagnosis of smear-negative pulmonary tuberculosis (PTB) in resource – poor countries in the absence of culture facilities, particularly under programme conditions, is challenging as this group of TB suspects is likely to include persons suffering from both tuberculosis and non-tuberculous respiratory infections. Secondly, their diagnosis is essentially made based on the presence of clinical symptoms supplemented by chest radiography. This practice might lead to over or under diagnosis.

The diagnosis of smear negative PTB cases is vital, as these cases are likely to break down to smear positive cases, if left untreated. A break down rate of about 28% in 6 months and 40% in two years has been reported[2,3]. Furthermore, the treatment regimen recommended in the programme is also different for smear–negative PTB cases; smear-negative PTB patients get 3 drugs in the initial phase as against 4 drugs in the treatment of smear–positive PTB cases[4]. Thus, there is a pressing need to develop tools to improve the accuracy of diagnosis of smear negative PTB.

WHO has recommended repeat sputum smear microscopy for TB suspects with persistent symptoms after adequate antibiotics[5]. This is not recommended as a routine procedure in Indian programme. Hence this step of repeat sputum smear microscopy needs to be evaluated under field conditions in India.

Smear negativity of sputum may also be due to limitations in collection or examination of poor quality sputum specimens. For good sputum

Correspondence: Dr. P.R. Narayanan, Director, Tuberculosis Research Centre, Mayor V.R. Ramanathan Road, Chetput, Chennai-600 031
E-mail: nrparanj@md2.vsnl.net.in

microscopy, prescribed methods of collecting sputum specimens, staining procedures and reading of sputum smears have to be accurately followed. The chance of getting a false negative result is high if any one of the above instructions is not attended to. Difficulty in expectoration may arise if there is associated airway obstruction[6]. Salbutamol has been reported to be effective in improving the quality of sputum in females who could not produce adequate sputum and thus the sensitivity of diagnosis by sputum microscopy[7].

.

With the objective of improving the accuracy of diagnosis of smear negative pulmonary tuberculosis, we undertook a randomized, double–blind, placebo-controlled study in an urban tuberculosis control programme (DOTS strategy) in south India to investigate the following: the role of repeat sputum smear microscopy following oral salbutamol supplement for TB suspects with persistent symptoms despite adequate antibiotics. This study will also throw light on utilization of available infra-structure for chest radiography in the peripheral health units as this is recommended in the diagnostic algorithm of the programme.

MATERIAL AND METHODS

Setting

Revised National Tuberculosis Control Programme (RNTCP) incorporating DOTS strategy was implemented in 10 Corporation zones of Chennai City in 1999 covering a population of about 5,000,000. All these units have a good outpatient attendance and programme staff were trained in RNTCP. The diagnostic algorithm for the diagnosis of tuberculosis is as follows: The physician advises tuberculosis suspects (cough of 3 weeks or more) to undergo three sputum smear examinations and sputum samples are collected on two days. Sputum specimens are examined by Ziehl-Neelsen's method. Patients with at least two positive smears for acid-fast bacilli are classified as smear-positive tuberculosis. Persons with all 3 negative smears are prescribed broad-spectrum antibiotics for 1-2 weeks (collected once in 2 days). If symptoms persist even after adequate antibiotics, suspects are expected to re-attend the health centres. Chest radiograph is recommended for persistent symptomatics and, if the radiographic findings are consistent with tuberculosis, the patients are classified as smear negative pulmonary tuberculosis and started on anti-tuberculosis treatment.

In order to maintain good quality sputum microscopy, a senior tuberculosis laboratory supervisor checks routinely all the positive slides and a sample of negative sputum smear slides.

Study Centre

One urban corporation zone catering to a population of 400,000 population was chosen. This included one microscopy centre and 7 treatment centres. This centre had one Medical officer, one senior treatment supervisor, one laboratory technician and one senior laboratory treatment supervisor. All of them have been trained in RNTCP and the laboratory was subjected to routine quality control under the programme. The average new OPD attendance was 6900 per month and sputum smear examinations were performed for about 95 – 100 chest symptomatics per month. This centre did not have an X-ray facility. The TB suspects with persistent symptoms after a course of antibiotics were referred to a neighbouring unit within 2 kilometers for chest radiography. Mass miniature radiographs were taken and results were written in the referral slip to be reported back to the study unit for further management.

At Corporation Study Centre (CSC)

In a double blind randomized placebo controlled clinical study (approved by institutional ethical committee), it was planned to enrol 200 smear-negative tuberculosis suspects attending the study centre. The sputum smear slides of these patients were once again checked and confirmed to be negative. All smear-negative tuberculosis suspects with persistent symptoms after adequate antibiotics who returned to the participating centre were screened. Patients with history of cardiac, renal or hepatic diseases or with a history of hypersensitivity to salbutamol or pregnancy were excluded. Eligible

patients were briefed about the study and informed consent was obtained from every person enrolled in the study. History of smoking habits and previous episodes of asthma were also noted.

At Tuberculosis Research Centre (TRC)

A statistician prepared, as per random numbers, envelopes with salbutamol or a similar looking placebo tablets. Patients enrolled into the study were administered either salbutamol 2 mg or placebo as per random covers plus cotrimoxazole and trimethoprim twice a day for the first 3 days, under supervision of a clinic nurse at the study centre. On the fourth and fifth days, 2 sputum specimens (spot and early morning) were collected for smear and culture examinations for *Mycobacterium tuberculosis* were performed by a modification of Petroff's method to estimate the actual proportion of TB patients in this series[8]. Even though in India culture examination for *M.tuberculosis* is not a routine practice under programme conditions, this was undertaken as the gold standard for TB diagnosis is positive culture for *Mycobacterium tuberculosis.*

Chest Radiography

The Corporation study centre referred suspects with 3 sputum smears negative for AFB to the neighbouring unit for chest radiography. Mass miniature radiographs were taken and results were written in the referral slips. Suspects with radiographic findings suggestive of TB reported back to the CSC for anti-tuberculosis treatment. The other suspects did not re-attend the CSC.

Treatment

The bacteriological results were intimated to the CSC for further management. Suspects with positive smear or culture for *Mycobacterium tuberculosis* were started on anti-tuberculosis treatment at the study centre as per RNTCP guidelines.

Analysis

After completion of intake, decoding was done at TRC and the repeat smear results of the two groups (placebo vs salbutamol) were compared in terms of yield of smear-positive results in sputum specimens of culture-positive patients. The data were analysed using "Epiinfo" and SPSS. The results were also correlated with smoking habits and asthmatic episodes.

RESULTS

Study population

In all, a total of 230 smear negatives suspects were enrolled in the study in one year 2001 (Fig. 1). Of these, 24 were excluded for various reasons: 13 failed to complete the exercise and 11 had previous history of hypersensitivity to salbutamol.

Pre- treatment characteristics

Of the remaining 206, 101 (M 46, F 55) were in salbutamol (S) group and 105 (M 60, F 45) in placebo (P) group (Table 1). The age distributions of patients were similar in both the groups (< 40 years age – S 69, P 65). There were 39 (P 20, S 19) smokers and 26 (S 8, P13) asthmatics.

Bacteriological investigations

Repeat sputum smear results

Fig 1 compares the smear and culture results of the repeat sputum samples of the two groups. In all, 10 (9.5%) of 105 patients in the P group and 16 (15.8%) of 101 in the S group were smear-positive for AFB after repeat sputum microscopy and the difference was not significant (P = 0.24).

Culture results

All the 26 (S 16, P 10) repeat smear-positive patients were also culture-positive for *M. tuberculosis.* In addition, another 14 (7 in each group) were positive only by culture. Thus, a total of 40 (S 23, P 17) of 206 smear-negative TB suspects were culture-positive TB cases. The proportion of smokers and asthmatics was similar in both the groups.

Table 1: Baseline characteristics of chest symptomatics enrolled in salbutamol (n=101) and placebo (n=105) groups.

	Placebo		Salbutamol	
Total No. of pts.	105	(%)	101	(%)
Sex				
Female	45	43	55	54
Male	60	57	46	46
Age				
<40	65	62	69	68
>=40	40	38	32	32
Repeat Smear*				
Negative	95	90	85	84
Positive	10	10	16	16
Smokers				
No	85	81	82	81
Yes	20	19	19	19
Asthma				
No	92	88	93	92
Yes	13	12	8	8
S+C+	10	9.5	16	15.8
S+C0	0	-	0	-
S0C+	7	6.7	7	6.9
S0C0	88	83.8	78	77.2

*P=0.25

Smear grading

With reference to the yield of positivity, the smear grading was similar in home sputum and clinic collection specimens. Eighty percent of them had one smear positive.

Chest radiography

Of 206 smear negative cases, 56 suspects had reported back to the study unit with report from an x-ray unit that chest radiographic findings were suggestive of TB and were started on anti-tuberculosis drugs (Fig 2). Among them both repeat smear and culture were positive for *M.tuberculosis* in 7 patients and additional 4 were culture positive. Both smear and culture were negative in 45 (80%) patients including two patients who in addition had tuberculous lymphadenitis.

Categorisation of smear negative patients

In all, 97 (47%) of 206 tuberculosis suspects were diagnosed as tuberculosis. However,

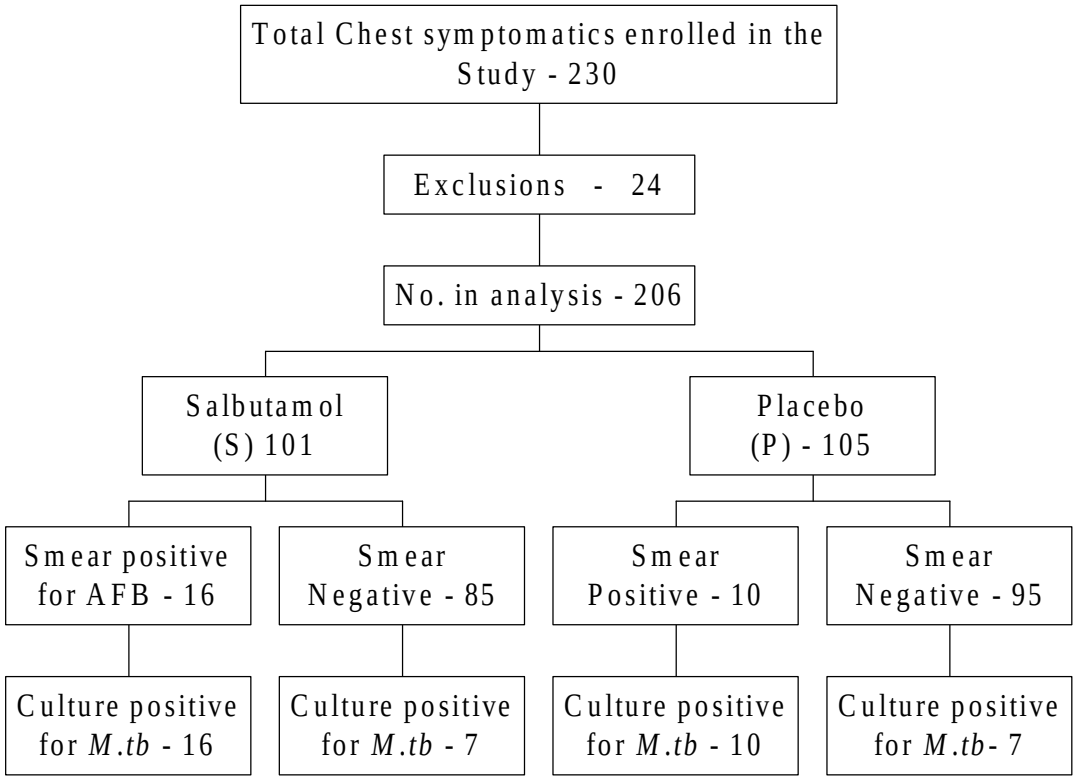


Fig.1: Patient enrolment with smear and culture results of salbutamol and placebo groups

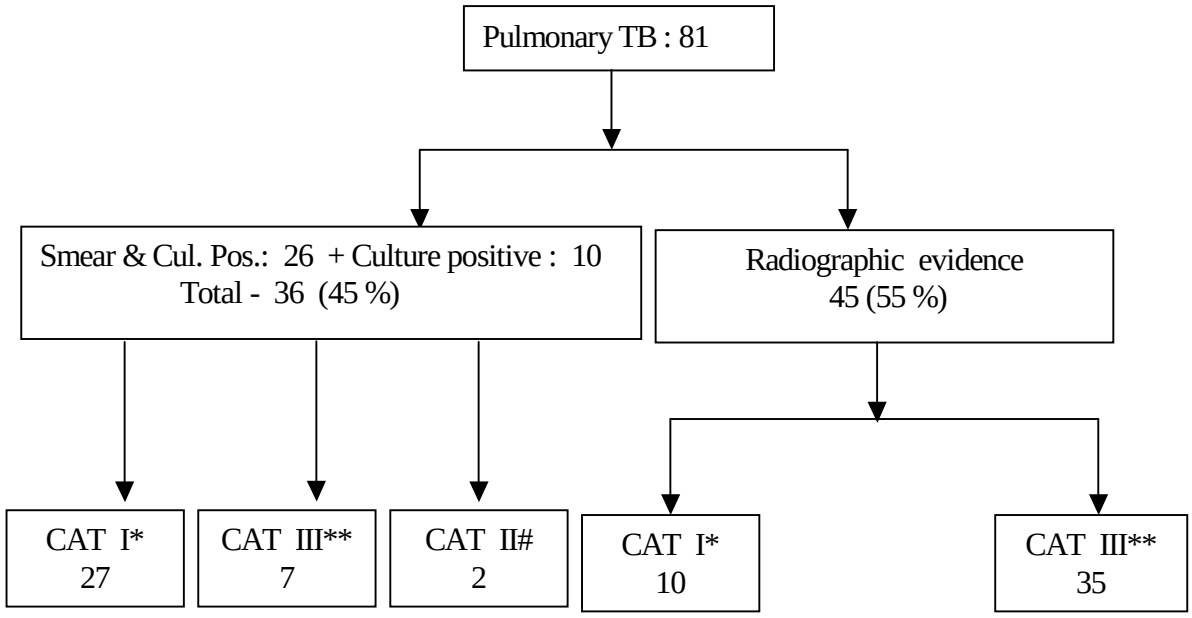
only 93 were started on anti-tuberculous treatment, 81 for pulmonary tuberculosis and 12 for extra-pulmonary tuberculosis. The remaining four-culture positives (4%) of 97 tuberculosis patients diagnosed were not started on anti-tuberculous treatment because when the culture results became available the patients could not be traced (Table 2).

DISCUSSION

The most important finding of this study is that repeat sputum smear microscopy after a course of antibiotics is very useful in getting confirmation of the disease among smear negative pulmonary TB cases. Of the initially smear-negative and culture (*Mycobacterium tuberculosis*) positive pulmonary TB patients, 65% (26 of 40) yielded positive sputum smears for acid-fast bacilli on repeat sputum examination and another 14 cases were only positive on culture. Thus, by repeat sputum microscopy, the disease was confirmed in 65% of the culture positive cases and this enabled patients getting appropriate treatment. In addition, among patients started on treatment for pulmonary tuberculosis at the CSC , repeat sputum microscopy was useful in confirming the disease in 45% of cases.

A study from South Africa had reported that sputum smear microscopy plus a trial of antibiotics improved the diagnostic accuracy among suspects[9] . In that study, the decision to start anti-tuberculosis treatment (ATT) was based on results of sputum smear microscopy plus non-response to a course of broad-spectrum antibiotics. By adopting this diagnostic algorithm, over diagnosis of patients was 20 % and under diagnosis was 20%. But with repeat sputum smear microscopy, all the smear positive cases were also culture positive and hence there was no over diagnosis. In our series, two thirds of culture positive cases in 206 TB suspects could be detected

Table 2: Repeat sputum results related to categorization of treatment



* 2 $(HRZE)_3$ / 4 $(HR)_3$ ** 2$(HRZ)_3$ / 4 $(HR)_3$ # 1 $(HRZES)_3$ / 2 $(HRZE)_3$ / 5 $(HER)_3$

H =Isoniazid, R = Rifampicin, Z = Pyrazinamide, E=Ethambutol, S = Streptomycin

Note: The number before the letters refers to the number of months of treatment.
The subscript after the letters refers to the number of doses per week.

by the additional step of repeat sputum smear examinations after a course of antibiotics. Thus, the diagnostic algorithm currently used in the Indian programme will yield better results if we include this additional step of repeat sputum smear microscopy after a course of antibiotics.

In India, under the DOTS strategy, chest radiograph is recommended as a routine procedure for sputum smear-negative PTB suspects with persistent symptoms after antibiotics,. This raises questions as to how far this procedure is feasible in the current setting and whether this procedure is being followed in health centres situated in the periphery. In this study, the smear negative suspects with persistent symptoms were referred to a neighbouring unit for taking chest radiograph as the facilities were not available in the CSC. Only the patients with chest radiograph suggestive of TB were referred back to CSC for treatment. Information on the proportion of tuberculosis suspects who had undergone chest radiography at the neighbouring centre and the results of the chest radiographs for all of them,were not available. This reflects the real situations of the peripheral health centres in India and the practical difficulties faced by them in adhering to the diagnostic algorithm.

Our study highlights the limitations in interpretations of chest radiographic findings in the diagnosis of tuberculosis. Out of 206 patients, 56 (27%) patients reported back with radiographic evidence suggestive of pulmonary tuberculosis of whom 80% were negative by smear and/or culture for *M.tuberculosis*. On the other hand, in this series, 4% of culture positive TB patients were missed and were diagnosed based on chest radiographic findings. Similar observations were reported from a study from Malawi, where 31% of 172 tuberculosis suspects diagnosed as normal or non-tuberculous based on X-ray findings had positive mycobacterial cultures for tuberculosis[10]. In addition, the chest

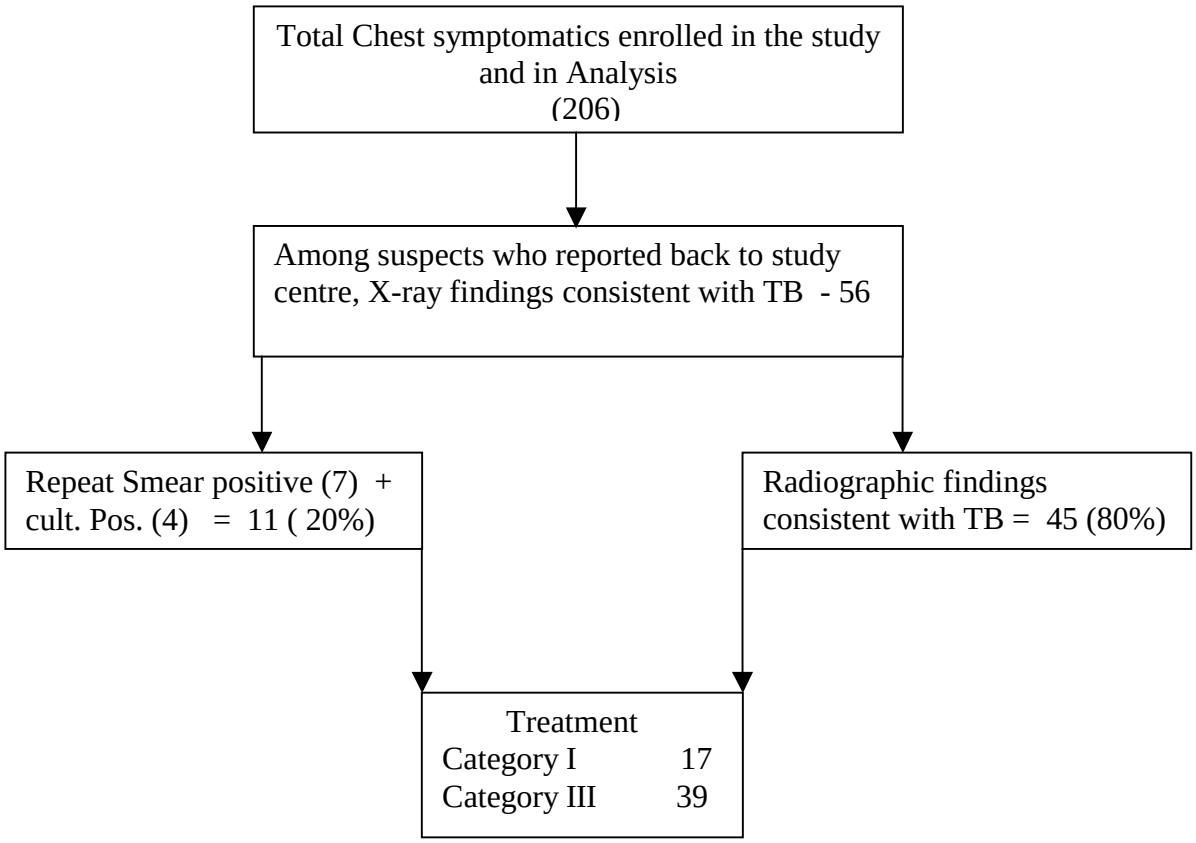


Fig. 2: Patient enrolment with smear and culture results of TB suspects with radiographic evidence of TB, who reported back to centre after antibiotics

radiograph is more expensive than sputum microscopy. From these findings, it appears that repeat sputum microscopy is a more reliable test, easy to perform and inexpensive compared to chest radiographs especially in resource – poor countries and in countries with high HIV co-infection with tuberculosis, as patients with HIV/TB co-infection quite often present with atypical radiographic findings leading to difficulties in diagnosis.

During the period of the study, the proportion of smear positive cases had increased to 15.3% of 1165 chest symptomatics investigated, from 10.6% of 2486 chest symptomatics investigated in the previous year. The increase has been about 50%, a significant contribution due to repeat sputum microscopy.

Our study has shown that the oral salbutamol therapy did not improve the efficiency of the diagnosis of smear-negative PTB patients (P=0.25). Contrary to an earlier report, our study failed to demonstrate improvement in the diagnosis of smear-negative PTB, after oral salbutamol therapy, even among smokers and asthmatics[7].

To conclude, this study has shown that in resource poor settings, repeat sputum smear microscopy after a trial of antibiotics, could improve the diagnostic accuracy of smear-negative PTB patients. On the other hand, oral salbutamol supplement was not beneficial in improving the diagnosis of smear-negative PTB. Therefore, we recommend repeat sputum examinations as a routine procedure to be included in the diagnostic algorithm, especially for developing countries like India where culture facilities are not available in the programme. It

has the advantage of both confirming the disease, helping in proper categorization and also in patients getting appropriate treatment.

ACKNOWLEDGEMENTS

The authors are grateful to Dr Thomas Frieden, formerly at regional office, South-East Asia, World Health Organization for encouraging us to do this study. We thank Dr Selvakumar , Bacteriology Department, for organizing bacteriological examinations for all the persons enrolled in this study. We are also thankful to Dr T Santha Devi, and Dr Aleyamma Thomas for their helpful suggestions in carrying out the study. The authors are grateful for the assistance and cooperation from Dr. Shanmugasundaram, Ms.Thirupura Sundari, Ms. Kasthuri and Ms. Loganayaki of corporation TB unit, Tondiarpet in conducting this study. The authors thank Ms. Sampoornam, Clinic Nurse, and are grateful to the patients who participated in this study.

REFERENCES

1. Styblo K. Epidemiology of Tuberculosis. VEB Gutsav Fisher Verlag Jena 1984; 79–80.
2. Gothi GD, Chakraborty AK, Krishnamurthy VV & Banerjee GC. Prevalence and incidence of sputum negative pulmonary tuberculosis and fate of pulmonary radiological abnormalities found in rural population. *Indian J Tuberc* 1978; **25**: 122-131.
3. Hong Kong Chest Service/Tuberculosis Research Centre/ British Medical Research Council. Sputum smear negative pulmonary tuberculosis. Controlled trial of 3 month and 2 month regimens of chemotherapy. First report. *Lancet* 1979; **1:** 1361–1364.
4. Khatri GR and Frieden TR. The status and prospects of tuberculosis control in India. *Int J Tuberc Lung Dis* 2000; **4**: 193-200.
5. WHO/TB/97.220. WHO Treatment of tuberculosis – Guidelines for national programmes.
6. Nefedova VB, Sokolava TB – "Role of Bronchospasm in development of bronchial obstruction in pulmonary TB. *Probl Tuberc* 1997; **1**: 34–38.
7. Murthy KJR, Yazdani A, Kiran A Lakshmi. Use of oral salbutamol in improving quality sputum microscopy in the DOTS strategy. *Int J Tuberc Lung Dis* 2000; **4(12)**: 1191–1192.
8. Tuberculosis Research Centre , Madras. A controlled comparison of a twice weekly and three once weekly regimens in the initial treatment of pulmonary TB. *Bulletin of the World Health Organisation* 1970; **43**: 143–155.
9. David Wilkinson, Kevin M. De Cock, A Wim Sturm. Diagnosing tuberculosis in a resource – poor setting ; the value of a trial of antibiotics. *Transaction of the Royal Society of Tropical Medicine and Hygiene* 1997; **91:** 422–424.
10. David Wilkinson, Newman W, Reid A, Square SB, Sturm AW, Gilks CF. Trial of antibiotic algorithm for the diagnosis of tuberculosis in a district hospital in a developing country with high HIV prevalence. *Int J Tuberc Lung Dis* 2000; **4(6)**: 513–518.