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Bacteriological study of sputum smears positive follow-up pulmonary tuberculosis patients attending Tuberculosis Centre, Yangon Division

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This study was carried out in Tuberculosis Centre, Yangon Division from December 2001 to January 2003. Sputum smear microscopy and drug susceptibility pattern of seventy follow-up pulmonary tuberculosis patients (46 Category I patients and 24 Category II patients) still sputum smear positive after initial intensive phase of anti-tuberculosis treatment were studied to determine the possible bacteriological factors related to slow rate of sputum conversion and to detect drug resistant cases among them. The *results showed that heavy initial bacillary load was the most common cause* for slow rate of sputum conversion after initial phase. Multi-drug resistant tuberculosis cases were detected in 4.6% (2 cases) of Category I follow-up patients and 12.5% (3 cases) of Category II follow-up patients. Resistance to one drug were seen in 22.7% (10 cases) of Category I patients and 29.1% (7 cases) of Category II patients. Based on the results of our study it is suggested that performing of drug susceptibility testing on follow-up pulmonary tuberculosis patients still sputum smear positive at the end of initial intensive phase treatment could detect drug resistance in the early stage and could be ultimately one of the means of reducing MDR-TB country wide.

INTRODUCTION

Tuberculosis (TB) is a major health problem in most developing countries.Approximately one third of the world's population has been infected with *Mycobacterium tuberculosis* and there are six to eight million new cases of disease and two to three million deaths each year [1].

South East Asia Region (SEAR) accounts for nearly 40% of all tuberculosis cases. Nearly 3 million cases and 700,000 deaths occur every year in SEAR, and 90% of morbidity and 95% of mortality are from five high burden countries including Myanmar [2].

In Myanmar, recent estimates suggest that 1.5% of population becomes infected with *tuberculosis every year and about 85,000* people progress to develop disease annually. About half of these have infectious pulmonary tuberculosis and continue to spread the disease [3]. Multidrug resistant tuberculosis (MDR-TB) is a serious threat to *National TB Programme (NTP) in both* developed and developing countries. In 1994-95, primary initial MDR-TB rate was 1.25% among out-patients of Zone 1 Zonal Centre, Yangon and acquired MDR-TB rate among re-treatment cases of Aung San TB *Hospital was 23.7% [4].*

The World Health Organization (WHO) TB Programme has adopted the Directly

Observed Treatment Short Course (DOTS) strategy since 1994. It consists of five components including the administration of standardized regimens with first line drugs under direct observation at least in the intensive phase regardless of the patient's drug susceptibility pattern [5].

National TB Programme in Myanmar was established in 1966. Short course chemotherapy was introduced in 1994 and DOTS strategy was started in 1997. The NTP fully intermitted regimen has been used in all DOTS implementing townships since 1999 [6].

In Guidelines for National TB Programme, WHO stated that under programme conditions in countries with a high incidence of TB, routine monitoring by sputum culture is not feasible or recommended and patients with sputum smear positive pulmonary TB should be monitored by sputum smear examination. Sputum conversion is the process whereby initially smear positive patients become smear negative and it is also a good response to treatment.

Therefore, in order to monitor sputum conversion and response to treatment, it was recommended that new sputum smear positive pulmonary TB patients (Category I) must have follow-up sputum smear check at the end of initial phase (second month), then during the continuation phase (fifth month) and in the last month of treatment regimen (sixth month). In relapse and retreatment smear positive pulmonary TB patients (Category II), sputum smear examination is performed at the end of initial phase (third month), during the continuation phase (fifth month) and at the end of treatment (eighth month).

If a patient has delay in smear conversion at the end of initial phase, then one-month prolongation of initial phase treatment is recommended regardless of patient's drug susceptibility pattern and recheck sputum examination is done after one month prolongation of initial phase treatment. For Category II patients if this sputum recheck showed positive, culture and sensitivity testing must be performed. [7].

Therefore, this study was carried out on follow-up pulmonary TB patients still sputum smear positive after initial phase treatment to determine the bacteriological factors ie. sputum microscopy pattern and drug sensitivity pattern related to slow rate of sputum conversion with the aim of detecting drug resistant cases early in the treatment course.

MATERIALS AND METHODS

Study area and study period

This study was carried out at the Tuberculosis Centre, Yangon Division, Aung San, Yangon from December 2001 to January 2003.

Collection of specimen

Sputum samples of seventy (70) follow-up pulmonary TB patients (46 Category I patients and 24 Category II patients) still sputum positive after initial phase treatment were collected and sent to Bacteriology Research Division, Department of Medical Research (Lower Myanmar). Relevant background data was also recorded.

Smear microscopy, culture and sensitivity

Sputum samples were again examined by direct microscopy after Ziehl Neelsen staining to confirm the smear microscopy. The number of acid-fast bacilli seen on microscopy was recorded as recommended by WHO. (Table 1) Then they were inoculated onto 3% Ogawa media and incubated for 8 weeks at 37°C. Positive cultures were tested for sensitivity to streptomycin, isoniazid, rifampicin and ethambutol on drug containing 1% Ogawa media using absolute concentration method for 4-6 weeks at 37°C according to Minimum Essentials of Laboratory Procedure for the Tuberculosis Control published

Table 1. Reporting on AFB microscopy (WHO)

Number of bacilli seen	Results reported
None per 100 oil immersion fields	Negative
1-9 per 100 oil immersion fields	Scanty, report exact number
10-99 per 100 oil immersion fields	1+
1-10 per 100 immersion fields	2+
>10 per oil immersion fields	3+

Table 2. Smear Microscopy Results of Category I follow-up patients in relation to different drug susceptibility pattern

Drug susceptibility pattern	No. of cases	Smear grading		
		First visit	Follow-up after initial phase	Follow-up after one month prolongation of initial phase treatment
Sensitive to HRSE (32 cases)	30	$2^+, 3^+$	1^+	-
	2	2^+	2^+	-
Resistant to HRS & HR (2 cases)	2	2^+	2^+	+
Resistant to H (8 cases)	8	2^+	1^+	-
Resistant to E (2 cases)	1	2^+	1^+	-
	1	2^+	2^+	-
Not done (2 cases)	2	2^+	2^+	-

Table 3. Smear Microscopy Results of Category II follow-up patients in relation to different drug susceptibility pattern

Drug susceptibility pattern	No. of cases	Smear Grading		
		First visit	Follow-up after initial phase	Follow-up after one month prolongation of initial phase treatment
Sensitive to HRSE (14 cases)	12	$2^-, 3^+$	1^+	-
	2	$2^-, 3^+$	2^+	-
Resistant to HRSE (1 case)	1	2^+	2^+	2^+
Resistant to HRS (2 cases)	1	1^+	2^+	1^+
	1	2^+	1^-	1^-
Resistant to H (3 cases)	3	2^+	1^-	-
Resistant to E (2 cases)	2	2^-	2^-	-
Resistant to S (2 cases)	1	2^-	1^-	-
	1	2^-	1^+	1^-

by the Research Institute of Tuberculosis, Japan 1986 [8].

Checking by smear microscopy after one month

The sputum smear microscopy results of study population were again checked after one - month prolongation of initial phase treatment.

Analysis of data

Smear microscopy results of diagnostic examination (first visit), follow-up after initial phase and follow-up after one month prolongation of initial phase were compared and analysed in relation to background data and drug susceptibility pattern.

RESULTS

Sputum microscopy at follow-up after initial phase

All of the tested samples were also positive on re-examination. Among them 39 (84.8%) of Category I patients and 18 (75%) of Category II patients had grade 1^+ smear positive smears. Seven (15.2%) cases of category I patients and 6 (25%) of Category II had grading 2^+ positive smears.

Drug susceptibility testing of Mycobacterium tuberculosis by culture method

Among 46 Category I patients, positive culture for *Mycobacterium tuberculosis* was obtained from 44 patients (2 isolates were contaminated). Drug susceptibility results on 44 Category I patients and 24 Category II patients are shown in Figure 1 and 2. Tested anti-tuberculosis drugs were isoniazid (H), rifampicin (R), streptomycin (S) and ethambutol (E).

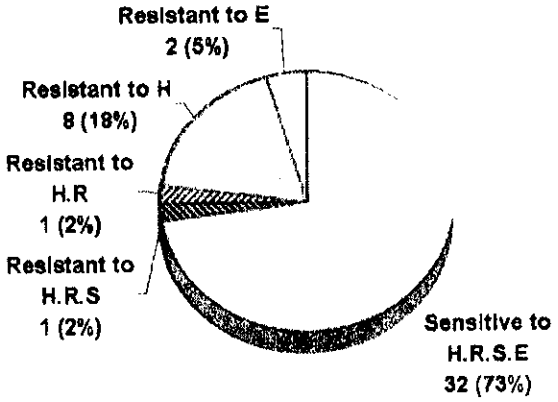

Fig.1. Drug susceptibility pattern of Category I follow-up patients

Sputum microscopy results in relation to different drug susceptibility pattern

Sputum microscopy results after the one-month prolongation of initial phase (i.e. third month in Category I patients and fourth month in Category II patients) were compared with those of diagnostic (first visit) and follow-up examination after initial phase treatment.

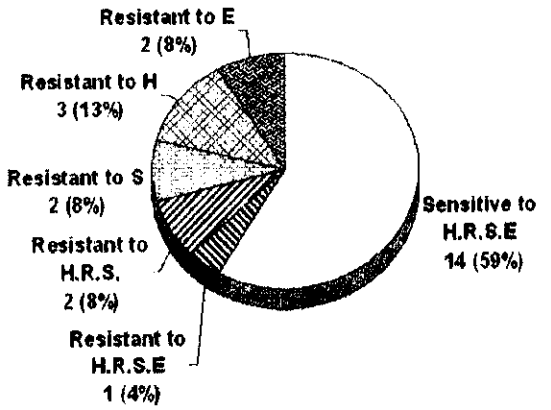

Fig.1. Drug susceptibility pattern of Category II follow-up patients

Table 2 and 3 show the smear microscopy results in relation to drug susceptibility pattern.

Analysis of sputum microscopy results in relation to drug resistant pattern and background data of follow-up patient

It was found that all patients in the study population were treated according to NTP fully intermittent regimen at TB Centre, Aung San or township health centers.

Out of 70 follow-up patients showing sputum positive on sputum re-check after initial phase treatment, 44 cases (95.6%) of Category I patients and all cases (100%) of Category II patients were found to have taken anti TB drugs regularly and correctly. Among Category I patients, 2 patients (4.4%) took the drugs in divided doses with meals but they achieved sputum conversion after completion of one month prolongation of initial phase treatment. They were also sensitive to all tested anti TB drugs. Five cases (20.9%) have extensive lung lesions and cavitations and they were resistant to one or two anti-TB drugs.

Among Category II cases, 2 cases (8.3%) of 2^+ smear positive follow-up patients resistant to 3 or 4 tested anti TB drugs had a history of massive haemoptysis and extensive pulmonary lesions but four cases

(16.7%) with cavitations and extensive lung lesions showed 1^+ or 2^+ positive smears in econd month follow-up and were sensitive to all anti TB drugs.

In both groups, all MDR-TB cases and one streptomycin resistant case were sputum positive on sputum re-check at one month after prolongation of initial phase treatment.

DISCUSSION

Tuberculosis continues to be a major health problem and is still one of the biggest killers in Myanmar. Sputum smear positive pulmonary TB patients are 4-20 times more infectious than smear negative patients and if untreated, they may infect 10-15 persons per year [9]. These are the group for whom bacteriological monitoring is essential, so they should be monitored by sputum smear examination. Culture of tubercle bacilli is necessary for carrying out sensitivity tests, which are of value in guiding retreatment of relapses and of patients in whom chemotherapy has failed and for epidemiological purposes to access the efficacy of TB control programme.

Data from TB Centre, Yangon Division in 2002 showed that out of 5544 registered new smear positive cases (Category I), 79% were smear negative on second month follow-up and 4.2% were converted to smear negative on third month follow-up. Those remaining smear positive cases at 3^{rd} month comprised 78 (1.4%).

In this study, among 70 follow-up patients still sputum positive after initial phase, 100% (46/46) of Category I follow-up patients and 95.8% (23/24) of Category II follow-up patients had initial bacillary load of 2^+ to 3^+ grading. However, after one month prolongation of initial phase treatment, 95.7% (44/46) of Category I patients and 83.3% (20/24) of Category II patients had smear conversion from positive to negative.

This indicated that heavy initial bacillary load may play an important role for slow rate of progress of sputum conversion after initial phase but tubercle bacilli were killed along with the treatment course as most of these cases had sputum conversion after one month.

Regarding the drug susceptibility pattern, 72.7% (32/44) of Category I and 58.3% (14/24) of Category II patients were sensitive to all tested four anti TB drugs (H, R, S, E). Multi-drug resistant TB MDR-TB (resistant to HRSE or HRS or HR) were seen in 4.6% (2/44 cases) of Category I patients and 12.5% (3/24 cases) of Category II patients. Resistance to one drug (H or E or S) was seen in 22.7% (10/44 cases) of Category I patients and 29.1% (7/24 cases) of Category II patients.

Drug resistance may also be one of the causes for positive smears after initial phase but it is a less frequent cause as only 5 cases of MDR-TB (2 cases of Category I and 3 cases of Category II) were observed among the study group.

Although drug resistance may be construed as a less frequent cause of slow rate of smear conversion, all MDR-TB and one streptomycin resistant case were shedding bacilli in the sputum after one month prolongation of initial phase treatment and these cases may be very infectious to the community at large.

As *Mycobacterium tuberculosis* is a slow growing bacterium, it takes at least 6 weeks for culture and another four weeks to get the drug susceptibility results. So by doing culture and drug susceptibility tests on those with positive smears on their follow-up after initial phase treatment, drug resistance could be identified earlier in the treatment course.

Drug susceptibility tests are laborious and need expertise and adherence to standard procedures in accordance with the reference laboratory. However, to spare labour and cost, drug susceptibility tests could be done

only on those with positive smears after one month prolongation of initial phase treatment.

In this study all the smear positive follow-up patients were taking anti TB treatment under NTP-Fully Intermittent Regimen through DOTS and almost all patients (95.6% of Category I patients and 100% of Category II patients) were found to be taking the drugs regularly and correctly. Only two cases (4.4%) of Category I patients were taking the drugs in divided dosage after meals. However, they were sensitive to all tested anti-TB drugs and gained sputum conversion after one month as they received thorough supervision of initial phase treatment afterwards.

Our finding highlights the fact that extensive pulmonary lesion was related to slow rate of sputum conversion after initial phase as tuberculous cavity lesion harbours millions of bacilli. Sputum microscopy may show positive although the patient's condition is improving because he is shedding dead bacilli. But it may also be associated with drug resistance as 5 cases of Category I patients and 2 cases of Category II patients with extensive pulmonary lesion were resistant to one or more anti-TB drugs.

WHO stated that the usual most frequent factor for delay in smear conversion is that the initial phase treatment was poorly supervised and the patient adherence was poor [7]. However, in our study, almost all the patients were thoroughly supervised and taking the drugs regularly and correctly under NTP fully intermittent regimen, so this factor did not play an important role for them. As this study mainly emphasized on bacteriological factors, the social factors, clinical picture and other relevant past history of these patients could not be analysed in detail.

Therefore, it was concluded that the most frequent cause for delay sputum conversion of follow-up patients of this study might be due to heavy initial bacillary load. The less frequent causes might be the fact that the patients may have extensive pulmonary lesion and cavitations and the patient may have drug resistant tubercle bacilli that does not respond to first line anti-TB treatment.

Our study also highlighted the occurrence of MDR-TB among smear positive follow-up patients as 4.6% in Category I patients and 12.5% in Category II patients. During 1994-1995 MDR-TB rate was 1.25% among isolates from 400 patients with new sputum positive pulmonary TB attending Union Tuberculosis Institute and acquired MDR-TB rate among retreatment cases of Aung San TB Hospital was 23.7% [10,4]. In 2000, it was reported that 23.3% of isolates from new pulmonary TB patients were resistant to at least one anti-TB drug and MDR-TB rate was 2.0% among isolates from 51 patients attending Zone I TB Centre [11].

This finding shows that there is a relatively high frequency of initial drug resistance among our patients. It may be due to undisclosed past exposure to anti-TB drugs and our study population also focused on sputum positive follow-up patients and the study site represented the major drainage of TB cases in Yangon. Carriage of drug resistant bacilli was more obvious in Category II patients than in Category I patients as expected. In a study carried out at Srinagarind Hospital, Thailand in 2000 pointed out that 2.4% of patients were infected with MDR-TB [12].

Our study also suggested that culture and drug sensitivity should be considered in patients with positive smears after initial phase provided facilities are available or if not they should be thoroughly monitored whether they get sputum conversion after one month prolongation of initial phase treatment, as those shedding bacilli in the sputum after one month have greater chance of drug resistant infection.

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