The Prevalence and Patterns of Resistance to Primary Line of Anti-Tubercular Drugs In Patients on Category-Ii Regimen of RNTCP

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Abstract : Background: RNTCP uses short course anti-tubercular (ATT) chemotherapy given intermittently under direct observation (DOTS). Isoniazid (H), Rifampicin(R), Pyrazinamide(Z), Ethambutol(E) and Streptomycin(S) form part of Category-I (2H3R3Z3E3 + 4H3R3) and Category-II (2H3R3Z3E3S3 + 1H3R3Z3E3 + 5H3R3E3) regimen, which are recommended for treatment of new and previously treated tuberculosis patients respectively. There is however concern regarding effectiveness of category-II regimen especially in failure cases.

Aim: Objective of the study was to determine the prevalence and pattern of anti-tubercular drug(ATT) resistance in category-II or retreatment patients.

Settings: Tertiary teaching hospital

Design: Non-randomised prospective study of all patients presenting to DOTS centre and started on category-II treatment. Material and Methods: In our study, all the patients registered under category-II at RNTCP centre over a one-year period were included. Sputum sample was collected for C/S mycobacterium tuberculosis while starting treatment and at the end of 3 months of treatment. Acid-fast bacilli staining of sputum was done by Zeihl-Nelson's Method, culture by modified Lowenstein-Jensen's medium and resistance testing was done by proportion method.

Results: 103 patients were registered under category-II. The culture results are available for 87 patients. Amongst, 65.5% were culture negative or pre-treatment susceptible to ATT. MDR-TB was observed in 10.3%, 8.2% and 23% of relapse, treatment after default (TAD) and failure subcategories respectively. Resistance to H, S, R, E, Z were 24.1%,17.2%,16.1%, 5.7% and 17.2% respectively. Emergence of resistance to Rifampicin was seen in 3.8% cases following category-II treatment.

Conclusions: 10.35% of all Retreatment regimen patients had Multi-Drug resistant Tuberculosis (MDR-TB). Percentage of MDR-TB was 10.3%, 8.2% and 23% among relapse, TAD and failures subcategories respectively Approval Ethics application site: Ethics Committee, King George Medical University.

Key-words: RNTCP, DOTS, Category 2, Retreatment regimen, Tuberculosis, Resistance, Antitubercular treatment.

Introduction

Tuberculosis (TB) causes enormous burden of disease and death around the world. Nearly one third of the global population (1) i.e., two billion people, are infected with mycobacterium tuberculosis and are at risk of developing the disease. More than eight million people develop active tuberculosis every year, and about two million die. More than 90%of global tuberculosis cases and death occur in developing world, with India having unique and unwanted honor of housing almost 30% of the total tuberculosis cases in the world, 2 million developing TB every year (2). Among adults it is the foremost cause of death from a single infectious agent, in the country killing more than AIDS, Malaria and other infectious diseases.

Category of treatment	Type of patient	Regimen
Category I	New cases	$2H_3R_3Z_3E_3 + 4H_3R_3$
Category II	Sputum smear-positive relapse Sputum smear-positive failure Sputum smear positive treatment after default Others	$2H_3R_3Z_3E_3S_3$ + $1H_3R_3Z_3E_3$ + $5H_3R_3E_3$

Category based treatment through DOTS is the recommended treatment. New smear positive pulmonary tuberculosis patients on Category – I regimen have good treatment outcome with a success rate of 83% or higher at national level. For previously treated patient on Category-II regimen, the success rate is low at 71%. In presence of isoniazid resistance [with or without streptomycin

resistance], regimen containing four or five drug is still effective. for example, Mitchinson and Nunn reviewed 12 trials of the British Medical Research Council and found only four failure among 154 patients [2.6%] infected with such strain and treated with four or five drug regimens. Even rifampicin monoresistance can be managed first line agents. Hence this is the basis of using retreatment regimen in patients who had earlier taken six months regimen of category-I. Retreatment Tuberculosis patients are further subclassified as relapse, treatment after default and failure. Relapse is a Tuberculosis patient who was declared cured or treatment completed by a physician, but who reports back to the health service and is now found to be sputum smear-positive. Treatment after default is a Tuberculosis patient who received anti-TB treatment for one month or more from any source and returns to treatment after having defaulted, i.e., not taken anti-TB drugs consecutively for two months or more, and who is found to be sputum smear-positive. Failure subcategory are Any TB patient who is smear-positive at 5 months or more after starting treatment. Failure also includes a patient who was treated with Category-III regimen but who becomes smear-positive during treatment. Among patients typed as 'Failures' and treated with retreatment regimen, risk of subsequent failure is higher, compared to other types.

In India, it is reported that 1-3.4% of new patients have multi drug resistant TB (MDR TB) and studies have shown resistance to isoniazid ranging from 34.5-67%, for streptomycin 25% and for Rifampicin 2.8-37.3%. There is thus a concern regarding the effectiveness of category II regimen. This could be known with help of study of culture and sensitivity patterns of sputum. With this background, the current study was carried out.

The aims and objectives of the study were: To know the prevalence and pattern of resistance to primary line of anti-tubercular drugs in patients of CAT-II regimen in RNTCP.

Subjects and Methods:

All the pulmonary tuberculosis retreatment patients initiated on category II regimen in RNTCP centre attached to department of pulmonary medicine, CSMMU, Lucknow, formed the study group. The standard treatment regimen of RNTCP in India for category-II consists of 2 months of Streptomycin (S), Isoniozid (H), Rifampcin (R), Ethambutol (E), Pyrizanamide (Z) followed by 1 month of RHEZ and further followed by 5 months of RHE [2 S3H3R3E3Z3 / 1H3R3E3Z3 / 5H3R3E3) given 3 times a week, throughout the 8 months. Patients whose sputum smear remained positive at end of intensive phase of three months treatment with R, H, Z and E was extended for one more month. The category II regimen was administered under direct supervision during the intensive phase, followed by supervised administration of the first weekly dose of combination where removing two doses issued for self-admission.

Three pre-treatment sputum specimens were collected from each patient within 1 weak of starting treatment and at the end of three months of treatment. It was transported to the reference laboratory attached to the department of microbiology for microscopy, culture and susceptibility tests.

Laboratory Bacteriological examination of sputum specimen & collection of sputum:

The patients were advised to give three sputum samples. The first sample was collected on the spot. To collect the overnight sputum sample (2nd sample) in the next morning a container was given to the patients and the 3rd sample was again collected on the spot. The sputum specimens were transported to the Department of Microbiology, CSMMU, Lucknow within 8 hours and the following examinations were undertaken.

Culture: Homogenisation and concentration of specimen was done by modified Petroffs method [3] and Staining for AFB was done by Zeihl Nelson's Method. Patients were categorized according to WHO guidelines for slide reporting [4].

No. of bacilli seen in a smear	Result Reported
No AFB per 100 oil immersion field	0
1-9 AFB per 100 oil immersion field	Scanty
10-99 AFB per 100 oil immersion field	(1+)
1-10 AFB per oil immersion field	(2+)
>10 AFB per oil immersion field	(3+)

Primary culture was done on Lowenstein Jensen's media slope and examined every week upto twelve weeks before reporting it as negative. Presence of any growth on slopes for presence of AFB was confirmed by Zeihl Nelson's staining. Tests of sensitivity to streptomycin, Isoniazid, Rifampicin, ethambutol and pyrazinamide were done by 1% proportion method. The Proportion Method enables precise estimation of the mutants resistant to a given drug. Several 10-fold dilutions of inoculum are planted on to both control and drug containing-media; at least one dilution should yield isolated countable (50-100) colonies. When these numbers re corrected by multiplying by the dilution of inoculum used, the total number of viable colonies observed on control medium, and the number of mutant colonies resistant to the drug concentrations tested may be determined. The proportion of bacilli resistant to a given drug is then determined by expressing the resistant portion as a percentage of the total population tested. The proportion method is currently the method of choice with critical drug concentrations as of isoniazid (0.2 µg/ml), rifampicin (40 µg/ml),

streptomycin (4 µg/ml), pyrazinamide (100 µg/ml) and ethambutol (2 µg/ml) were carried out to declare resistance to each drug.

DEFINITIONS OF RESISTANCE-PROPORTION METHOD

Drug		Concentration (mg/1)	Proportion
Streptomycin		4	1% or more
Isoniazid		0.2	1% or more
Rifampicin		40	1% or more
Ethambutol		2	1% or more
Pyrazinamide	100		1% or more
(pH of media adjusted to 4.9)			

Sputum was sent at the third month of treatment for AFB smear and culture sensitivity for those patients with positive sputum smear at third month sputum was sent at the fourth month for culture and sensitivity. The definition and type of patients (relapse, TAD, failure and others) were according to the criteria laid down by RNTCP.

Results and Outcome:

In our study 103 patients were registered out of which 6 patients didn't raise sputum and 2 died before sputum could be sent for culture sensitivity. Thus, we could send culture sensitivity of 95 patients. Out of which culture sensitivity outcome for mycobacterium tuberculosis of 87 patients is available. It was observed in our study that despite previous tuberculosis treatment, 44.8% of the study cohort was pre-treatment susceptible to all five first line ATT drugs (Table 1). 24.12% were resistant but non Multi-Drug resistant tuberculosis (MDR-TB) which is defined as resistance to isoniazid and rifampicin, which are two major drugs for treatment of Tuberculosis. 20.7% were culture negative. Only 10.35% patients were MDR. Thus only 34.5% of the patients were actually having resistant bacilli (Table 2).

	Total No.	%age
Total no. of patient with known culture status	87	100
Culture negative	18	20.69
Susceptible for all drugs	39	44.83
Resistance to atleast 1 drug	30	34.48
One drug only	8	9.19
H =	3	3.44
Z =	3	3.45
R =	1	1.15
S =	1	1.15
Two drugs only	11	12.64
HR =	3	3.44
HS =	2	2.30
HE =	1	1.15
RZ =	2	2.30
SR =	1	1.15
HZ =	1	1.15
SZ =	1	1.15
Three drugs	6	6.89
SHZ	4	4.59
SHR	2	2.30
Four drugs	3	3.45
SHEZ	1	1.15
SHRZ	1	1.15
SHRE	1	1.15
Five drugs	2	2.30
SHREZ	2	2.30

Table 1: Resistance profile

The prevalence of MDR-TB in retreatment patents in the study was 10.35% and it was higher (23%) among failure cases.

C/S status	**Total No.**	**%age**
Culture negative	18	20.7
Susceptible	39	44.83
Non MDR	21	24.12
MDR	9	10.35
Total	**87**	**100**

Table 2: Culture sensitivity outcome of all category II patients

The overall rates of resistance to S,H,R,E & Z were 17.2%, 24.14%, 16.09, 5.75 & 17.2% respectively (Table 1).
Among subcategories- Relapse, Treatment after default and Failure, Multi drug resistant Tuberculosis rate in failure subcategory was 23% (Figure 1,2,3).

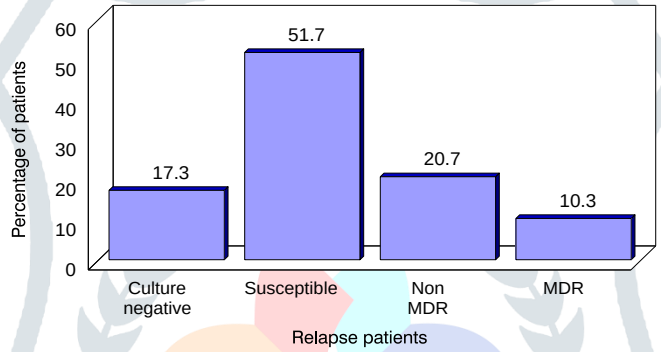

Figure 1: Culture results in Relapse

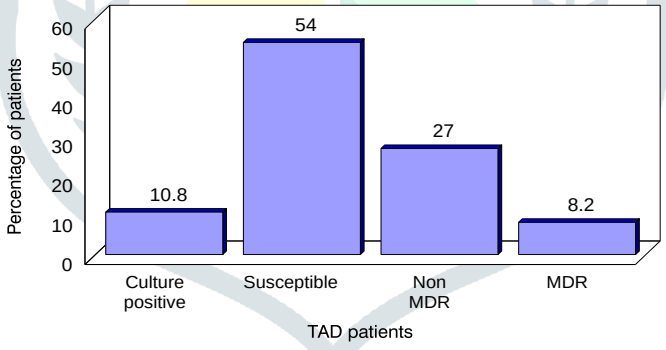

Figure 2: Culture results in Treatment after Default

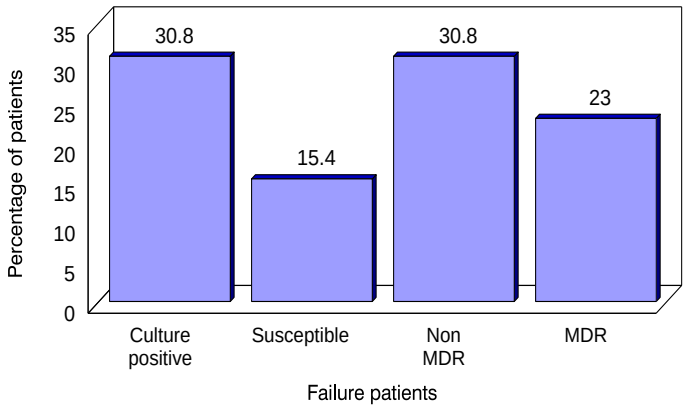

Figure 3: Culture results in Failure

Emergence of resistance to Rifampicin was seen in 3.8% of cases suggestive of the fact that category-II regimen does not lead to

development of significant acquired resistance.

Discussion:

A total of 103 patients were registered in the study, out of which 6 patients didn't raise sputum and 2 died before sputum could be sent. We could send culture of 95 patients out of which culture sensitivity outcome for Mycobacterium tuberculosis is available of 87 patients. Of the 103 patients, 88 were sputum positive while, 15 patients belonged to others group. Of the 88 sputum positive patients, 42 were TAD, 30 were relapse and 16 belonged to failure subcategory. Of the 87 patients with known culture sensitivity pattern, 20.7% were culture negative, 44.83% had susceptible T.B. bacilli, while 24.12% were non MDR and 10.35% were MDR. Percentage of MDR patients in relapse and TAD subcategory was 10.3% and 8.2% respectively, while it was 23% among failures.

In similar studies, Pauline Joseph et al.5 had reported MDR-TB rates of 11%, 9% and 22% among relapse TAD and failures subcategories respectively. Espinal et al.6 had reported 19% MDR-TB among re-treatment cases while Sophia Vijay et al.7 had reported MDR-TB among 12.8% of retreatment cases. Santha et al.8 in his study on failures subcategory of retreatment cases reported MDR-TB rate of 17%.

In our study, Drug resistance to Isoniazid was maximum and was present in 24.14% cases followed by Streptomycin and Pyrazinamide in 17.24% cases each. Resistant to Rifampicin was seen in 16.1% followed by Ethambutol as in 5.75% cases. In similar studies by S.S. Trivedi et al.9 and N.K. Jain et al.10 had reported higher rates of resistance. They had reported rates of 34.5-67%, 26-26.9% and 2.8-37.3% respectively for H, S and R. However, Sophia Vijay et al.7 had reported similar rates. They had reported rates of 27.4%, 23%, 15.5%, 6.6% respectively to H, S, R and E.

The main shortcoming of the study was small sample size and as the study was conducted in a tertiary care centre it may not represent the community as a whole.

In conclusion, among patients who were put on retreatment regimen in our study, 20.7% were culture negative, 44.83% had susceptible T.B. bacilli, 24.12% were non MDR and 10.35% were Mult-Drug resistant Tuberculosis (MDR-TB). Percentage of MDR-TB was 10.3%, 8.2% and 23% among relapse, TAD and failures respectively. It is suggested that Drug Sensitivity Tests (DST) should be done on all patients who are put on the retreatment regimen through rapid probe assay as per World Health Organization (WHO) guidelines and appropriate regimen should be started as early as possible for better treatment outcomes and to reduce transmission of MDR-TB.

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