Efficacy of the 6-month thrice-weekly regimen in the treatment of new sputum smear-positive pulmonary tuberculosis under clinical trial conditions


ABSTRACT

Background. Under the Revised National Tuberculosis Control Programme of India, patients with new smear-positive pulmonary tuberculosis are treated with a thrice-weekly regimen of antitubercular drugs (2H₃R₃Z₃E₃/4H₃R₃ [H isoniazid, R rifampicin, Z pyrazinamide and E ethambutol]) for 6 months. We conducted a retrospective analysis of the efficacy and tolerability of this regimen under clinical trial conditions in HIV-negative patients with newly diagnosed smear-positive pulmonary tuberculosis.

Methods. We retrospectively analysed the data on patients assigned to the control regimen (2H₃R₃Z₃E₃/4H₃R₃) in two clinical trials during 2001–06 at the National Institute for Research in Tuberculosis, Chennai, India.

Results. Of the 268 patients treated with this regimen, data for efficacy analysis were available for 249. At the end of treatment, of 249 patients, 238 (96%) had a favourable status. Treatment failure occurred in the remaining 11: 7 in whom the organisms were initially drug-susceptible and 4 with initial drug resistance. Of the 238 patients who had a favourable status at the end of treatment, 14 (6%) had recurrence of tuberculosis during the following 24 months. In the intention-to-treat analysis, 245 (94%) of 262 patients had a favourable status at the end of treatment. Of the 28 patients with initial drug resistance, 24 (86%) had a favourable outcome. Only 4 of these 24 patients were found to have recurrence of tuberculosis in 2 years of follow-up. Among the 221 patients initially infected with drug-susceptible organisms, drug resistance did not develop in any of the 7 patients in whom the treatment failed or the 10 who had recurrence of tuberculosis. Further, 5 of the 7 patients in whom the treatment failed continued to excrete drug-susceptible bacilli at 6 months. Adverse drug reactions were observed in 38 (14%) of the 262 patients. Only 3 (1.1%) needed a modification in the treatment.

Conclusion. This thrice-weekly 6-month regimen of antitubercular drugs, when administered under full supervision, is associated with a high rate of favourable treatment outcomes in HIV-negative patients with newly diagnosed sputum smear-positive pulmonary tuberculosis. There are few adverse drug reactions in these patients.


INTRODUCTION

India contributes one-third of the global burden of tuberculosis (TB). The Revised National Tuberculosis Control Programme (RNTCP) of the Indian government is based on the WHO-recommended Directly Observed Treatment Short course (DOTS) strategy. Under the RNTCP, patients with new smear-positive pulmonary TB are treated with a 6-month thrice-weekly regimen starting with 2 months of isoniazid (H), rifampicin (R), pyrazinamide (Z) and ethambutol (E), followed by 4 months of H and R (2H₃R₃Z₃E₃/4H₃R₃). This regimen is administered under direct observation during the first 2 months and supplied once a week thereafter for self-administration. The cure rate among new smear-positive pulmonary TB patients under the RNTCP is 68%–85%. There is a subsequent recurrence of TB among 11%–12% of these patients.

The National Institute for Research in Tuberculosis (NIRT) [formerly known as the Tuberculosis Research Centre (TRC)], in Chennai investigated the efficacy of the above regimen in two clinical trials during 2001–06. These trials compared the efficacy of the 6-month control regimen (2H₃R₃Z₃E₃/4H₃R₃) with that of thrice-weekly 4-month regimens containing quinolone. The
first trial compared it with a regimen of ofloxacin (20H, R, Z, /20H, R, ) and the second with a regimen of moxifloxacin (2G, R, Z, /2G, H, R, ). Both these trials were approved by the Scientific Advisory Committee and the Institutional Ethics Committee, and informed consent was obtained from the study participants. The study methods used for both the trials were the same. Both trials were stopped midway on the recommendation of the Data and Safety Monitoring Board due to the high recurrence rates of TB in the test regimens. We retrospectively evaluated the efficacy and safety of the 6-month control regimen (2H, R, , E /4H, R, ) used in these trials.

**METHODS**

Patients eligible for enrolment in the study were those who had newly diagnosed sputum smear-positive pulmonary TB (or who had been previously treated for TB for not more than one month, or not more than 7 days in the month preceding enrolment); who were of the age of 18 years or above; who weighed not less than 30 kg; and who were willing to undergo supervised treatment. Moribund patients, those with diabetes mellitus, serious nontuberculous diseases or extra-pulmonary TB, or those who were seropositive for HIV were excluded.

**Pre-treatment assessment, including investigations**

A detailed history was taken of the specific anti-TB therapy received previously. This was followed by a clinical examination. Pre-treatment screening included four (two spot and two overnight) sputum specimens. Smears were prepared from raw sputum and stained with auramine rhodamine and read under fluorescence microscopy and graded as follows: >6 bacilli/high power field (HPF) (1+), 6–100 bacilli/HPF (2+), and >100 bacilli/HPF or large clumps (3+). The sputum was decontaminated and concentrated before culture for mycobacteria by the modified Petroff’s method. The cultures were graded on the basis of the quantum of growth in Lowenstein–Jensen (LJ) medium, as follows: actual number up to 19 colonies, 20–100 colonies (1+), >100 colonies (2+) and confluent growth (3+). Positive cultures were identified as *Mycobacterium tuberculosis* by standard methods.

Drug susceptibility tests for H and R were done in LJ medium, using the minimum inhibitory concentration (MIC) method. MICs of ≥5 mg/L and of ≥128 mg/L were defined as resistance for H and R, respectively.

The following investigations were also carried out: a chest X-ray; urine examination for albumin, glucose, bile pigments, acetyl isoniazid and rifampicin; haemogram; liver and renal function tests; measurement of random blood glucose and serology for HIV.

**Treatment regimen and follow-up**

The patients received E (1200 mg), H (600 mg), R (450 mg, or 600 mg for those weighing >60 kg) and Z (1500 mg) for the first 2 months, and H (600 mg) and R (450 mg, or 600 mg for those weighing >60 kg) for the next 4 months. All drugs were administered thrice a week, under supervision, as a single dose for the entire duration of the treatment. The intensive phase was not extended if the sputum smears were positive at 2 months.

The patients were examined every month by a physician, who recorded their adherence to treatment, assessed their clinical response and recorded any adverse drug reactions. Three sputum specimens (two overnight and one spot) were examined every month by microscopy and culture. A chest X-ray, haemogram and biochemical tests were done at 2 months and at the end of treatment. The patients were followed up every month for 2 years after the completion of treatment. At each visit, two sputum specimens (one overnight and one spot) were examined by microscopy and culture, and processed as described earlier. Patients who defaulted were visited at home by the staff of the clinic and motivated to continue their treatment.

**Definitions of treatment outcome**

**Bacteriological status at the end of treatment.** It was considered favourable if

(i) all six sputum cultures were negative during the last 2 months of treatment, or
(ii) one culture was positive at 5 or 6 months but subsequent cultures became negative without additional chemotheraphy.

It was considered unfavourable (treatment failure) if at least two cultures were positive in the last 2 months of treatment, with one of the cultures growing 20 colonies or more, or if the sputum was persistently positive, or if there was radiographic or clinical deterioration or death due to any cause.

**Bacteriological recurrence.** Among those patients whose response was favourable at the end of treatment, recurrence of TB was defined as either (i) the production of two positive sputum cultures in a 2-month period, with one of the cultures growing 20 colonies or more; or (ii) a finding of positive sputum cultures during four consecutive monthly examinations, with none of the cultures yielding 20 colonies or more.

**Statistical analyses**

The proportion of patients with different outcomes was calculated. The primary analyses of the outcomes of interest were both by intention-to-treat and efficacy. The efficacy analysis was performed on the basis of the response at the end of treatment, along with recurrences of TB. Kaplan–Meier analysis was carried out to compare change between smear and culture. All statistical analyses were carried out using SPSS software version 14.0 (SPSS Inc, Chicago, Illinois).

**RESULTS**

Of the 268 patients treated with the regimen (Table I, Fig. 1), 19 were excluded from the outcome analyses (two had mycobacteria other than tuberculosis [MOTT]; one had no positive sputum cultures; one had received previous anti-TB treatment for more than one month; two had multidrug-resistant TB [MDR-TB]; and 13 had missed more than 20% of treatment, or one month of treatment continuously).

Of the remaining 249 patients, 221 (89%) had drug-susceptible TB and 28 had bacilli resistant to either H (n=27) or R (n=1). At the end of treatment, of the 249 patients, 238 (96%) had a favourable outcome and 11 (4%) had treatment failure (Table II). Of those who had a favourable outcome at the end of treatment, 14 (6%) had recurrence of TB in the following 24 months (Table III).

**Sputum smear and culture conversion**

The sputum conversion results (based on three specimens each month) for the 249 patients are shown in Table IV. At 2, 3 and 4 months, the proportion of patients with negative cultures was significantly higher than that of those with negative smears, the differences being 21%, 17% and 8%, respectively (Fig. 2), and these differences were statistically significant (log rank test.
TABLE II. Bacteriological status at the end of treatment (n=249) (efficacy analysis)

<table>
<thead>
<tr>
<th>Initial bacteriological status</th>
<th>Favourable</th>
<th>N (%</th>
<th>Unfavourable</th>
<th>Treatment failure</th>
<th>N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All six cultures negative</td>
<td>221 (93)</td>
<td>8</td>
<td>7</td>
<td>221 (93)</td>
<td>8</td>
</tr>
<tr>
<td>H/R-susceptible</td>
<td>206 (93)</td>
<td>4</td>
<td>3</td>
<td>206 (93)</td>
<td>4</td>
</tr>
<tr>
<td>H/R-resistant</td>
<td>8 (4)</td>
<td>23</td>
<td>1</td>
<td>23 (82)</td>
<td>1</td>
</tr>
<tr>
<td>All</td>
<td>229 (92)</td>
<td>9</td>
<td>11 (4)</td>
<td>229 (92)</td>
<td>11</td>
</tr>
</tbody>
</table>

* Including one patient with pre-treatment rifampicin resistance

Fig. 1. Flow diagram of patients from eligibility to analysis stages. MDR-TB multidrug-resistant tuberculosis H isoniazid R rifampicin

TABLE I. Characteristics of 249 sputum-positive pulmonary tuberculosis patients at enrolment

<table>
<thead>
<tr>
<th>Variable</th>
<th>Category</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>Men</td>
<td>181 (73)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>≤40</td>
<td>190 (76)</td>
</tr>
<tr>
<td></td>
<td>&gt;40</td>
<td>59 (24)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>&lt;35</td>
<td>28 (11)</td>
</tr>
<tr>
<td></td>
<td>35–40</td>
<td>62 (25)</td>
</tr>
<tr>
<td></td>
<td>41–45</td>
<td>70 (28)</td>
</tr>
<tr>
<td></td>
<td>46–50</td>
<td>62 (25)</td>
</tr>
<tr>
<td></td>
<td>&gt;50</td>
<td>27 (11)</td>
</tr>
<tr>
<td>Initial home sputum smear grading</td>
<td>1+</td>
<td>77 (31)</td>
</tr>
<tr>
<td></td>
<td>2+</td>
<td>125 (50)</td>
</tr>
<tr>
<td></td>
<td>3+</td>
<td>47 (19)</td>
</tr>
<tr>
<td>Initial home sputum culture grading</td>
<td>≤1+</td>
<td>45 (18)</td>
</tr>
<tr>
<td></td>
<td>2+</td>
<td>11 (4)</td>
</tr>
<tr>
<td></td>
<td>3+</td>
<td>193 (78)</td>
</tr>
</tbody>
</table>

TABLE III. Bacteriological recurrence in patients followed up for 24 months (n=238) (efficacy analysis)

<table>
<thead>
<tr>
<th>Pre-treatment drug sensitivity</th>
<th>n (%)</th>
<th>Month of recurrence post-treatment</th>
<th>n (%)</th>
<th>11–14</th>
</tr>
</thead>
<tbody>
<tr>
<td>H/R-susceptible</td>
<td>214</td>
<td>10 (5)</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>H/R-resistant</td>
<td>24</td>
<td>4* (17)</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>All</td>
<td>238</td>
<td>14 (6)</td>
<td>4</td>
<td>1</td>
</tr>
</tbody>
</table>

* Including one patient with pre-treatment rifampicin resistance

p<0.05. The proportions at 5 and 6 months were similar and high (>90%) in both groups.

Status at the end of treatment in patients who initially had drug-susceptible TB

At the end of treatment, 214 of 221 (97%) patients who initially had drug-susceptible bacilli had a favourable outcome (Table II). Of these 214 patients, 206 had all six cultures negative at the fifth and sixth months of treatment. Eight patients had one positive culture either at the fifth (5) or sixth month (3). Of these 8 patients, 3 had 1+ culture and 5 had 1 colony on culture. Among the 8 patients, 7 had negative cultures during the subsequent months and remained bacteriologically quiescent, while one had recurrence of TB at the eighth month. All 7 patients who had treatment failure received more than 80% of chemotherapy. Of these 7 patients, 2 needed a change of treatment due to clinical and radiological deterioration, and 5 continued to excrete drug-susceptible bacilli.
Bacteriological recurrence in patients who initially had drug-susceptible TB

Of the 214 patients who initially had drug-susceptible TB and who had a favourable outcome at the end of treatment, 10 (5%) had recurrence of TB (Table III), 8 of them within the first 4 months. The sputum cultures at recurrence were still drug-susceptible in 9 patients and the tenth patient developed TB lymphadenitis.

Status at the end of treatment in patients with initially H- or R-resistant bacilli

Of the 28 patients who were H- or R-resistant pre-treatment, 24 (86%) had a favourable bacteriological outcome at the end of treatment (Table II), including the patient with initial R resistance. One patient had one positive culture (1+) at the sixth month and he subsequently had recurrence of TB at the eighth month. Of the 4 patients with treatment failure in this group, R resistance emerged in 2, while the other 2 continued to have H-resistant organisms.

Bacteriological recurrence in patients with initially H/R-resistant bacilli

Of the 24 drug-resistant patients (23 to H and 1 to R) who had a successful outcome at the end of treatment and were followed up for 24 months after treatment (Table III), 4 (3 H-resistant, 1 R-resistant) had recurrence of TB, 3 of them within 4 months. The drug susceptibility profile of the bacilli at recurrence was the same as that during pre-treatment in 3 patients (2 H-resistant, 1 R-resistant), and in the remaining one (initially H-resistant), the bacilli were susceptible to H.

Intention-to-treat analysis

This was done for 262 patients (249 from per-protocol analysis and 13 patients excluded from the efficacy analysis because they had received <80% of the prescribed treatment). Excluding 3 patients who were lost to follow-up, there remained 259 patients (230 with drug-susceptible TB and 29 with initial resistance to H/R). Of these, 245 (94%) had a favourable status at the end of treatment and 14 (6%) had recurrence of TB during the follow-up period of 24 months.

Adverse reactions to drugs

Of the 262 patients treated with the regimen, 38 (14%) had some adverse drug reaction, the majority (26; 10%) being gastrointestinal. A modification of the scheduled treatment was required in only 3 patients (1.1%). In one patient, the administration of H and R was stopped due to hepatitis. In the other 2 patients, each of whom had an episode of seizures during the continuation phase, H and R were temporarily withheld and later resumed without any further problems.

DISCUSSION

Of the 249 patients treated with the 6-month thrice-weekly regimen recommended by the RNTCP, 238 (96%) had a favourable outcome at the end of treatment, and of these, 6% had recurrence of TB over the following 24 months. Even among patients with an initial resistance to H or R, 86% had a favourable outcome and only 4 of 24 patients had recurrence of TB. None of the 7 patients who had drug-susceptible TB and in whom the treatment failed, and none of the 10 who had recurrence of TB, developed drug resistance. Further, 5 of the 7 patients who had treatment failure, continued to excrete drug-susceptible bacilli at 6 months. The continued excretion of drug-susceptible bacilli despite supervised chemotherapy has already been reported by this centre. 13

Sputum culture-negativity at 2 months and post-treatment TB recurrence are reliable markers of the sterilizing potential of a drug regimen.14,15 Negativity of cultures at the second month was 82% and the recurrence rate 6% in our analysis of the 6-month thrice-weekly regimen discussed here. The corresponding figures for 6-month thrice-weekly regimens (6H R Z E, and 6H R Z S) from a clinical trial from Hong Kong were 90% and 2%, respectively, for the first regimen, and 90% and 1%, respectively, for the second. In contrast, another study analysing a 6-month thrice-weekly non-pyrazinamide regimen (6H R S E) in Hong Kong found a culture-negativity rate of 76% at 2 months and a relapse rate of 8%.16 However, the assumption that a high rate of negativity of cultures at 2 months will result in a low recurrence rate is not always true. We have observed that in a 4-month ofloxacin regimen (2 OHRZ daily/2 HR twice-weekly), a culture-negativity rate of 92% at 2 months was associated with a TB recurrence rate of 13% in 24 months.17 So it is not only the culture-negativity at 2 months that determines the recurrence rate, but also the drugs prescribed and the duration of their administration in the continuation phase.

In our analysis, the culture-negativity rates were significantly higher than the corresponding smear-negativity rates during the initial months of treatment. Rapid culture conversion during the early days of chemotherapy is attributable to the early bactericidal activity of H.18,19

The smear conversion rates observed in our study were low (61% at 2 months and 80% at 3 months) compared to the rate observed in the RNTCP—90% at 3 months.18 This is probably due to the different definitions of smear conversion used for our analysis (3 negative smears) and in the RNTCP (2 negative smears). An additional reason may be that our study used the fluorescent auramine rhodamine stain, which is more sensitive than the Ziehl–Neelsen stain used in the programme.19,20,21 At the end of 2 months, 21% of patients had positive smears with corresponding negative cultures. This could explain the fact that despite not extending the intensive phase for patients who were smear-positive after 2 months of treatment, in contrast to the RNTCP recommendations, smear-negativity increased from 61% at the end of the second month to 80% at the end of the third month.

In field trials under the RNTCP, the 6-month thrice-weekly regimen was associated with significant default rates.12,23 Treatment default was due to reasons related to the DOTS provider and could have been prevented.13,23 Therefore, if the default rate is lowered, the cure rate can be enhanced. Six-month, thrice-weekly, fully supervised chemotherapy produced excellent results in China between 1991 and 1996. Thanks to a well-executed DOTS strategy, more than 90% of patients received the scheduled treatment (defaulter rate 0.2%–2.2%) and the cure rates were high (91%–97%).20,26

Under the RNTCP, the cure rate among the 6.2 lakh new smear-positive pulmonary TB patients registered in 2009 was 84.9%.18 A recent systematic review has shown that while regimens utilizing R only for the first 1–2 months were associated with significantly higher rates of failure, recurrence and acquired drug resistance, regimens that used R for 6 months showed little evidence of difference in rates of failure or recurrence with daily or intermittent schedules of treatment. 27 The RNTCP does not routinely perform sputum culture and drug susceptibility testing before the start of TB treatment and hence, the initial drug susceptibility profile of these patients is not known. Even though
the methods used in the source trials and the procedures in the RNTCP are different (in particular, treatment in the RNTCP is directly observed only for the first 2 months and partially observed for the next 4 months), this analysis indicates that if the 6-month thrice-weekly regimen is administered under full supervision for the entire duration, it has the potential to prove successful for patients with newly diagnosed pulmonary TB.

Limitations
The principal limitation of this study is that it is a retrospective analysis of a portion of the data from two randomized clinical trials that were prematurely terminated. The data were obtained under ideal trial conditions and hence, may not be reproduced in toto for use under a programme. Further, the treatment outcome was based on the results of sputum cultures, which are unavailable under the RNTCP. Genotyping of cultures was not performed to differentiate between reactivation and re-infection. Finally, treatment defaulters are excluded from the efficacy outcome analysis in a clinical trial, whereas they are included in the RNTCP.

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REFERENCES
2 Khatri GR. The Revised National Tuberculosis Control Programme: A status report on first 1,00,000 patients. Indian J Tuberc 1999;46:157–66.
4 Vijay S, Balasangameshwarra VH, Jegannatha PS, Saroja VN, Kumar P. Treatment outcome and two and a half years follow-up status of new smear positive patients treated under RNTCP. Indian J Tuberc 2004;51:199–208.
16 Hong Kong Chest Service/British Medical Research Council. Controlled trial of 4 three-times-weekly regimens and a daily regimen all given for 6 months for pulmonary tuberculosis. Second report: The results up to 24 months. Tubercle 1982;63:89–98.