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Viability and drug sensitivity of *M. Leprae* isolated from long-term WHO/MDT treated multibacillary leprosy patients

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Introduction

The ALERT Leprosy Control Programme introduced WHO/MDT in Ethiopia in January 1983. Paucibacillary patients are treated for 6 months with self-administered daily dapsone and rifampicin once monthly under supervision. Multibacillary patients are treated for a period of at least 2 years or until the skin smears become negative. Treatment consists of self-administered dapsone, clofazimine and rifampicin and clofazimine monthly under supervision.

In 1990, the ALERT leprosy control and the ALERT Hospital Services observed multibacillary patients who were suspected to be unresponsive to the WHO/MDT regimen. The basis of this concern was that by the yearly skin slit examination, the bacteriological index (BI) did not decrease by 1 log unit per year, despite adequate compliance and above 80% attendance rate, with the WHO-recommended MDT. Using the mouse foot pad assay system, we tested for viable bacilli from some of these patients from 1992 to 1993.

Materials and methods

Forty-one patients, who were still on chemotherapy, were enrolled in the study. The patients were subjected to a 4–6 mm punch biopsy and their tissue biopsy was processed for mouse foot pad inoculations. Depending upon the bacterial yield, up to 10^4 acid-fast bacteria (AFB) from each biopsy were inoculated to each mouse foot pad whenever possible. In the absence of identifiable bacilli, 30 μl of the suspension was inoculated to each pad of BALBIC mice and harvested a year later.

'Multiplication' means that the number of AFB harvested pad was $\geq 10^5$ AFB per foot pad. Thus viability of bacilli is based upon viable bacteria to multiply.

When growth was observed, mouse-to-mouse passages were carried out for sensitivity studies to dapsone, clofazimine and rifampicin. Therapy was stopped in these patients at the beginning of 1994, and future follow-up to assess clinical status and bacteriological status was planned.

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Table 1. Viability of *M. leprae* isolated from MB leprosy patients with poor BI reduction on long-term treatment with WHO/MDT (1992–1993)

				Passage and sensitivity to		
				DDS (%)	GLOF (%)	RIF (%)
Average RX in years	No. of patients	Average BI at testing	Viability test results	00·0001, 0·001, 0·01	0, 0·0001, 0·001, 0·01	0·003, 0·03
2–3 years	17	1. 4·5	0/8 Total 5/17	Passage was not possible due to small quantity of bacterial available for inoculation.		
		2. 4·8	0/8			
		3. 4	0/8			
		4. 4·7	0/8			
		5. 3·8	0/6			
		6. 5	4/4			
		7. 2·7	4/4			
		8. 3	1/7			
		9. 1·4	2/8			
		10. 4·7	1/8			
		11. 3·4	0/8			
		12. 1·4	0/5			
		13. 4	0/8			
		14. 2·5	0/8			
		15. 1·5	0/8			
		16. 4·7	0/8			
		17. 3·5	0/8			
> 3 < 5 years	15	1. 3·4	0/6 Total 3/15	2/2 Sensitive		
		2. 1·2	0/7			
		3. 1·2	0/7			
		4. 1·5	0/6			
		5. 2	2/2			
		6. 3·6	6/6			
		7. 5·2	2/2			
		8. 3·7	0/8			
		9. 2·2	0/6			
		10. 3·2	0/3			
		11. 2·2	0/8			
		12. 1	0/8			
		13. 0·5	0/8			
		14. 2	0/8			
		15. 2·5	0/8			
> 5 years	9	1. 2·7	0/7 Total 3/9	Not Done		
		2. 4	0/6			
		3. 2·2	1/7			
		4. 1·2	2/8			
		5. 1.4	1/7			
		6. 1·2	0/7			
		7. 1·5	0/8			
		8. 4	0/8			
		9. 1·2	0/8			

Results

Eleven biopsies showed viable bacilli and in two specimens, the strains were sensitive to the three drugs (clofazimine, dapsone and rifampicin). For the remaining nine strains, the inoculum was not sufficient to carry out drug sensitivity testing. Multiplication was not observed in 30 biopsy specimens (Table 1). Three years later, the 11 patients with proven growth were recalled for clinical and bacteriological assessments. One out of the 11 cases died and another one was lost to follow-up. Nine patients were clinically inactive and their skin slit smears were negative for AFB (Table 2).

Discussion

A trial comparing 18 monthly and 30 monthly doses of the World Health Organization recommended multibacillary therapy in 305 multibacillary leprosy patients in Malawi is described. Patients were randomly allocated to one of the two regimens following the 18th supervised dose of WHO/MDT.

The mean follow-up period was 3 years. No relapse was observed in either group.[1]

Several studies demonstrated that biopsies, or nasal secretions taken from patients having received a single dose of 600 mg of rifampicin usually failed to give growth of *M. leprae* in foot pads of mice,[2–6] whereas it took 3–6 months of daily treatment to obtain the same results with either dapsone alone or clofazimine.

In this study, we observed that the isolation of viable bacilli is not necessarily related to the duration of MDT or to the average BI. It also appears that there was a rapid clearing of bacilli following the discontinuation of therapy in some of our patients. Viable bacilli can be isolated from some patients after completion of WHO/MDT, but most patients can eliminate these bacilli without further chemotherapy and do not seem to be at great risk of relapse.

Table 2. Average BI of the nine multibacillary patients with poor BI reduction on long-term treatment with WHO/MDT and with proven viable bacilli

Exp. no.	Pre-treatment BI	Duration of treatment at time of enrolment (years)	BI status at time of enrolment	No. of AFB incubated	BI status after 3 years follow-up period
1	4	5	2·2	Not identified	0
2	5	4·4	5·2	10^4	0
3	5	4	2	Not identified 30 μl inc. of susp.	0
4	5	3·6	3·6	10^4	0
5	5	5	1·4	Not identified 30 μl inc. of susp.	0
6	6	4	2	10^4	0
7	6	2	4·7	10^4	0
8	6	7·2	1–2	Not identified 30 μl inc. of susp.	0
9	5	2	5	10^4	0

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