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Studies on dapsone-resistant *Mycobacterium leprae* in leprosy patients of Gudiyatham Taluk, the leprosy control area of the Schieffelin Leprosy Research and Training Centre, Karigiri. 2. A progress report

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Summary The 1580 LL and BL leprosy patients in a community of 480,000 persons in South India were studied for the occurrence of dapsone-resistant *Mycobacterium leprae*, between March 1978 and February 1981. Patients with a BI $\geqslant 2+$ were biopsied for mouse inoculation, even if they were improving on dapsone monotherapy. Between 89 and 116 patients per 1000 patients screened were estimated to harbour dapsone-resistant *M. leprae*.

Introduction

Gudiyatham Taluk of North Arcot District in Tamil Nadu, the leprosy control area of the Schieffelin Leprosy Research and Training Centre, covers an area of approximately 1320 sq km with a population of 480,000 (1981 Census). The region is hyperendemic for leprosy, and in December 1977, 6880 patients were on the treatment register at 44 peripheral clinics within the control area. Dapsone monotherapy has been extensively used in this area since 1963, and fairly accurate records of patients have been maintained systematically throughout this period.

The objectives of the study were: 1, to determine the number of registered patients who harbour dapsone-resistant *Mycobacterium leprae*; and 2, to identify risk factors associated with the occurrence of dapsone-resistant *M. leprae*.

Materials and methods

The denominator chosen for the study was all LL and BL cases on the treatment register maintained by the Department of Epidemiology and Leprosy Control of

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this institution at the end of December 1977, who resided within the control area. Every patient in the denominator was clinically examined by a medical officer, and skin smears were taken from 4 routine sites as well as from other sites at which there was evidence of activity.

Patients with a BI $\geqslant 2+$ at any one site were biopsied, preferably from the site with the highest index (avoiding the face). In order not to underestimate the number of patients harbouring dapsone-resistant *M. leprae*, biopsy was performed on all patients with a BI $\geqslant 2+$, and not only on those showing evidence of active disease, as in the studies reported to date.[1–13] It must be emphasized that those biopsied included patients improving on dapsone monotherapy, who would not ordinarily be suspected of harbouring dapsone-resistant *M. leprae*. Biopsies were usually taken in the field and transferred to the base laboratory on wet ice for mouse foot-pad studies, which were performed by methods already described.[14, 15]

The patients whose *M. leprae* failed to grow even in untreated mice, and those in whom the test did not detect resistant *M. leprae*, were rescreened and biopsied again if eligible.

Results and interpretation

All 1580 registered LL and BL patients residing within the area were enumerated in December 1977. The screening began in March 1978, and the activities undertaken during the next 3 years are summarized in Table 1. Of the total, 1431 patients were screened in the first year, forming a cohort that was subjected to annual screening, and biopsied when eligible.

In the first year 149 patients evaded screening. A 10% random sample of these patients were subsequently screened, and none was found eligible for biopsy. The

Table 1. Numbers of patients screened annually

	Year of survey		
	1978–79	1979–80	1980–81
Enumerated	1580	1431	1320
Migrated or died during the previous year	—	56	48
Resistant bacilli demonstrated in previous year	—	33	27
Eligible for screening	1580	1342	1245
Acutally screened	1431 (90·6%)	1320 (98·4%)	1208 (97·1%)

149 patients were therefore not included in any subsequent procedures or analysis.

As shown in Table 2, 9 patients among the 1431 screened had been shown earlier by mouse inoculation to harbour dapsone-resistant *M. leprae*. Table 2 also shows the number of patients found eligible for biopsy (BI $\geqslant 2+$) during each year of the study, and the number of patients subjected to biopsy. The large proportion of biopsies done during the third year of survey did not reflect an increase in the number of patients attaining eligibility for biopsy during that year, but resulted from an improved operational capacity for handling biopsy specimens. A total of 188 patients were found eligible for biopsy, of whom 142 have thus far been subjected to the procedure.

The results of biopsy and mouse inoculation are presented in Table 3. Of the 188 patients eligible, 46 escaped biopsy for a variety of reasons. Of the 142 mouse foot-pad studies carried out, the results of 17 are still not available. In 26 studies the inoculated *M. leprae* failed to multiply in both control and dapsone-treated mice. Dapsone-resistant *M. leprae* were detected in 89 studies. The resistant *M. leprae* in 81 of these studies manifested resistance to the highest concentration of dapsone used (0·01 g%). 10 studies did not detect any dapsone-resistant *M. leprae*. It is of great interest to note that an eleventh study, which on one occasion did not detect dapsone-resistant *M. leprae*, was repeated on the same patient after a period of 12 months. On the second occasion, dapsone-resistant *M. leprae* were detected, that manifested resistance to the highest concentration of dapsone used.

Thus, of the 188 patients eligible for biopsy and mouse inoculation, the results of mouse foot-pad studies have so far been obtained for only 99. Because 89 patients for whom no results are available comprise so large a fraction of the total, it is necessary to make some assumptions regarding them, in order to estimate the number of patients harbouring dapsone-resistant *M. leprae*. It appears reasonable to assume that among the 46 patients not subjected to biopsy and the 17 for

Table 2. Numbers of patients with positive smears and numbers biopsied annually

No. of patients	Pre-1978*	Year of survey 1978–79	1979–80	1980–81	Cumulative†
Smear positive	—	336	330	179	—
BI $\geqslant 2+$	9	114	86	82	188
BI $\geqslant 2+$ with clinical relapse	9	46	49	34	87
Biopsied for mouse inoculation	9	26	28	80	142

* 9 patients among the 1431 screened had already been shown by mouse inoculation to harbour dapsone-resistant *M. leprae*.

† A patient who appears to more than 1 year is counted only once in the cumulative total.

Table 3. Results of biopsies and mouse inoculation

	Number of specimens				
		Year of survey			
	Pre-1978	1978–79	1979–80	1980–81	Cumulative
Biopsied	9	26	28	80	142
No growth of *M. leprae*	—	—	—	26	26
No DDS-resistant *M. leprae* detected	—	2*	1	8	10
DDS-resistant *M. leprae* detected	9	24	27	29	89
Resistant to DDS, at mouse diet concentration of:					
0·0001 g%	—	—	—	1	1
0·001 g%	—	2	2	3	7
0·01 g%	9	22	25	25	81
Study in progress	—	—	—	17	17

* One of these patients was later biopsied again and shown at that time to harbour resistant organisms; he is included only once in the cumulative totals.

whom results are awaited, the proportion who harbour dapsone-resistant *M. leprae* is the same as among those for whom results are available. The 26 patients whose organisms failed to multiply in control mice are problematic, however. It could be argued that these patients harbour no dapsone-resistant *M. leprae*, because such a large proportion of the organisms from these patients had been killed during dapsone treatment that no organisms grew even in untreated mice. However, it is possible that viable dapsone-resistant *M. leprae* were present, although the inoculum contained too few to produce growth in the mouse foot-pad. To allow for this uncertainty, a separate estimate of the total number of patients harbouring dapsone-resistant *M. leprae* has been made for each of the alternative possibilities.

It has already been pointed out that some patients who showed improvement on dapsone monotherapy were biopsied only because they had a BI $\geqslant 2+$; these patients would not ordinarily have been suspected of harbouring dapsone-resistant *M. leprae*. They differ markedly from the rest of the patients biopsied, in showing a decrease in BI in successive smears at the time of biopsy. Until the significance of this difference is more fully understood, it appears important to maintain the distinction between this group of patients and the rest. Therefore, the 188 patients eligible for biopsy have been divided in 2 groups: 142 who showed an increase in BI in successive smears at the time of biopsy; and 46 who showed a decrease in BI.

Table 4 shows the estimation of the total numbers of patients harbouring

Table 4. Estimation of total number of patients harbouring dapsone-resistant *M. leprae*

	Number of patients		
	Successive smears show increasing BI	Successive smears show decreasing BI	Total
Eligible for biopsy	142	46	188
No results available*	27	36	63
No growth of *M. leprae*	22	4	26
Resistant *M. leprae* detected	84	5	89
No resistant *M. leprae* detected	9	1	10
Predicted additional number with resistant *M. leprae*:			
alternative no. 1†	$\frac{84}{(93+22)} \times 27 = 20$	$\frac{5}{(6+4)} \times 36 = 18$	38
alternative no. 2‡	$\frac{84}{93} \times 49 = 44$	$\frac{5}{6} \times 40 = 33$	77
Total number with resistant *M. leprae*:			
alternative no. 1	$84+20=104$	$5+18=23$	127
alternative no. 2	$84+44=128$	$5+33=38$	166

* Includes patients not biopsied, and those whose results are pending.

† *M. leprae* that failed to grow in mice assumed not resistant.

‡ *M. leprae* that failed to grow in mice assumed susceptible and resistant in same proportions as those that multiplied in mice.

dapsone-resistant *M. leprae* in each of the 2 groups. The assumption has been made that none of the 26 patients whose *M. leprae* failed to multiply in mice harbours dapsone-resistant organisms, whereas, among the remaining 63 patients for whom no results are available, the proportion who harbour resistant organisms is the same as among the 99 patients for whom results are available. With these assumptions, a total of 104 patients from the first group and 23 patients from the second group, altogether 127 patients, were estimated to harbour dapsone-resistant *M. leprae*.

Instead, if among the 26 patients whose *M. leprae* failed to grow in control mice, the proportion who harbour resistant bacilli is considered to be the same as the proportion among the 99 patients whose results are available, then, by similar calculations, an alternative estimate is obtained. According to this alternative estimate, 128 patients from the first group and 38 patients from the second group, altogether 166 patients, were estimated to harbour dapsone-resistant *M. leprae*.

The number of registered LL and BL patients residing in Gudiyatham Taluk,

who harbour dapsone-resistant *M. leprae*, is therefore estimated to be between 127 and 166 patients, of a total of 1431 patients screened annually. Expressing these figures as fractions, between 89 and 116 per 1000 patients screened are estimated to harbour dapsone-resistant *M. leprae*. It appears reasonable to assume that the true figure must fall somewhere between these two estimates.

Discussion

As reported earlier,[15] the crude estimate of the prevalence of dapsone-resistant leprosy in Gudiyatham Taluk after the first year of this study was 23 per 1000. This may be explained partly by our earlier inability to test in mice the *M. leprae* of all the patients eligible for biopsy during a given year, and partly by the fact that biopsy during the first year was done only on patients who were deteriorating, by smear and clinical criteria.

The unexpected finding that patients who were improving on dapsone monotherapy were also shown by the mouse foot-pad test to harbour dapsone-resistant *M. leprae* raises problems of interpretation. It would appear premature to make a decision on this until a detailed analysis of our data can be completed.

Analysis of risk factors has also been deferred. However, careful records have been kept of the treatment and progress of all the patients screened, from their date of diagnosis. An analysis of the prevalence of dapsone resistance, and its causation and consequences in the individual and in the community, will be undertaken in subsequent publications.

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References

[1] Pettit JHS, Rees RJW. Sulphone resistance in leprosy: an experimental and clinical study. *Lancet*, 1964; **ii,** 673–4.

[2] Pettit JHS, Rees RJW, Ridley DS. Studies on sulfone resistance in leprosy. 1. Detection of cases. *Int J Lepr*, 1966; **34:** 375–90.

[3] Pearson JMH, Rees RJW, Waters MFR. Sulphone resistance in leprosy—a review of one hundred proven clinical cases. *Lancet*, 1975; **ii:** 69–72.

[4] Roy Chaudhry SB, Desikan KV. Sulphone resistance in leprosy: a report of three cases. *Lepr India*, 1975; **47:** 283–9.

[5] Jacobson RR. Sulphone resistance in Hansen's disease. *J Christian Med Assoc India*, 1974; **49:** 553–4.

[6] Peters JH, Shepard CC, Gordon GR, Rojas AV, Elizondo DS. The incidence of DDS resistance in lepromatous patients in Costa Rica: their metabolic disposition of DDS. *Int J Lepr*, 1976; **44:** 143–51.

[7] Taylor PM, Chacko CJG, Job CK. Study of sulphone resistance in leprosy patients in India. *Lepr Rev*, 1976; **47:** 5–11.

[8] Pearson JMH, Ross WF, Rees RJW. DDS-resistance in Ethiopia—a progress report. *Int J Lepr*, 1976; **44:** 140–2.

[9] Levy L, Rubin GS, Sheskin J. The prevalence of dapsone-resistant leprosy in Israel. *Lepr Rev*, 1977; **48:** 107–12.

[10] Pearson JMH, Cap JA, Haile GS. Dapsone-resistant leprosy and its implications for control programmes. *Lepr Rev*, 1977; **48:** 83–94.

[11] Pearson JMH, Haile GS, Barnetson R StC, Rees RJW. Dapsone-resistant leprosy in Ethiopia. *Lepr Rev*, 1979; **50:** 183–99.

[12] Sardari Lal, Jaganath C, Garg BR, Paneerselvam R. Secondary sulphone resistance in leprosy—report of a case. *Lepr India*, 1980; **52** (2): 299–301.

[13] Baquillon G, Ferracci C, Saint Andre R, Pattyn SR. Dapsone-resistant leprosy in a population of Bamako (Mali). *Lepr Rev*, 1980; **51:** 315–19.

[14] Rees RJW. Drug resistance of *M. leprae*—particularly to DDS. *Int J Lepr*, 1967; **35:** 625.

[15] Balraj V, Jesudasan K, Chacko CJG, Christian M, Taylor PM, Fritschi EP, Job CK. Prevalence of secondary dapsone resistance in Gudiyatham Taluk, the leprosy control area of the Schieffelin Leprosy Research and Training Centre, Karigiri. 1. Preliminary report. *Int J Lepr*, 1980; **48:** 397–401.