Lepr Rev (1999) **70,** 281–286

Presence of *M. leprae* in tissues in slit skin smear negative multibacillary (MB) patients after WHO-MBR

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Accepted for publication 1 July 1999

Summary This study looked for *M. leprae* in the lymph node, nerve and skin of multibacillary (MB) leprosy patients who become slit skin smear negative after the completion of WHO-MBR. Twenty-five WHO-MBR-treated multibacillary leprosy patients were studied; borderline lepromatous (BL) leprosy ($n = 11$) and lepromatous (LL) leprosy ($n = 14$)). Fifteen patients had reaction (erythema nodosum leprosum 11, upgrading reaction 4) either at presentation or during therapy. All patients attained slit skin smear negativity after WHO-MBR (range 24–39 months. Sixteen (64%) patients with multibacillary leprosy showed fragmented bacilli in skin and nerve biopsy or lymph node aspirates after WHO-MBR. Lymph node aspirates alone revealed *M. leprae* in seven patients, followed by nerve in two and skin in one patient. Four cases showed *M. leprae* at all sites followed by nerve and skin or lymph node in one case each. A pretreatment bacteriological index (BI) of 4+ or more was significantly associated with the presence of *M. leprae* at the end of treatment. Also, significantly more lymph node aspirates contained *M. leprae* in comparison with nerve or skin biopsies. All seven cases in whom treatment was extended beyond 24 months showed *M. leprae* in tissues even after attaining slit smear negativity. In conclusion, *M. leprae* persist in tissues after 2 years of WHO-MBR and patients with an initial BI of 4+ or more need to be closely followed up after stopping MDT.

Introduction

The aims of therapy in leprosy are to interrupt the transmission of infection and to eliminate all viable *M. leprae* from the body as rapidly as possible. Ever after treatment with monotherapy and multidrug therapy regimens, bacilli linger in protected sites like Schwann cells, perineural cells, smooth muscle, cells of blood vessel walls, erector pili of the skin and

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0305-7518/99/070281+06 $1.00

dartos tunica of the scrotum. Rarely, Kupffer cells in the liver and macrophages in the lymph nodes, bone marrow and spleen may also harbour *M. leprae*. These persisting bacilli survive in the host despite adequate antimicrobial treatment.[1–3] The study of persisters is important because persistence of bacilli is one of the major reasons for relapses in leprosy following both monotherapy and multidrug therapy, irrespective of the regimen used and the duration of treatment. The WHO study group on chemotherapy or leprosy has recommended stoppage of MDT after 24 months in all multibacillary cases irrespective of slit skin smear status.[4] This needs to be critically evaluated if persisters are present. This study was carried out to document *M. leprae* in the lymph nodes, nerves and skin in multibacillary leprosy patients who become slit skin smear (SSS) negative after the completion of 24 months or more of WHO recommended multibacillary regime (WHO-MBR).

Materials and methods

Twenty-five multibacillary (MB) patients attending the leprosy clinic of Department of Dermatology, Venereology who had become SSS negative after treatment with WHO-MBR for 24 doses or more were recruited for the present study. SSS from five sites (both ear lobes and three skin lesions) and a skin biopsy was carried out in all patients before and after treatment. In addition, nerve (radial cutaneous/sural) biopsy and fine needle aspiration cytology of inguinal or femoral lymph nodes were carried out after treatment as described previously.[5,6]

Sections from the skin and nerve were stained with haematoxylin and eosin stain and evidence of active leprosy was looked for. The above were also stained with Ziehl–Neelsen stain and studied for presence of *M. leprae*. Smears from lymph node aspirates were similarly studied. Two observers independently assessed for persisting bacilli and looked for any solid staining bacilli. Percentage, mean and Student's *t*-test were used for statistical analysis.

Results

PATIENTS

Of the 25 patients 17 were males and eight females. The age range was 16–72 (mean age 39 years). Duration of the disease ranged from 1 month to 7 years (mean duration 24 months). There were 14 lepromatous leprosy (LL) and 11 borderline (BL) lepromatous patients. Fifteen (60%) patients (nine males and six females) had reaction either at presentation or during the course of their treatment. Three of the four patients with type 1 reaction and all the 11 patients with type 2 reaction required systemic prednisolone. Two patients also received colchicine and pentoxyphyline besides prednisolone. All patients showed regression of lesions at the end of therapy.

PRE- AND POST-TREATMENT SLIT SKIN SMEAR

All patients were SSS positive prior to instituting WHO-MBR and 15 had BI 4+. Eighteen patients attained slit skin smear negativity at 24 months, three patients at 30 months and the remaining four attained smear negativity after 30 months (range 31–39 months).

HISTOPATHOLOGY FINDINGS

Skin

The study of pre- and post-treatment biopsies showed resolution of granulomas at the end of treatment. All biopsies showed atrophy of epidermis, paucity of skin appendages and increased collagenization of dermis. Mild perivascular and periappendageal lymphocytic infiltrate was noticed in all patients and scanty foam cells in 15 (60%) patients. Six (24%) patients showed *M. leprae*.

Nerve

All nerve biopsies showed fibrosis and varying amount of lymphocytic infiltrate around the vessels and in the nerve tissues. Scanty foam cells in perivascular locations were seen in 20 (80%) biopsies. *M. leprae* were present in eight (32%) biopsies both in Schwann cells and in foam cells.

Table 1. Clinical profile of patients treated with WHO-MBR and presence of *M. leprae* in tissues after MDT

Subject no.	Age/ Sex	Diagnosis	BI	MI	Duration of disease	Reaction type	Drugs required	Duration of		*M. leprae* in	
								MDT	LN	Nerve	Skin
1	43/F	LL	5	2%	2y	II	P+C	49m	+	+	+
2	48/F	LL	4	5%	2y	II	P	38m	+	–	–
3	35/F	LL	4	4%	6y	II	P	24m	+	+	+
4	17/F	LL	4	2%	1·5m	II	P	24m	NP	–	–
5	22/M	BL	4	2%	1y	I	–	24m	+	+	–
6	27/F	LL	4	1%	5m	II	P	24m	NP	–	–
7	25/M	LL	4	1%	1y	II	P	30m	+	–	–
8	35/F	BL	4	1%	3y	–	–	24m	–	+	–
9	58/F	LL	4	1%	6m	–	–	46m	–	–	+
10	40/M	LL	4	1%	2y	II	P	26m	+	–	–
11	28/M	LL	4	0%	2m	II	P+C	44m	+	+	+
12	30/F	LL	4	0%	1·5y	II	P+PF	28m	+	+	+
13	40/M	LL	4	0%	1y	II	P	24m	NP	–	–
14	40/M	BL	4	0%	2y	I	P	24m	NP	+	–
15	70/M	LL	4	0%	5y	II	P	24m	+	–	–
16	33/M	LL	2	0%	7y	–	–	24m	–	–	–
17	72/M	BL	1	0%	10d	–	–	24m	NP	–	–
18	55/M	BL	1	0%	2m	–	–	24m	–	+	+
19	16/M	BL	1	0%	2y	–	–	24m	+	–	–
20	56/M	BL	1	0%	1·5y	–	–	24m	NP	–	–
21	46/M	BL	1	0%	1y	–	–	24m	+	–	–
22	34/M	BL	1	0%	4y	–	P	24m	NP	–	–
23	60/M	BL	1	0%	3y	–	–	24m	+	–	–
24	21/M	LL	1	0%	4y	–	–	24m	NP	–	–
25	38/F	BL	1	0%	1·5y	I	P	24m	NP	–	–

y = year, m = month, dis = disease, d = day, P = prednisolone, C = colchicine, PF = pentoxiphyline, NP = not possible, LN = lymph node.

Table 2. Presence of *M. leprae* at different sites after WHO-MBR

Tissue	No. studied	No. positive for *M. leprae*
Lymph node	16	12[a]
Nerve	25	8[a]
Skin	25	6[b]

[a] Lymph node versus nerve, $P = 0{\cdot}02$.
[b] Lymph nodes versus skin, $P = 0{\cdot}004$.

CYTOLOGY FINDINGS

Lymph node

Fine needle aspiration cytology in lymph nodes was possible in 16 of the 25 patients. Reactive lymphoid cells were seen in all patients and foamy macrophages in eight (50%) patients. *M. leprae* were seen in 12 (75%) aspirates both within and outside the macrophages. All aspirates with foamy macrophages showed *M. leprae*.

The clinical profile and presence of *M. leprae* in nerve and skin biopsies and lymph node aspirates is summarized in Table 1.

PRESENCE OF LEPRA BACILLI AFTER WHO-MBR

Sixteen (64%) WHO-MBR-treated patients showed fragmented bacilli in tissues. Lymph node aspirates alone revealed *M. leprae* in seven patients followed by nerve in two patients and skin in one patient. Four cases showed bacilli at all the sites followed by nerve and skin or lymph node in one case each. The presence of bacilli in lymph nodes was significantly higher than in nerve ($P = 0{\cdot}02$) and skin ($P = 0{\cdot}004$) (Table 2). *M. leprae* were present in lymph nodes when nerve and skin biopsies were negative in seven and eight patients, respectively. *M. leprae* were also more frequent in nerves compared to skin, but this was not statistically significant (Table 2).

A significant correlation ($P < 0{\cdot}05$) was found between the bacillary presence after

Table 3. Pretreatment slit skin smear in patients with presence of lepra bacilli after WHO-MBR

Pretreatment BI	No. studied	No. of patients with *M. leprae* after WHO-MBR			Total no. with *M. leprae* in tissues
		Lymph node	Nerve	Skin	
4+ to 6+	15	9	7	5	12*
1+ to 3+	10	3	1	1	4*

* P value <0·05.

WHO-MBR and pretreatment bacteriological index (BI) of 4+ and above (Table 3). *M. leprae* were detected in tissues of all seven patients who received WHO-MBR for more than 24 months for attaining slit smear negativity (Table 1).

All the four patients with multiple site (i.e. lymph node, nerve and skin) positivity had lepromatous leprosy and severe type 2 reaction which required systemic prednisolone. All had BI of 4+ or more at the beginning of treatment. Three (75%) of these patients were females and all were below 50 years of age.

Discussion

The long-term efficacy of any therapeutic regimen in leprosy is assessed by its relapse rate. Job[7] proposed various reasons for relapse, namely persisting bacilli, drug resistant *M. leprae*, reinfection, misdiagnosis of multibacillary cases treated as paucibacillary and inadequate therapy or non-compliance. Amongst these reasons, bacterial persistence plays a significant role in the causation of relapses.[8]

Various workers have demonstrated viable *M. leprae* in various human tissues after both monotherapy and multi-drug therapy irrespective of drug regimen and duration of therapy. Katotch *et al.*[9] found persisting bacilli in 16% of highly bacilliferous (BI 4+ to 6+) BL/LL patients after 2 years of modified WHO regimen. This figure fell to 5% with continuation of treatment for another year, i.e. at 3 years. No viable bacilli were found after 36–45 months of treatment. In contrast, using immunosuppressed mice, Shetty *et al.*[10] found viable bacilli in 12 (46%) out of 26 patients after 24 months or more of WHO-MBR. Persisters were demonstrated twice as frequently in nerve (46%) compared to skin (23%). Similar results reported by other workers.[11,12]

Sivaprasad *et al.*[13] found *M. leprae* in 9/11 (81·9%) skin biopsies and all the 11 lymph node biopsies after 5 years of dapsone monotherapy supplemented with 2 years of multidrug therapy. Also, it was found that *M. leprae* disappeared from the skin lesions much earlier than from the draining lymph nodes. The author therefore suggested that *M. leprae* persist in the macrophages of the regional lymph nodes much longer than in the skin. Similar observations were made in the present study. Despite the limitation of not studying viability of *M. leprae*, our study shows the presence of *M. leprae* at different sites even after attaining slit skin smear negativity. Finding of *M. leprae* in lymph nodes, nerve and skin are not surprising because they are known sites for persisters. However, it has been shown that fragmented bacilli may be viable[14] and therefore may cause relapse. Moreover, there are reports of relapse in patients with high initial BI (4+ or more).[7] In this group of patients, one needs to be cautious in stopping MBR at the end of 2 years despite BI continuing to fall even after stopping treatment and results are comparable in both groups when treatment is stopped at 2 years or continued.[15] The Marchoux chemotherapy study group[8] has suggested that treatment should not be stopped in patients with BI of 2+ or more at the end of 2 years of therapy. The current recommendation of stopping WHO-MBR at the end of 1 year even in highly bacilliferous patients (BI 4+ or more) who show clinical improvement needs to be evaluated in view of the above findings.

The role of corticosteroids in the presence of *M. leprae* in tissue after 24 months or more of WHO-MBR could not be confirmed as the association did not attain statistical significance. Moreover, the majority of such patients had a high initial BI and severe disease.

References

[1] Water MFR, Rees RJW, McDougall AC, Weddell AGM. Ten years of dapsone in lepromatous leprosy: clinical, bacteriological and histopathological assessment and the finding of viable bacilli. *Lepr Rev*, 1974; **45**: 288–298.

[2] Ramu G, Desikan KV. A study of scrotal biopsy in subsided cases of lepromatous leprosy. *Leprosy in India*, 1979; **51**: 341–347.

[3] Toman K. Bacterial persistance in leprosy. *Int J Lepr*, 1981; **47**: 205–217.

[4] WHO technical report series-847, WHO study group on chemotherapy of leprosy. WHO 1994.

[5] Kaur, Kumar B, Gupta K. Fine needle aspiration of lymph nodes in leprosy. A study of bacteriologic and morphologic indices. *Int J Lepr*, 1973; **41**: 94–101.

[6] Kaur S, Sharma VK, Basak P, Kaur Radotra BD. Concurrent skin and nerve histology in leprosy. *Lepr Rev*, 1993; **64**: 110–116.

[7] Job CK. Histopathological features of relapsed leprosy. *Ind J Lepr*, 1995; **67**: 69–80.

[8] Marchoux Chemotherapy Study Group. Relapses in multibacillary leprosy patients after stopping treatment with riframpin-containing combined regimens. *Ind J Lepr*, 1992; **60**: 525–535.

[9] Katoch K, Ramu G, Ramanthan US, Sharma VD, Shivannavar CT, Katoch VM. Results of a modified WHO regimen in highly bacilliferous BL/LL patients. *Int J Lepr*, 1989; **57**: 451–457.

[10] Shetty VP, Suchitra K, Uplekar MW, Antia NH. Higher incidence of viable *Mycobacterium leprae* within the nerve as compared to skin among multibacillary leprosy patients released from multi-drug therapy. *Int J Lepr*, 1997; **68**: 131–138.

[11] Shetty V, Naik SM, Uplekar M, Antia NM. Lepra viability in skin and nerve after MDT and their sensitivity to a anti-leprosy drugs. *Int J Lepr*, 1993; **61**: 4-A.

[12] Job CK, Jayakumar J, Aschoff M, Nathan MM. Viability of Myobacterium leprae in skin and perpheral nerves and persistence of nerve destruction in multibacillary patients after 2 years of multidrug therapy. *Int J Lepr*, 1996; **64**: 44–50.

[13] Sivaprasad N, Snehalatha S, Lobo D, Aschoff M, Job CK. Viability of *Mycobacterium leprae* in lepromatous patients after five years of dapsone monotherapy with two years of multidrug therapy. *Ind J Lepr*, 1995; **67**: 427–433.

[14] Karat ABA, Jamuet I, Albert R, Kumar ASJ. Experiments in cultivation of *M. leprae* in monkeys and footpads of mice—an interim report of six years of study. *Leprosy in India*, 1973; **45**: 138.

[15] Pattyn SR, Bourland S, Grillone G, Ghys P. Combined regimens of one year duration in the treatment of multibacillary leprosy I. Combined regimen with rifampicin administered during one year. *Lepr Rev*, 1989; **60**: 109–118.