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Original Article

A Retrospective Study of Morphological Indices in Leprosy Patients on MB-MDT in a Tertiary Care Hospital of North India

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Slit skin smear (SSS) examination, is a basic, cost effective, diagnostic test for confirmation, classification and assessment of response to treatment in multi bacillary (MB) leprosy patients. The retrospective study was undertaken to see the change in Morphological Index (MI), which is the percentage of viable uniformly stained bacilli out of the total bacilli present in the tissue i.e. the Bacteriological Index (BI) at the time of completion of twelve months of chemotherapy. From Aug 2017 to Dec 2019, 49 patients clinically suspected of multibacillary leprosy and those already on Multibacillary Multidrug treatment, referred to the Microbiology Department, were examined over several visits by slit skin smear microscopy. These 49 MB patients (40 males and 9 females) were followed up for 2 to 6 visits, yielding 249 sets of smears. MI fell significantly in 47/49 patients (95.9%) at the time of treatment completion. It was observed that *Mycobacterium leprae* lose their acid fastness due to chemotherapy and presumably non viable bacilli appeared fragmented and granular thereby making it an objective and sensitive indicator for the clinician. Our study endorses the relevance of MI in monitoring the effect of therapy in leprosy cases.

Keywords : Multibacillary Leprosy, Slit Skin Smears, Morphological Index, MI, Monitoring Treatment, North India

Introduction

National Leprosy Eradication Programme (NLEP), launched in 1983 was a spectacular success. NLEP in partnership with various state governments and non governmental organizations successfully eliminated leprosy as a public health problem in India (WHO 2016-20a). The point prevalence of the disease fell from a high of 57.8/10,000 population in 1983, to <1/10,000 population by the end of December 2005. India with a global disease burden of around 60%, has registered a decline in new case detection since 2017-2018 by nearly 15000 cases. The country has also reported reduction in the numbers of Grade 2 disability

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detected in new cases from 5245 to 3666 and new paediatric cases to less than 10,000 (9227) from more than 10,000 previously (WHO 2019), the two leading indicators to assess the public health impact of leprosy related health services. The world is steadily marching towards its goal of being leprosy free.

WHO has revised the case definitions since 2017 through Monitoring and Evaluation Guide to the Global Strategy (WHO 2016-20b, Monitoring Guide), as follows:

Paucibacillary (PB) case: a case of leprosy with 1 to 5 skin lesions, without demonstrated presence of bacilli in a skin smear;

Multibacillary (MB) case: a case of leprosy with more than five skin lesions; or with nerve involvement (pure neuritis, or any number of skin lesions and neuritis); or with the demonstrated presence of bacilli in a slit-skin smear, irrespective of the number of skin lesions.

Slit skin smear (SSS) examination for detection and quantification of acid fast bacilli (AFB) by assessment by Dharmendra & Ridley scales have been part of clinical practice in leprosy since 1960s. Besides this detection and semiquantitative assessment, measurement of percentage of solid staining acid fast bacilli has been a commonly used procedure which shows correlation with viable bacilli present in the lesions. This percentage of solid staining bacilli is referred to as morphological index (MI) and has been used to monitor the response to therapy in MB cases since 1969 (Browne 1969). Due to operational reasons our national programme had nearly stopped using SSS examination for many years. Now the SSS examination is once again increasingly being used for bacteriological and molecular detection including drug resistance. The aim of the study was to re-establish the procedure in our settings by investigating the decrease in the burden of solid stained AFB (indirectly live bacteria in a patient) at the time of treatment completion by grading slit skin smears on the basis of morphological index (MI).

Patients and Methods

During the study period, Aug 2017 to Dec 2019, 75 patients including both paucibacillary (PB) and multibacillary (MB) leprosy had attended the Leprosy Clinic of our Tertiary Care Hospital of Govt Medical College, Amritsar.

Clinical profile of both paucibacillary and multibacillary cases is summarised in Tables 1, 2, 3. These patients were classified as per Ridley-Jopling (1966) and Indian Association of Leprologists criteria (IAL 1982) (Table 1). Morphological characteristics are presented in Table 2. Nerves involved are summarised in Table 3.

A subset of 49 patients classified as borderline lepromatous and polar lepromatous were further evaluated. 249 sets of slit skin smears (from 49 patients on multiple visits), were examined in the Microbiology laboratory of Government Medical College Amritsar, referred by Skin and V.D. Department of the same institution from the following types of cases (Table 4) :-

- Patients clinically suspected of multibacillary leprosy,
- Hospital registered leprosy patients on multi drug therapy,
- Patients released from treatment reporting for follow up,
- and those suspected of disease relapse,

Their age and gender classification is presented in Table 5.

All the MB cases received one year MB-MDT regimen comprising of rifampicin, clofazimine and dapsone (NLEP 2009).

Each set, at our centre, is eight smears taken from various sites such as earlobes, eyebrows,

Table 1 : Classification of patients visiting Leprosy clinic from August 2017 – December 2019 (Ridley & Jopling 1966; IAL 1982)

1.	Tuberculoid (TT)	02
2.	Borderline Tuberculoid (BT)	11
3.	Borderline (BB)	02
4.	Borderline Lepromatous (BL)	02
5.	Lepromatous (LL)	16
6.	Pure Neuritic	05
7.	LL with Erythema nodosum leprosum (ENL)	15
8.	Histoid	01
9.	Erythema nodosum leprosum (ENL)	03
10.	BT to BB	05
11.	BL to LL	01
12.	BB to BL	03
13.	BT to LL	01
14.	BL to Indeterminate (I)	01
15.	BT to BL	04
16.	BL with Type 1 reaction	02
17.	BL with ENL	01
	Total	75

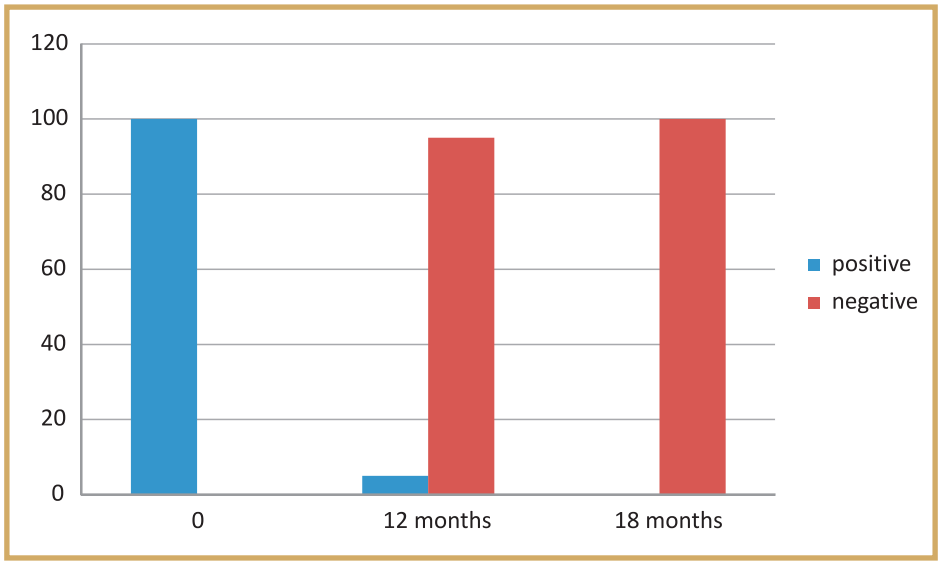

Fig 1 : Fall in Morphological indices in patients on Multi bacillary - Multi Drug Therapy.

Table 2 : Morphology of skin lesions in patients presenting in Leprosy clinic from August 2017 to December 2019

1.	No skin lesion	04
2.	Hypopigmented patches <5	07
3.	Multiple patches , plaques , nodules	04
4.	Multiple papules , plaques , nodules	06
5.	Multiple plaques	15
6.	Multiple papules , plaques , nodules ,erosions	03
7.	Multiple patches , plaques ,ulcers	06
8.	Multiple patches , plaques ,papules	12
9.	Papules , plaques , erosions ,bullae	01
10.	Plaques, Patches , Erosions	01
11.	Nodules , Plaques , Ulcers	03
12.	Multiple Nodules , patches	09
13.	Multiple Ulcers	03
14.	Single Plaque	01
	Total	75

Table 3 : Study cases attending Leprosy clinic classified according to nerve involvement

1.	Thickened Ulnar nerve (UN), Radial cutaneous nerve (RCN)	14
2.	Thickened Ulnar nerve, Lateral popliteal nerve (LPN)	07
3.	Thickened Ulnar nerve, Lateral popliteal nerve, Radial cutaneous nerve (RCN)	06
4.	Thickened Ulnar nerve, Lateral popliteal nerve, Posterior tibial nerve (PTN)	05
5.	No nerve involvement	04
6.	Thickened Ulnar nerve	11
7.	Thickened Ulnar nerve, Common peroneal nerve (CPN), Posterior tibial nerve	01
8.	Thickened Ulnar nerve, Radial cutaneous nerve, Posterior tibial nerve	05
9.	Thickened Radial cutaneous nerve	05
10.	Thickened and tender ulnar nerve	03
11.	Thickened UN, RCN, CPN	02
12.	Thickened RCN, LPN, PTN and tender PTN	02
13.	Thickened Infraorbital nerve, UN, LPN	05
14.	Thickened Sural nerve	01
15.	Thickened UN, GAN, LPN, PTN	01
16.	Thickened UN, RCN, LPN, PTN	02
17.	Thickened LPN	01
18.	Thickened GAN	01
	Total	75

forehead, chin, buttocks, thighs and two active lesions. These SSS were stained by Ziehl Neelsen technique being routinely used in the Microbiology Department of our medical college hospital. Percentage of solid staining bacilli (morphological index) was calculated as per known published criteria (Browne 1969, MRL).

This retrospective study of smears from leprosy cases and their clinical correlates was approved by Institutional Ethics Committee (GMC/IEC/SS/21/006) Dated 05/ 02/ 2021).

Results

Important results are summarised below :

(i) Morphological indices in the beginning of treatment ranged from 17% to 100% in SSS from these 49 patients.

(ii) Fall in morphological index after twelve months of MDT, was observed for 47/49 (95.9%) patients implying satisfactory response at treatment completion (Fig. 1).

(iii) There was only one patient with one fresh lesion positive for acid fast bacilli on the back of his trunk, eleven months after his treatment, during the study period.

(iv) The fall in MI was not uniform to zero within 5-6 months post therapy with rifampicin,

Table 4 : Distribution of patients according to their inclusion criteria

INCLUSION CRITERIA	NUMBER OF PATIENTS
New patients clinically suspected of leprosy	14
Patients registered for MB MDT therapy	31
Patients for follow up after completion of chemotherapy	0
Suspected patients of relapse	4
Total	49

Table 5 : Age and gender distribution of patients positive for acid fast bacilli on slit skin smear examination

Age	Number of patients	Male Positive	Female Positive
< 10	1	1	0
10-20	6	5	1
21-30	14	12	2
31-40	12	8	4
41-50	7	6	1
51-60	5	4	1
61-70	3	3	0
71-80	0	0	0
81-90	0	0	0
>91	1	1	0
	49	40	9

dapsone and clofazimine. MI from SSS from 19 patients took 6-12 months to become negative. There was no clear corelation of time between two end points i.e initial burden of solid bacilli present to zero MI, during multi drug therapy. MI in these 19 patients ranged from 32% to 81%.

Discussion

Slit skin smear (SSS) examination of multi bacillary leprosy patients, is a cost effective diagnostic test for confirmation of the disease, its classification and assessment of response to chemotherapy. The number of bacilli in the site examined as assessed in the smears is called the Bacteriological Index (BI) and it has been observed that this drops slowly for the first twelve months of treatment as both dead and live bacilli in tissues are being counted.

Browne (1969) described a standardized procedure for studying the morphology of stained acid fast lepra bacilli and this has been extensively used during the last more than 50 years. Morphological Index (MI) is expressed as the percentage of uniformly stained bacilli calculated after counting 200, red staining elements lying singly. This indicates whether the patient is responsive to therapy or has defaulted or become resistant to chemotherapy. Generally speaking, this index may be anywhere between 25%-75% at the commencement of multi drug chemotherapy and falls to zero within five to six months of treatment on rifampicin, dapsone and clofazimine (Mahajan 2013, Gautam & Jaiswal 2019, MRL, Leiker et al 1971, Rees et al 1970, Sehgal & Joginder 1990).

In our study also, in 95.6% of cases MI fell steadily and significantly towards the end of twelve months of treatment completion. However, at treatment completion MI was not zero for 5 out of these 49 patients. But when they were subsequently followed up at six monthly or yearly intervals, all became negative and cleared out, there was no evidence of AFB in their slit skin smears.

Patients presenting on follow up, generally remained well clinically and there was no sign of acid fast bacilli in smears taken from them. One patient in our study had a history of one fresh lesion eleven months after he had successfully completed treatment. His BI and MI at treatment completion was 3 and 24% respectively down from 5 and 56% initially and was one of the patients to have cleared solid bacilli at 18 months after start of treatment.

Six to eight smears from various sites were examined from each patient and the average of Biological Index and Morphological indices for all smears was calculated and reported to the physician. However, two patients four and five months into their treatment, respectively, showed one or two smears highly positive and rest of the set of slides showed scanty or no bacilli. Since the index is calculated as a mean for all the smears, it pulled down the average MI, BI dramatically and deceptively, showing a fall in the index. This rose again the next time when sample collection improved or a different area examined, giving a discordant result. To correct this operational error, site/slide specific index was reported, if there was a wide disparity in the BI of slides sent to the lab, with a request to repeat sample if possible. Warndorff made a similar observation that averaging the MI/BI from all the sites scraped and sampled was a compromise and it understates the disease severity, hence all the smears should be reported individually. A special mention should also be made of the highest value of indices observed (Warndorff 1980). This would be a more accurate assessment of disease status.

Modern medicine extends various diagnostic tests to the physician to aid his/her diagnosis and guide his / her choice of treatment. Unfor-

tunately, with leprosy, there has been no such luck. Slit skin smear microscopy, determination of bacteriological and morphological indices remained the mainstay of laboratory diagnosis for more than half a century. However, due to operational reasons this SSS examination was nearly abandoned. During the recent 5-10 years, bacteriological examination is being emphasized again (Mahajan 2013, WHO 2018).

Since the 1990s many studies of serological tests, biological markers (anti PGL 1) and molecular assays have been published. Lateral flow and Enzyme linked Immuno sorbent assays have shown poor diagnostic sensitivity and accuracy for paucibacillary disease, an area where the need for diagnostics is acute (WHO 2018). Polymerase chain reaction which showed better sensitivity and better diagnostic accuracy has not been standardized as ready to use procedures, there are no commercial kits available and it is not feasible to implement them in field settings (WHO 2003, 2018).

Based on available evidence, Guideline Development Group for diagnosis, treatment and prevention of Leprosy, WHO found no clear cut advantage of serological tests and PCR over and above clinical examination with or without microscopy of slit skin smears in the current scenario (WHO 2003, 2018).

In research and tertiary settings, PCR is a perfect tool for early detection of leprosy especially paucibacillary disease when the bacilli in skin are scanty. DNA based techniques, however, do not differentiate between live and dead bacilli, so is of no help in therapeutic follow up of a patient on chemotherapy (WHO 2018). As there is additional use of detection of drug resistance and success of such tests in tuberculosis at Microscopy centre levels (TrueNat) shows the way for implementation of molecular assays in leprosy as well.

The term, Leprosy elimination has confused the general public and many in the medical profession as well. It has led to leprosy receding from their minds and vision. The war is still on. The disease has not been eradicated. There is ongoing transmission in the community and the detection capacity does not match the rate of disease occurrence (WHO 2003, 2012, 2018).

Treatment completion rate, is a core indicator and crucial to the interruption of transmission of disease (WHO 2003, 2012). It is an indication of quality of services provided, supervised and reflects the accessibility of leprosy related services.

Conclusion

The determination of Morphological Index on slit skin smears is an available objective parameter to help the physician to presume microbiological cure of the disease at treatment completion. It gives a bacteriological basis and reassurance to entire chain of healthcare staff involved. Medical colleges with manpower and a good microscope should not abandon it till more sensitive tests become available for clinical practice.

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