

Selection of Sites for Slit-skin Smears

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The results of slit-skin smears from 18 untreated lepromatous leprosy patients showed high bacteriological index (BI) and morphological index (MI) in the ears, fingers, face, buttocks and toes. Need for standardization of site of smearing is stressed. The ears, fingers, face and buttocks are suggested as standard sites for slit-skin smearing for diagnosis, follow-up and assessment of chemotherapy.

Introduction

The bacteriological status of leprosy patients is usually assessed by the slit-skin smear method introduced by Wade (1963). A detailed description of the method is given by Cochrane (1964). The bacteriological index (BI) is used as a semiquantitative assessment of the bacterial load in the sites from which smears are taken (Ridley 1964). The morphological index (MI) is the percentage of solid-stained (living bacilli) found in the smear (Waters and Rees, 1962). The MI is assumed to be a sensitive index for assessment of the efficiency of chemotherapy and for the detection of early mycobacterial resistance to antileprosy drugs. The viability of *Mycobacterium leprae* in slit-skin smears can also be estimated by the mouse footpad technique (Pearson, 1975). However, the MI, in spite of all its limitations (Chatterjee, 1973; Chang, 1977), remains the only method for routine estimation of viability of *M. leprae* in slit-skin smears.

With the increasing importance of dapsone resistance (Pearson *et al.* 1976, 1977; Waters, 1977), new antileprosy drugs and new combinations of the existing drugs are needed. To obtain a meaningful result from such drug trials there is real need for standardization of the methods of assessment of drug effectiveness.

This study was undertaken to determine the difference in BI and MI in the smears from different sites on lepromatous leprosy patients. Only untreated patients were studied because these are the ones who might usually be selected in drug trials (Rees, 1975). Six sites which showed the highest BI and MI are suggested as standard sites for slit-skin smearing.

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Material and Method

Slit-skin smears are taken routinely from all new leprosy patients attending the outpatient clinic in the Addis Ababa Leprosy Hospital. Besides the six routine sites, namely, ear lobe, face, arm, back, buttock and legs, smears were taken from the dorsum of the index finger and the big toe. There was no selection of obvious lesions at the site of these smears. They were stained by a standard Ziehl-Neelsen technique (heat fixation, carbol fuchsin for 10 min, differentiation in 10% sulphuric acid for a few sec, counterstaining with brilliant green for 5 min). All smear preparation, staining and reading was done by the same technician who does the routine laboratory work. The BI is recorded for each site according to Ridley's scale (Ridley 1964) and the MI as the percentage of the morphologically solid-stained bacilli found in the smear.

Results from 18 newly diagnosed untreated lepromatous leprosy patients seen over the period May 1978 to January 1979 were examined. All the patients have an average BI of 2 or more.

TABLE 1
Bacteriological and Morphological Indices from 18 Untreated Lepromatous Leprosy Patients

Indices	Ears	Fingers	Faces	Buttocks	Toes	Legs	Arms	Backs
BI	4.5*	4.5	4.2	4.2	4.0	3.8	3.5	3.0
	\pm 0.1	\pm 0.1	\pm 0.1	\pm 0.1	\pm 0.1	\pm 0.1	\pm 0.1	\pm 0.2
MI	14.3	13.0	12.5	11.8	10.8	10.2	10.6	8.3
	\pm 0.5	\pm 0.9	\pm 1.0	\pm 1.1	\pm 0.9	\pm 0.2	\pm 1.0	\pm 0.8

* Mean of 18 readings \pm standard deviation.

Results

The mean values of BI and MI from the different sites of the 18 patients are shown in Table 1. Comparing all 8 sites the BI and MI were highest in the smears taken from the ears. The finger smears gave BI and MI results as high as the ears in most of the patients and were always higher than in the other sites. Smears from the face and buttocks showed similar BI and MI. The BI and MI of the smears from the toes were lower than those of the fingers but were always more than those from the back which gave the lowest results. There was a direct correlation between BI and MI in sites in all the patients.

Discussion

Slit-skin smears have been used by different workers both for diagnosis and assessment of efficiency of drug therapy. There has been no uniformity in the selection of skin sites for slit-scraping or in the number of sites scraped. The nasal-scraping which was at one time used routinely (Browne, 1966, 1967;

Dharmendra, 1967; Doull, 1961) is no longer in general use. The main reasons for exclusion of the nasal smear are the possibility of contamination of smear by non-pathogenic acid fast bacilli (Cochrane, 1965) and hence the possibility of giving a false positive result, variation in the results from different areas within nasal cavity (Davey and Barton, 1973) and the inconvenience the nasal cavity smears caused to the patients.

The number of sites smeared by different leprologists vary widely. Davison (1961) used four sites for follow-up of his patients. Dharmendra (1967), Browne (1959, 1966), recommended six sites as routine for diagnosis. Six sites were also used by Leiker and Carling (1969) and Garrod and Ellard (1968) for assessment of efficiency of drug therapy. Jopling (1965) recommended eight sites as routine for diagnosis. Eight sites were also used by Doull (1961), Browne (1965, 1966, 1967) for drug assessment and follow-up of patients. Cochrane (1964) recommended eleven sites for diagnosis, while others (Bryceson and Pfaltzgraff, 1973) recommend six to eight sites without specification. Levy (1969) recommended only one site to be smeared.

As leprosy workers differ in their selection of the number of sites smeared, they also differ in the specific sites smeared. Dharmendra used the ear, cheek, chin, right arm, left thigh and nose. Browne (1959) used six sites including the ear lobe, the forehead, cheek, and three active lesions. Cochrane (1964) used two ear lobes, forehead, chin, cheeks and six smears from suspicious lesions. Jopling (1965) recommended two ear lobes and six active-looking lesions.

It has been noticed (Levy 1969) that there is a wide variation both in BI and MI from different sites. Browne (1967) stressed the variation of result due to the technique of taking the smear. In a study by Gideon and Job (1965) the ears were found to have the highest BI followed by the chin, with similar results for the buttocks in males and thighs in females. The arm, chest and back showed the lowest results. Similar findings were repeated by Padma (1965).

Although serial biopsies had been recommended for the assessment of bacteriological changes (Leiker, 1971), the direct skin-slit scrape method has been shown (Izumi, 1971) to be sensitive enough to be used for bacteriological and morphological indices.

The results in this study confirm the previous finding of Gideon and Job (1965) and Padma (1965). The ears, fingers, face, buttocks give the highest BI and MI. There is correlation between BI and MI which confirm the findings of Levy (1969). Smears from the fingers show approximately 30-fold more bacilli than the back smears (BI 4.5 vs 3) and the difference in number of viable bacilli is approximately 50-fold (MI 14.3 vs 8.3). The fingers provide uniformity of sites and are more likely to yield comparable results with repeated smearing. They have also been shown (Ridley *et al.*, 1976) to be the site at which bacilli were most frequently detected.

It is evident that the highest numbers of viable bacilli are in the ears, fingers, face and buttocks. These results provide a rational basis for the selection of standard sites for skin smears. However in relapses and drug resistant cases it is important to smear newly developing lesions which may be in unexpected sites. We recommend that if six sites are to be smeared they be routinely taken from the two ear lobes, the face, the buttocks and two fingers. The problem of variation in the technique of smear taking (McDougall, 1975)

and the staining problem (Ridley and Ridley, 1971) could be overcome by the use of a single well-trained technician for all patients under study. The use of the recommended standard sites for skin smears should make the BI and MI reliable indices for assessment of new patients and response to antileprosy drugs.

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