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Selection of Sites for Slit Skin Smears in Untreated and Treated Leprosy Patients[1]

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The skin is one of the most easily accessible organs which contains the largest number of bacilli in leprosy. The bacteriological status of leprosy patients is generally assessed by slit-skin smears ([15,23]) taken by the method of Wade ([23]). The bacterial index (BI) is a semiquantitative assessment of the bacterial load; whereas the morphological index (MI) is the percentage of solid-staining bacilli in smears. The MI is assumed to be a routine, sensitive index for assessment of the efficacy of antileprosy chemotherapy and detection of early mycobacterial resistance to dapsone and relapse ([14]).

Ridley, *et al.* ([22]), in a series of long-treated lepromatous patients, found the fingers to be the most suitable site where bacilli were most frequently detected, and the bacterial load and solid-staining bacilli were highest in number. Studies by Ahmed, *et al.* ([1]) and Hiramalini, *et al.* ([11]) supported these findings. Kim ([17]) studied 16 sites in 39 treated lepromatous patients and found bacilli from fingers when all other sites were negative. Warndorff van Diepen ([24]), however, failed to demonstrate high BI and MI in the fingers in either untreated or treated patients as compared to the ears and other peripheral sites. The appearance of solid bacilli in fingers has been shown to precede clinical relapse ([3]).

Most leprosy control projects recommend only a few smears for routine monitoring of therapeutic response, and it is important that the sites chosen are those most likely to be productive. Very few studies are available ([15, 16, 24]) which give information about the selection of the most rewarding sites for making slit-skin smears in long-treated leprosy patients, and there is no universal agreement on this site selection.

The aim of the present study was to compare bacterial load and morphology at various sites in untreated and treated, bacillary-positive, lepromatous leprosy (LL) patients in order to identify the most productive site for sampling and to identify the site which will become positive first with solids in case of relapse.

MATERIALS AND METHODS

Bacillary-positive leprosy patients (polar or subpolar LL of Ridley-Jopling classification) fresh untreated or under treatment with dapsone monotherapy or multidrug therapy (MDT), consisting of dapsone, rifampin, and clofazimine, were taken for study. The patients were attending a leprosy clinic from January 1980 to December 1985 at the Postgraduate Institute of Medical Education and Research, Chandigarh, India. Age and sex were not considered relevant for inclusion in the study. Duration of treatment varied from 6 months (MDT) to 7 years (dapsone monotherapy). All new patients after October 1982 have been put on MDT as per WHO recommendations. All patients were responding to treatment; some had become bacteriologically negative by the usual earlobe smears but none had signs of relapse.

Data are presented for readings of 1000 slit-skin smear sets taken from 558 leprosy patients. There were 319 sets of smears (1595 smears) from untreated patients and 681 sets (3405 smears) from treated patients.

The smears were taken from five sites: both earlobes; right elbow; dorsal aspect of middle phalanx of the left middle finger (terminal phalanx was not taken because many patients had total resorption/ulceration), the adjoining finger was used if the middle finger was missing; and the dorsum of the middle phalanx of the middle toe of the right foot. The patients wore shoes or chappals

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TABLE 1. *Mean BI and MI values ± standard deviations at various sites from untreated and treated leprosy patients.*

Site	Untreated patients (319 smear sets)		Treated patients[a] (681 smear sets)	
	BI	MI	BI	MI
Right ear	2.38 ± 1.66	2.43 ± 3.80	0.70 ± 1.37	0.37 ± 0.73
Left ear	2.42 ± 1.67	1.86 ± 2.65	0.72 ± 1.49	0.42 ± 0.76
Right elbow	1.93 ± 1.57	1.58 ± 2.41	0.63 ± 1.48	0.27 ± 0.63
Left finger	1.63 ± 1.61	1.67 ± 2.63	0.65 ± 1.54	0.32 ± 0.63
Right toe	1.35 ± 1.63	1.59 ± 2.07	0.52 ± 1.60	0.21 ± 0.65

[a] One-hundred-three patients in the treated group did not show bacilli at any site.

(dorsum of foot almost uncovered); some remained barefoot.

The slides were stained by the Ziehl-Neelsen method with slight modification, i.e., decolorization was done with acid alcohol (1% HCl in 70% alcohol) for 2 min and counterstained with 0.3% methylene blue for 2 min. The BI was estimated according to the method of Ridley ([21]), and the MI was calculated according to the method of Waters and Rees ([25]). The smears were screened by two independent observers not aware of the site of the slit smear, and the mean of the readings taken. Any gross discrepancy (BI varying by more than two logs and MI varying by more than 5%) was rechecked after taking a fresh smear; if the discrepancy persisted, the reading was discarded.

RESULTS

Untreated group. As is evident from Table 1, the earlobes gave significantly higher ($p < 0.05$) BI values compared to the toes and fingers. The MI was also significantly higher from the earlobes as compared to toes, elbows, and fingers. In five patients, sites other than ear lobules yielded bacilli.

Treated group. In 103 sets of smears (515 smears), no bacilli were detected at any site and so these smears are not included in the calculations. The period after which all sites became negative varied from 6 months (with MDT) to 3 years (dapsone monotherapy). All other sites yielded similar BI and MI values; the figures were lowest from elbows and toes but were not different statistically.

In 20 long-treated patients (more than 1 year of MDT or 3 years of dapsone monotherapy), bacilli were detected at sites other than earlobes, e.g., elbows, fingers, and knees.

In 28 patients, sites other than ear lobules, e.g., fingers and elbows, gave the highest MI, and in 20 patients, solids were seen from other sites but not from ear lobules. This observation lends weight to the importance of peripheral sites other than ears or any single site.

DISCUSSION

There is no uniformity of opinion regarding the selection and number of slit-skin smear sites for the demonstration of bacilli. One to 11 sites have been recommended by various workers ([2–9, 14, 18, 19]).

The ears have been found to have the highest BI, followed by nearly similar results from the buttocks in males and the thighs in females. The arms, chest, and back have been shown to have the lowest BI ([10, 20]). Recently, Ahmed, *et al.* ([1]) found higher BI from the earlobes, buttocks, face, fingers, and toes; whereas legs, arms, and the back were found to have the lowest BI. Hiramalini, *et al.* ([11]) found fingers (terminal phalanax better than the middle) and uncovered toes to be the most productive sites, even better than ear lobules. The eyebrows were found to have the highest BI, but fingers were the most productive site in long-treated patients when other usual sites had become negative ([17]). Following the initial studies ([1, 11, 14, 17, 22]) stressing the importance of fingers as primary sites for getting solid bacilli when the other sites were negative, two studies by Kaur, *et al.* ([15, 16]) reported the highest BI and MI in the earlobes but stressed the importance of sampling other peripheral sites as well in order to get maximum information. In a detailed study on 244 long-term-treated leprosy patients, Warndorff van Diepen ([24]) found more ba-

TABLE 2. *Sites with highest BI and MI in the untreated and treated patients.*

Site	Untreated patients (319 smear sets)		Treated patients[a] (681 smear sets)		
	Highest BI	Sole site showing bacilli	Highest MI	Sole site showing	
				Bacilli	Solids
Right ear	27	3	7	11	7
Left ear	29	3	8	16	5
Right elbow	16	2	6	9	5
Left finger	12	3	13	15	13
Right toe	6	0	9	10	2

[a] One-hundred-three patients in the treated group did not show bacilli at any site.

cilli in earlobes compared to all other sites, including fingers, and stressed the difficulty of making a slit-skin smear from fingers because the site bled more and was inadvisable in laborers and others of the working classes.

From the present study, it is apparent that earlobes yielded the highest number of bacilli compared with other sites in the untreated patients. The MI was also found to be the highest in the earlobes compared to other sites in the untreated patients. These findings are in agreement with the studies of Gideon and Job ([10]), Padma ([20]), Ahmed, *et al.* ([1]), and Warndorff van Diepen ([24]).

However, in the treated patients, as the bacterial population goes down in response to therapy, the bacilli do not disappear in any uniform fashion or from all sites simultaneously. It is very difficult to say which site remains more productive longer than any other since all sites yielded bacilli a sufficient number of times to qualify for inclusion in any study. Similarly, solids among scanty bacilli were also recovered from all sites, with the ear lobules and fingers the most productive.

On the basis of our observations it is recommended that, in addition to the standard earlobe smears, it is rational and imperative to study multiple sites, especially fingers and toes since these sites may show bacilli over a long period in treated patients.

The persistence of bacilli at peripheral sites is generally explained as due to the coolness of these parts of the body. A lower concentration of drugs due to peripheral vascular abnormalities and the presence of numerous cutaneous nerves over the dorsal aspects of the fingers and toes hiding bacilli probably increases the chances of bacterial positivity at these sites.

SUMMARY

A total of 1000 slit-skin smears were taken from multiple sites (both ear lobules, right elbow, dorsal aspect of middle phalanx of the left finger, and dorsum of middle phalanx of the right foot) from 558 leprosy patients. There were 319 sets (1595 smears) from untreated patients and 681 sets (3405 smears) from the treated group. The duration of treatment varied from 6 months (multidrug therapy) to 7 years (dapsone monotherapy). The ear lobules gave significantly higher values for the bacterial index (BI) compared to toes and fingers in the untreated group. The morphological index (MI) was also significantly higher from the ear lobules compared to toes, elbows, and fingers. In five patients from the untreated group, bacilli were found in some other sites when the earlobes did not reveal any. In the treated group, all sites yielded similar BI and MI values, the figures being lowest from elbows and toes but not different statistically. In 20 long-treated patients, bacilli were detected at sites other than the ear lobules. In 28 patients, sites other than the ear lobules gave a higher MI and in 20 patients, solid bacilli were seen at sites other than the ear lobules.

RESUMEN

Se hicieron un total de 1000 extendidos de linfa cutanea obtenida por el método de la incisión dérmica a partir de múltiples sitios (ambos lóbulos de las orejas, codo derecho, dorso de la falange media de la mano izquierda, y dorso de la falange media del pie derecho) en 558 pacientes con lepra. Hubieron 319 juegos (1595

extendidos) correspondientes a pacientes no tratados y 681 juegos (3405 extendidos) correspondientes a pacientes tratados. La duración del tratamiento varió de 6 meses (terapia múltiple) a 7 años (monoterapia con dapsona). Los lóbulos de la oreja dieron valores significativamente más elevados de índice bacteriano (IB) que los obtenidos de los dedos del pie o de la mano en el grupo no tratado. El índice morfológico (IM) también fue significativamente mayor en el lóbulo de la oreja que en el codo o en los dedos de los pies y manos. En 5 pacientes del grupo no tratado se encontraron bacilos en algunos otros sitios aún cuando no se encontraron en los lóbulos de las orejas. En el grupo tratado, todos los sitios dieron valores similares de IB y de IM, correspondiendo los valores más bajos a los codos y a los dedos de los pies aunque las diferencias no tuvieron significancia estadística. En 20 pacientes con tratamiento prolongado también se encontraron bacilos en otros sitios además de los lóbulos de las orejas. En 28 pacientes otros sitios diferentes a los lóbulos de las orejas tuvieron los mayores IM y en 20 pacientes se observaron bacilos sólidos en sitios diferentes a los lóbulos de las orejas.

RÉSUMÉ

Chez 558 malades de la lèpre, on a prélevé un total de 1000 frottis cutanés ("slit-skin smears") en de multiples endroits, à savoir les lobes de l'oreille, le coude droit, la face dorsale du doigt (médian) gauche, le dos d'une deuxième phalange au niveau du pied droit. On a étudié 319 séries (1595 frottis) provenant de malades non traités, et 681 séries (3405 frottis) de malades traités. La durée du traitement a varié de 6 mois (polychimiothérapie) à 7 ans (monothérapie par la dapsone). Les lobes de l'oreille ont fourni des valeurs significativement plus élevées pour l'index bactériologique (IB), lorsqu'on comparait ces valeurs à celles obtenues au niveau des orteils et des doigts, dans le groupe non traité. L'index morphologique (IM) était également significativement plus élevé dans les lobes de l'oreille que dans les orteils, le coude ou les doigts. Chez 5 malades appartenant au groupe non traité, on a observé des bacilles à d'autres endroits, alors que le lobe de l'oreillen'en révélait aucun. Dans le groupe traité, tous les endroits ont livré des valeurs semblables tant pour l'Index Bactériologique que pour l'Index Morphologique, les chiffres étant cependant plus faibles pour les prélèvements effectués au coude et aux orteils (ces résultats n'étaient cependant pas différents au point de vue statistique). Chez 20 malades traités depuis longtemps, des bacilles ont été décelés à des sites de prélèvement autres que les lobes de l'oreille. Chez 28 malades, l'Index Morphologique était inférieur au niveau des lobes de l'oreille par rapport à d'autres sites. Chez 20 malades, on a observé des bacilles solides à des endroits de prélèvement autres que les lobes de l'oreille.

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