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Original Research Article

An observational study on bacteriological and morphological index on slit skin smear of biopsy proven cases of leprosy from WHO recommended sites versus hidden sites

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Abstract

Background: The bacteriological and morphological status of leprosy patients is assessed by Slit Skin Smear (SSS) method. Bacteriological index (BI) is the semi quantitative index for measuring bacterial load and morphological index (MI) is for assessment of chemotherapeutic efficacy and bacterial resistance.
Aims: To evaluate Bacteriological index and Morphological index on Slit Skin Smear in world health organization (WHO) recommended sites vs other reported hidden sites in untreated and relapsed cases of leprosy.

Methods: 75 patients with biopsy proven leprosy, not on treatment or relapsed cases, irrespective of age, sex and duration of disease were included. Smears were taken from 3 WHO recommended sites (two lesional sites and one ear lobe) and 5 other reported hidden sites (left ear lobe, right hand middle phalynx, right elbow, left eyebrow and left foot great toe). Slides were stained using Ziehl-Nelson method. BI and MI were evaluated using Ridley's algorithmic scale.
Results: Ear lobes gave uniformly higher values of BI and MI as compared to elbows, fingers and toes. Slit Skin Smear was found to be positive from only peripheral sites in 8 patients. While in 5 cases were positive only from the lesional sites whereas 2 patients out of 75 were positive only from earlobes. Morphological indices were better observed in untreated cases. Ear lobules gave higher morphological index. Maximum number showed bacilli in the lesions and ear lobules followed by toes, elbow, eyebrows and fingers. In 25 out of 75 patients, no bacilli could be seen at any sites.
Conclusion: In a few cases bacilli were seen only in peripheral hidden sites while the WHO recommended sites were negative. Thus, it is important to take SSS from hidden sites.

Keywords: Hansens disease, slit skin smear, mycobacterium leprae

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Introduction

Leprosy is a chronic infectious disease caused by mycobacterium species such as, Mycobacterium leprae and Mycobacterium lepromatosis. It mainly affects the skin, eyes, nose and peripheral nerves. Its prevalence supervenes in many countries including India. India hosts 63% of world's leprosy population [1] It is also called as Hansen's disease, after the scientist who discovered M. leprae in 1873 [2]. Leprosy still remains a public health challenge.

More than 70% new cases of leprosy of world are detected in India [3].

Slit skin smear is a useful, cost-effective tool in the diagnosis of Hansen's disease. It is useful in estimating the bacillary and morphological index of the acid-fast bacilli and is important in determining the type and severity of disease as well as assessing the response of treatment.

The skin smear is a valuable, cost-effective tool in the routine management of the patient with Hansen's disease. The smear is a means of estimating the number of acid-fast bacteria present, reported as the Bacterial Index (BI) and is important in determining the type and severity of disease as well as assessing the response to treatment.

Being nearly of 100% specificity when performed expertly, slit-skin smear remains the simplest diagnostic technique available until new cutting-edge diagnostic tools become available for routine bedside use. However, the interest has been declining for learning this simple test among all the persons involved in leprosy work even in the teaching/training institutes. This is perhaps due to confusion over number and sites of smears, and its declining usefulness in WHO recommendations/guidelines.

Materials and Methods

Study design: Hospital based prospective observational study.

Study place: Department of Dermatology, Venereology and Leprology, Muzaffarnagar Medical College, Muzaffarnagar, UP.

Duration of study: 18 months.

Sample size: 75 cases.

Sampling technique: Simple random sampling.

Statistical analysis and software: Suitable statistical significance test was used for statistical analysis along with SPSS17/20 Statistical 2 software.

Inclusion criteria: Consenting patients of all age groups with leprosy, untreated cases, patients who had earlier completed the course of multi drug therapy (MDT) and came again with relapse, patients willing for examination and procedure.

Exclusion criteria: Patients on MDT, patients not willing to undergo examination and procedure, patients not willing to be a part of study, patients with pure neuritic or indeterminate leprosy.

Materials required: Gloves, swabs and spirit, scalpel handle and surgical blades, band aid, spirit lamp, slide box, watch or clock, Tissue paper staining rods, pipette, slide rack, two 10ml syringes.

Chemicals required

1% Carbol fuchsin solution, 1% acid alcohol, 0.2% methylene blue solution.

Procedure

Slit skin smear is a test in which a sample of material is collected from a tiny incision in the skin and then stained for M. Leprae, an acid-fast bacillus.

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It is done to diagnose skin smear positive multibacillary leprosy in a suspect, relapse in a previously treated case, evaluate the bacillary load and in classification of new patients.

Steps

A detailed history of every patient along with the complaints, duration of illness was taken. The socioeconomic factors such as occupation, education, income was recorded. Family history was taken. The general physical examination and systemic examination was conducted in all cases, along with sensory, motor and nerve examination.
Smears were taken from 8 sites, out of which

1) WHO recommended sites are: 2 lesional sites and one ear lobe.
2) Other peripheral sites (2^{nd} earlobe, right mid phalanx, right elbow, left eyebrow and left foot great toe).

All microscopic slides were pre-cleaned in 70%alcohol to remove amorphous debris and wiped with clean tissue.
Skin was cleansed with 70% alcohol and air-dried. A fold of skin was pinched or compressed and made relatively avascular, in order to obtain a bloodless field.
An incision 3-5 mm long and 2-3 mm deep was made with a #15 Bard-Parker blade. Mild pressure to maintain relative avascularity was continuously applied until an adequate smear was obtained.
The material was then transferred to the cleaned microscopic slide. A moderately thick smear with a visible uniform opacity was made in a circular manner, no larger than 5-7mm, beginning peripherally and ending in the center, leaving a central button 2-4 mm which could be easily focused upon with the microscope. Slides were properly labelled.

Staining
Primary staining

Slides were dried at room temperature, by placing on staining racks and poured with 10% formalin for 15 mins for fixation, then gently rinsed under tap water. Ziehl-Neelsen method was used for staining. 1% Carbol-fuchsin was poured over the smear for 20mins, after that decolorized with 2% acid alcohol (1% HCL and & 70% alcohol) for 1min, slides were then thoroughly, gently rinsed with tap water. Counterstaining with 2% alkaline methylene blue for 30secs to 1 min was done thereafter. Lastly gently rinsed with tap water and air dried.

Microscopic examination of skin smears: The stained smears were examined under a microscope using oil immersion objective(X100) and 100 fields were examined to determine total number of bacilli. Four separate quadrants of smear were examined and averaged to establish the bacterial index.

Reporting the bacterial index: The results were reported on a numerical code 0 to 6+ using Ridley's Algorithmic scale. Bacilli are seen as red rods against the blue background. Live Bacilli are solid bacilli which are uniformly stained, having length 4 times greater than breadth, sides are parallel, ends may be rounded, straight or pointed. Dead bacilli stain irregularly and may appear as granular or fragmented. The bacilli may be seen singly or in small groups called as globi as shown in Figure 1 and 2.

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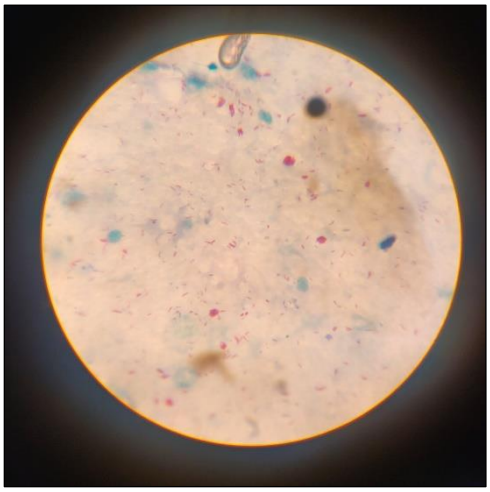

Fig 1: Multiple singly lying solid and fragmented bacilli, from site left foot great toe with BI(+4) and MI(50%)

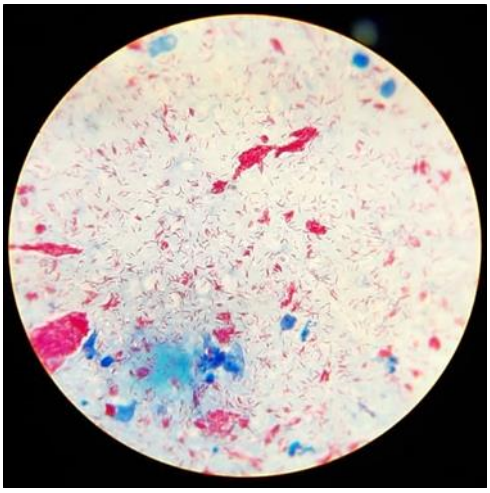

Fig 2: SSS showing abundant bacilli on slit skin smear

Morphological index: The morphological index is expressed in percentage and was calculated by counting the percentage of solid-staining acid-fast rods as only the solid staining bacilli are viable.

Results: Results were obtained by using appropriate statistical method, that is, by calculating the percentage, mean and standard deviation.
Slit skin smear was found to be positive in 50 cases that is around 66.6% of cases whereas it yielded a negative result in approximately 33.3% of cases (Table 1).
Of the total cases examined, 42 (56%) cases were of multibacillary type while 33(44%) were of paucibacillary type based upon the definition given by WHO (Table 2).
The highest incidence observed was of borderline tuberculoid pole while least that of tuberculoid or borderline lepromatous pole (Table 3).
Amongst the peripheral sites highest positivity was observed in left foot great toe followed by left earlobe, left eyebrow, right elbow, right hand middle finger in decreasing order.
In 8 out of 75 cases SSS was exclusively positive from the peripheral sites, highest from great toe of left foot in 33 cases (44%) followed by left ear lobe in 28 cases (37.33%), left eyebrow 21cases (28%), right elbow 20 cases (26.67%) and least from right hand middle phalanx in 16 (21.33%) cases (Figure 3, Table 4).
There were two cases that were only positive from either of the earlobes and 8 cases (10.6%)

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that were exclusively positive from peripheral sites. Whereas 5 cases showed SSS positivity only from lesional sites, which is about 6.6%.

Table 1: Results of Slit Skin Smear

Slit Skin Smear Result	Total Number of Cases	Total Percentage
Negative	25	33.3%
Positive	50	66.6%
Total	75	100%

Table 2: Distribution of types of leprosy

Type	Total Number	Total Percentage
Multibacillary	42	56%
Paucibacillary	33	44%
Grand Total	75	100%

Table 3: Distribution of poles of leprosy

On Biopsy	Total Number
Borderline Borderline	15
Borderline Lepromatous	12
Borderline Tuberculoid	22
Lepromatous Lepromatous	14
Tuberculoid Tuberculoid	12
Grand total	75

Table 4: Slit skin smear positivity amongst peripheral site

Slit Skin Smear Positive		
Peripheral sites	**Total count**	**% distribution**
LT. Earlobe	28	37.33
RT. Hand Middle Phalynx	16	21.33
RT. Elbow	20	26.66
LT. Eyebrow	21	28
LT. Foot Great Toe	33	44

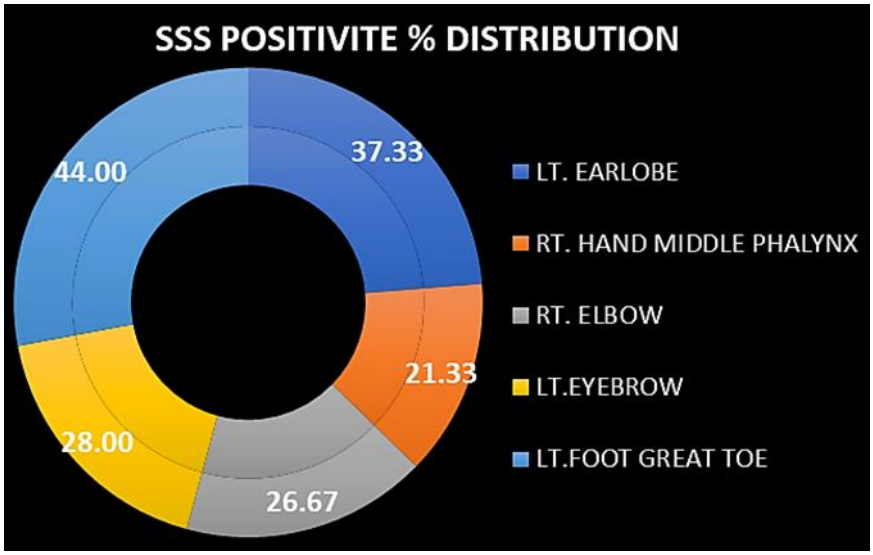

Fig 3: Showing the positivity of slit skin smear at various sites

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Discussion

Leprosy is diagnosed clinically on the basis of presence of two or more of the three cardinal clinical features i.e., hypopigmented or erythematous anesthetic patch on skin, thickened/tender peripheral or cutaneous nerves supplying the affected area and acid-fast bacilli in the slit skin smear. The clinical applicability of this has been extensively reviewed. Despite the high sensitivity of the clinical methods, delay in the diagnosis of more than 80% of new cases of leprosy was reported [3]. There are different opinions regarding the choice and number of sites for slit skin smear examination for demonstrating bacilli. The literature identifies about one to eleven sites for this purpose by various authors [4] (Davidson, 1961 [5], Dharmender, 1967 [6], Browne, 1959 [7], 1966 [8], 1967 [9], Levy 1969 [10], Jopling 1971 [11]). However wide variations were observed in both values of BI and MI.

In this study it is evident from the results that the ears have been undoubtedly found to bear the highest bacillary load however a few studies have been done to reestablish the importance of slit skin smear examination from various peripheral sites as seen in our study. Also, from this study we established that occasionally there were higher incidence of BI and MI in peripheral sites, especially in lepromatous leprosy. The percentage is though small but is significant in determination of leprosy in cases which are negative on SSS from WHO recommended sites.

In the present study it was observed that high bacillary load was observed in lesional site and bilateral earlobes which are the WHO recommended sites but there were also cases that showed bacilli only from the peripheral sites. In 8 patients out of 75 it was exclusively positive from the peripheral sites, highest from Left foot great toe in 33 cases or 44% followed by Left ear lobe 28 cases (37.33%), Left eyebrow 21cases (28%), Right elbow 20 cases (26.67%) and least from Right hand middle phalanx in 16 cases (21.33%) of all the cases.

The number of sites smeared by different leprologists vary widely. *Davison* (1961) [5] used four sites for follow-up of his patients. *Dharmendra* (1967) [6], *Browne* (1959, 1966) [7], recommended six sites as routine for diagnosis. Six sites were also used by *Leiker* and *Carling* (1969) [11] and *Garrod and Ellard* (1968) for assessment of efficiency of drug therapy. *Jopling* (1965) [11] 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 [12].

In the study conducted by *Haider Abu Ahmad et al*. [13] the results of slit-skin smears from 18 untreated lepromatous leprosy patients showed high BI and MI in the ears, fingers, face, buttocks and toes.

In another study conducted by *B Kumar, S Kaur* [14] the ear lobules gave significantly higher values for the BI compared to toes and fingers in the untreated group. The 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.

S kaur, H Darshan et al. [15] conducted a study on 46 patients in which multiple sites, especially peripheral sites are recommended for study of skin slit smears to discover persistent bacilli.

Conclusion

A total of 600 Slit Skin Smear were taken from 75 patients from various WHO recommended sites (i.e., lesional skin and right earlobe) and peripheral or hidden sites which include left

earlobe, right hand middle phalanx, right elbow, left eyebrow and great toe of left foot. It was observed that bilateral earlobes have significantly higher values of bacillary index. And the morphological index was also significantly higher from bilateral earlobes as compared to elbows, toes and fingers.

In 12 patients the SSS was positive only from the WHO recommended sites. Whereas in 8 patients out of 75 it was exclusively positive from the peripheral sites, highest from great toe of left foot followed by left ear lobe, left eyebrow, right elbow and least from right hand middle phalanx, while the WHO recommended sites were negative.

Five cases were positive only from the lesional sites whereas two cases showed SSS positivity only from earlobes when lesional sites showed no bacilli.

In 25 patients of biopsy proven leprosy, Slit Skin Smear was negative from all the sites. And thus, reflects the limitation of SSS being used as a diagnostic method.

In this study approximately 10.6% cases SSS were exclusively positive from the peripheral sites reflecting the presence of high bacillary load in the cold extremities. In clinically suspected cases of leprosy even if the SSS from the WHO recommended sites is negative, repeat smears may be taken from the hidden sites in order to avoid missing the diagnosis. Thus, the importance of taking smears from reported hidden or peripheral sites has been emphasized upon.

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