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Review Article

Slit-skin smear in leprosy: lest we forget it !

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Diagnosing and classifying leprosy solely on the basis of skin lesions as per WHO operational classification may lead to over or under diagnosis and inadequate treatment particularly of pauci-lesional multibacillary cases with consequent risk of resistance, relapse and progressive horizontal transmission. Announcing elimination of leprosy as public health problem in India under NLEP was probably ambitious aspiration. However, such a strategy is perhaps not justified scientifically at the moment in view of new case detection rate not showing significant decline. The fact remains that it is still highly desirable to provide sustained quality leprosy services to all individuals through general health services and good referral system. 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. Various technical aspects of slit-skin smear testing are reviewed here keeping in view the need of leprosy workers in referral/teaching institutes.

Keywords : *Bacteriological index, Morphological index*, Multibacillary leprosy, *Mycobacterium leprae*, Paucibacillary leprosy, Ziehl-Neelsen's staining.

Introduction

Demonstration of *Mycobacterium leprae* in skin-slit smears (SSS) was an essential component of multidrug therapy program in the beginning. Yet most leprosy control program managers and supervisors, medical or non-medicals lacked requisite experience in smear taking, fixing, staining or scoring. They were not able to encourage or encourage adequately their field staff/technicians to maintain quality work and safety standards in most parts of the world. This prompted Georgiev and McDougall (1988) to suggest for abandoning SSS in leprosy control programs.

WHO in its 6th technical report of 1988 for citing reasons of low standard of bacteriological reports in control programs too modified the classification of leprosy to include all smear positive cases in multibacillary group to prevent their under treatment. However, with highly simplified operational classification of leprosy as paucibacillary or multibacillary on the basis of number of skin lesions in the 1980s reduced the significance of mandatory use of slit-skin smear

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examination. Bhushan et al (2008) further demonstrated superiority of WHO operational classification of leprosy over SSS in their study and its diagnostic value in patients having negative smears despite repeated examinations by an expert.

However, classifying leprosy solely on the basis of skin lesions is fraught with over or under diagnosis and inadequate treatment of pauci-lesional MB cases with consequent risk of resistance, relapse and progressive horizontal transmission. Delayed diagnosis is another problem despite high sensitivity of clinical methods as has been reported in 80% cases of leprosy at Hospital of Tropical Diseases, London (UK) during 1995-99 (Lockwood and Reid 2001).

Despite its low sensitivity (10-50%, depending upon expertise of laboratory workers), SSS remains gold standard for all diagnostic techniques due to specificity of nearly 100% (Report of the International Leprosy Association Technical Forum 2002). This led Poricha et al (2011) to suggest for designing simplified and more relevant test system: 1) combining skin smear test with sputum microscopy; 2) reporting smears as positive or negative correctly and grading simply as mild (1-100 bacilli/oil field), moderate (100-100 bacilli/oil field) and massive (>1000 bacilli/ oil field); 3) reducing the smear sites to two, and; 4) provision for better logistics, and practical guidelines with clear directives on nature of work to be assigned specifically to each worker.

Although WHO no longer consider SSS essential to make diagnosis as quality control in its field program may not be possible, ILEP Medical Commission 1988 favored the shifting of processing and reporting of smears to referral centers to maintain high quality. However, the program itself has weak referral system and it is not unusual for workers, even in training institutes, to have even a waning interest to learn this simple procedure. This is perhaps due to confusion over number and sites of smears and its declining usefulness in WHO recommendations/guidelines. Various technical aspects of slit-skin smear testing are reviewed here keeping in view the need of residents/leprosy workers in referral/teaching institutes.

Whom to smear, Number and Sites of smear

Skin smears should be taken from all patients suspected or diagnosed to have leprosy, before commencement of multidrug therapy (MDT), and who have relapsed after release from treatment or as per program scheme. Smears are taken from suspected skin lesions and particularly from the most active-looking edge of the lesion and from sites with a high bacterial load or bacterial persistence over a long period. Such sites with the highest probability of demonstrating AFB are the earlobes, forehead, chin, extensor surface of the forearms, dorsal surface of the fingers, buttocks and extensor surface of knees. There has been a difference of opinion regarding selection and number of skin smear sites from time to time. From 6-8 sites (earlobes, eyebrows, chin, cheek during 1960s), 7 sites (two earlobes, nasal smear, 4 lesions (WHO Expert Committee on leprosy 1980)), 6 sites (both earlobes and 4 lesions (Rees and Young 1994)), 5 sites (two ear lobes, right elbow, left finger, right toe (Kumar and Kaur 1986), 4 sites (right ear lobe, right forehead, chin, left buttock in males or upper thigh in females (Job and Ponnaiya 2010) and any 2 sites (Poricha et al 2011) for slit-skin smear have been recommended by several workers over the years. The importance of smears from dorsa of fingers in the diagnosis of long treated lepromatous cases and impending relapse too has been highlighted in the past (Jopling and McDougall 2000). However, WHO's recommendation of 1988 for minimum of three sites (one ear lobe and two active lesions) for smears, and in the case of single leprosy lesion the two smears taken from

Table 1 : Equipments, Materials and Stains required for Slit-skin smear Tray

S.No.	Equipments and Materials	Stains	Others
1.	Gloves	1% Carbol fuschin	Light microscope
2.	Swabs and spirit	5% Sulfuric acid or 1% Acid alcohol (1% hydrochloric acid in absolute ethyl alcohol)	Immersion oil and Blotting papers
3.	Bard Parkar Scalpel- Handle and new Blades (size 15)	1% Methylene blue	Slide box
4.	Medicated dressing strips		Sink with running water, Staining rods
5.	Spirit lamp		Safe disposal bins
6.	Microscope glass slides		
7.	Marking pencils		
8.	Record register		

diametrically opposite active edge of the lesion (World Health Organization 1992) appears reasonably practicable and should be adhered to avoid problem of too many or too less. Smears may be prepared from peripheral skin over finger or toes, elbow, wrist or knee in lepromatous leprosy having no defined lesions. However, it must be remembered that a single slit-skin smear examination performed reliably and accurately from the most active lesion is sufficient for the diagnosis. The smears from forehead, cheek, chin, buttocks or nasal mucosa are no longer recommended for cosmetic and practical reasons. The same sites are used for follow-up smears and in relapsed cases along with new relapse lesions.

Technique of Smear taking, Staining and Reading

Technical guidelines prepared by Drs. Groenen, Saunderson and Baohong Ji on behalf of ILEP Medico-Social Commission for smear taking in leprosy are available online (www.ilep.org.uk/fileadmin/uploads/Documents/Learning_Guides/lg3eng.pdf), and all standard textbooks on leprosy describe them adequately with few variations. It is advisable to maintain a slit-skin smear tray (Table 1) for ready use, and replenished after every use.

In Ziehl-Neelsen (ZN) stained smears the viable *M. leprae* are seen against blue background as uniformly and intensely red stained bacilli having length 4 times greater than breadth; they are described as solid-stained (S) bacilli. Dead lepra bacilli stain irregularly and are described as fragmented (F) or granular (G). The total number of the bacilli are measured using Ridley's logarithmic scale and bacteriologcal index (BI) is calculated (Table 2), albeit, best is to report the highest BI. The morphological index (MI), the percentage of solid-staining bacilli, and Ridley's

Table 2 : Ridley's logarithmic scale for Bacteriological Index (BI)

6+	Many clumps of bacilli in an average field (over 1000)
5+	100-1000 bacilli in an average field.
4+	10-100 bacilli in an average field.
3+	1-10 bacilli in an average field.
2+	1-10 bacilli in 10 fields.
1+	1-10 bacilli in 100 fields.

Note: Record the BI for each smear separately and calculate average or best is to report the highest BI. A simplified reporting.

SFG index (Ridley 1971) requires high standard of smear preparation and microscopy and is unsuitable for routine reporting.

Comments

Bacilli are abundant in lepromatous leprosy (BI 5+ or 6+), can not be demonstrated in tuberculoid leprosy, and may have intermediate counts in borderline leprosy patients. Presence of clumps of bacilli (globi) indicates high BI, presence of viable bacilli, and may be seen in new, untreated or relapsed lepromatous cases. Over 99.9% live bacilli get killed from action of rifampicin. Thereafter BI reflects only the presence of dead bacilli with occasional live ones. As the dead bacilli can be cleared from the body by natural mechanisms of the host, BI in skin smears start falling after one year of multidrug therapy roughly as 0.6-1.0 log per year and continue even after stopping the treatment and precisely is the basis for fixed duration MDT. Changes in the MI are rapid and it falls to 0 within 5 weeks following treatment with rifampicin containing multidrug therapy. Thus MI is more sensitive parameter of therapeutic failure, non compliance, drug resistance, or relapse.

A positive slit-skin smear is not only the 3rd cardinal sign and confirms the diagnosis of leprosy, bacteriological examination is an essential screening procedure for all patients in whom the diagnosis of leprosy is suspected. It helps in: 1) diagnosis or excluding the diagnosis of leprosy; 2) the classification of leprosy within the Ridley and Jopling spectrum and between the two treatment groups (paucibacillary and multibacillary); 3) monitoring of the response to treatment in skin smear positive patients. Additionally, it is useful to study distribution of *M. leprae* in skin and in ascertaining infectivity and severity of the disease. *M. leprae* is usually present in massive numbers in the dermis of multibacillary leprosy patients (1 gm of skin tissue in lepromatous leprosy contains as many as 7000 million leprosy bacilli). As it requires 10^4 bacilli/gm of tissue for reliable detection by ZN staining (Banerjee et al 2011), smears may be negative in paucibacillary leprosy lesions where *M. leprae* are scantly present. However, it must be remembered that negative smears will not exclude leprosy automatically.

Directorate General of Health Services, Central Leprosy Division, New Delhi has issued guidelines on strengthening of skin smear labs for leprosy control activities and programs. However, continuous supervision and monitoring for the collection and processing of slit-skin smears are necessary especially at referral centers in order to ensure uniformity, reliability and high levels of quality and performance standards. Announcing elimination of leprosy as public health problem in India under NLEP was probably ambitious aspiration. However, such a strategy can not be justified scientifically at the moment in view of new case detection rate not abating significantly. In such a scenario the importance of slit skin smear examination should not be undervalued in spite of all the drawbacks. The fact remains that it is still highly desirable to provide sustained quality leprosy control services to all individuals through general health services and good referral system. It will be rather prudent to identify our shortcomings and knowledge gaps in various aspects of leprosy transmission, microbiology, and treatment. The program managers and the staff at peripheral centers must be encouraged to coordinate with referral centers maintaining specialized services in dermatology (for diagnosis, SSS, histopathology), ophthalmology (for eye care), physiotherapy (for managing disability), foot care, reconstructive surgery, etc for integrated services in leprosy care. Sadly, poor coordination between program managers and referral centers, mostly medical colleges in any state, is too stark to be ignored.

The cumbersome reading/reporting process, casual approach in organizing SSS, tendency of the leprosy elimination program to avoid the difficult, and overtone of public health approach were perhaps real reasons behind abandoning this inexpensive diagnostic method. Clubbing of SSS reporting with other infectious disease control programs such as sputum microscopy (Poricha et al 2011) will perhaps solve the problem to an extent provided quality and performance standards can be maintained. Moreover, an underpaid lab technician is unlikely to have much patience in examining weakly positive smears. This is especially true in the resource limited developing world with nearly 80% of the global case load and where modern sophisticated diagnostic tests are unlikely to be available in the near future for routine bedside practice (Lini et al 2009, Stefani et al 2009, Kerkeni et al 2011). This relatively inexpensive diagnostic method despite inferior operating characteristics still retains practical value as the world moves toward the eventual global eradication of leprosy.

Quality smears are a matter of leadership, concern and quality control for which provision of adequate material and logistics, regular training and supervision of laboratory staff, maintaining their equipment and making a system of quality control by random checks of smear results by establishing regional reference laboratories are of paramount importance.

A slit-skin smear examination from the single most active lesion and simplified reporting of smears as 'positive' or 'negative' will perhaps be more practical in view of fixed-duration MDT for all patients in spite of varied bacterial load from 1-1000 bacilli/high field. However, it is a significant observation that in the fields where slit-skin smear is not routinely practiced bacteriological relapse, that occurs much before the clinical relapse, mostly remains undetected during short follow-up (Girdhar and Girdhar 2002).

Last but not the least, from the point of public health all advantages of fixed-duration MDT in MB patients with initial high bacterial load are debatable in view of the persistence of source of infection and its horizontal transmission possibly due to early bacteriological relapse and high rates of clinical relapse (2.04-3.4% per 100 person-year), persistence of viable drug-sensitive *M*. *leprae* in nerves or other tissues (nearly in 1/3rd of patients (Girdhar and Girdhar 2002, Girdhar et al 2000, Jamet and Ji 1995), a poor cell-mediated immunity and macrophage function to deal with these dormant yet active bacilli, and virtually no post-MDT follow up. On the other hand, the need of SSS is emphasized especially in institutional patients with BI $\geq$ 4+ where extended MDT remains desirable when cure is aimed. As for risk of transmission of HIV and hepatitis B infections to the patient or the health care provider, strict adherence to universal precautions for their control and prevention is essential for all leprosy workers as well.

Points to remember

Classifying leprosy solely on the basis of skin lesions is fraught with under diagnosis and inadequate treatment.

Slit-skin smear is useful in diagnosis, classification, monitoring of treatment and disease severity. Classify and treat all smears positive patients as multibacillary leprosy. Lepra reactions are common among patients with high BI.

Perform slit-skin smear examination in all patients suspected to have leprosy or relapse of leprosy, and before starting MDT.

Make smears from three sites-one earlobe and two from the lesion(s). However, remember a single slit-skin smear examination from the single most active lesion and simplified

reporting of smears as 'positive' or 'negative' will be more practical. Never send any patient while smear sites are still bleeding.

Remember to label the slides with patient's name/registration number, and sites of smears taken. The slide should be examined immediately and stored away from sunlight for cross checking/records.

Remember results of Ziehl-Neelsen staining at 60°C (hot method) are better than at 22°C (cold method) but avoid over heating the stain. Addition of 'Tween 80' will reduce staining time to 5 minutes.

Calculate average or more practical is to report highest BI. MI is more sensitive parameter of therapeutic follow up, needs quality smears and better left for the referral/research centers.

Remember new cutting-edge diagnostic tools are not going to be available for bedside use anytime soon. Slit-skin smear is still the most useful diagnostic method available.

Remember, negative smears will not exclude leprosy automatically. Develop skill as quality smears are matter of leadership, concern and quality control.

Remember to follow guidelines on universal precautions for prevention of HIV and other infections. Dispose-off soiled swabs, blades etc carefully, preferably by burning.

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