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Slit-skin smear for the classification of leprosy; are we wasting time and resource?

Wondmagegn Demsiss[1], Saskia van Henten[2], Kudakwashe Collin Takarinda[3], Edward Mberu Kamau[4], Seid Getahun Abdela[1]

[1] College of Medicine and Health Sciences, Wollo University, Dessie, Ethiopia
[2] Institute of Tropical Medicine, Antwerp, Belgium
[3] Center for Operational Research, International Union Against Tuberculosis and Lung Disease, Paris, France
[4] UNICEF/UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR), World Health Organization, Geneva, Switzerland

Abstract
Introduction: Leprosy is a chronic neglected tropical disease, classified into two groups: multibacillary (MB) and paucibacillary (PB) leprosy based on the number of skin lesions and nerve involvement. A positive skin slit smear (SSS) result automatically puts a patient in the MB category. Although guidelines do not recommend routine use of SSS for classification and diagnosis of leprosy, it is performed for most patients in Ethiopia. However, the added value of performing SSS for the classification of leprosy on top of clinical classification is unclear.
Methodology: A cross sectional study was done using routine laboratory and clinical data from September 2018 to January 2020 at Boru Meda General Hospital, Ethiopia. All newly diagnosed leprosy cases were included. Descriptive statistics were performed to calculate frequencies and proportions.
Results: We included 183 new leprosy patients in our study, of which 166/183 (90.7%) were MB patients and 17/183 (9.3%) were PB patients. All clinical PB cases and 150/166 (90.4%) clinical MB patients had SSS done. All PB patients had negative SSS result and 68 (45.3%) clinical MB patients had a positive result. Based on the SSS, no patient with a clinical classification of PB was reclassified to MB.
Conclusions: SSS microscopy was performed routinely for all leprosy cases without changing the classification and management of patients in Boru Meda Hospital. Therefore, we recommend restricted and rational use of the SSS for PB cases in which SSS could change management.

Introduction

Leprosy, also known as Hansen's disease, is a chronic neglected tropical disease of public health importance, and one of the leading causes of infectious disability [1]. Leprosy is caused by *Mycobacterium leprae*, which affects the skin and peripheral nerves leading to loss of sensation and tissue damage through repeated injuries [2]. This may result in neuropathic ulcers and self-amputation [3]. Despite the availability of effective treatment, the global leprosy incidence remains high, with more than 200,000 new cases every year for the past five years across the different regions of the world [4].

Global statistics show that 94% of new leprosy cases were reported from 14 countries, five in Africa including Ethiopia with the remaining 5% reported by 92 other countries worldwide [5]. Ethiopia is among the top 20 countries in the world by new leprosy case detection from the world with more than 3200 new cases in 2019, which is new leprosy case detection rate of 28.56 per 1,000,000 population [6]. Ethiopia is also among the top 7 countries in the world in terms of patients with grade two disabilities [7].

According to the Ethiopian [8] and World Health Organization (WHO) [9] guidelines, leprosy is diagnosed on the basis of the presence of at least one of the following signs: definite loss of sensation in a pale (hypo-pigmented) or reddish skin lesion, a thickened or enlarged peripheral nerve with or without tenderness, and the presence of acid-fast bacilli in a slit-skin smear (SSS) [8]. According to both guidelines, SSS should only be performed for doubtful cases, where the diagnosis or classification is unclear [9].

The classification of leprosy was based on various evolving methods since the 1930s. In 1988 the WHO expert committee for leprosy advised all smear-positive

cases to be considered multibacillary (MB), which was changed to pure clinical classification in 1998 as per the recommendation of the same organization due to the poor sensitivity of SSS and its inaccessibility. The clinical classification of leprosy patients is based on the number of skin lesions and nerve involvement. Patients with one to five skin lesions and only one enlarged nerve are classified as paucibacillary (PB), while patients with six or more skin lesions or a positive SSS result (regardless of the number of lesions) or enlargement of more than one nerve are classified as MB [10-13].

The 2018 WHO leprosy guideline developing group recommends the same 3-drug regimen with rifampicin, dapsone and clofazimine for all PB and MB patients, which is believed to reduce the impact of misclassification of MB cases and consequences associated with undertreating of MB cases as PB [9]. However, according to the Ethiopian national guideline for leprosy, PB patients should be treated with rifampicin and dapsone for 6 months and MB leprosy should be treated with a 3-drug regimen of rifampicin, dapsone and clofazimine for 12 months [8]. A misclassification of either MB to PB or PB to MB has clinical implications. The first scenario could result in undertreatment which could lead to relapse, whereas the latter scenario could result in overtreatment and side effects particularly in countries that follow the two-drug regimen such as Ethiopia [14].

Although the guidelines recommend the conditional use of SSS, others [15,16] are recommend SSS for all patients.

SSS is performed routinely for almost all leprosy-suspected patients in Boru Meda Hospital, providing a unique opportunity to study the added value of SSS in the diagnosis and management of leprosy. Specifically, we wanted to investigate whether SSS was used to reclassify cases clinically classified as PB to MB. The results from this study may be used for the development of a more rational and cost-effective strategy of using the SSS.

Methodology

General setting

Ethiopia is the second most populous nation in Africa with the current population estimated to be more than 118,000,000 [17]. It occupies a total area of 1,100,000 square kilometers. Leprosy prevention and control in Ethiopia started in the 1950s. The country has five leprosy referral centers distributed across the country, including Boru Meda Hospital, located in the northeastern part of Ethiopia.

Specific setting

Boru Meda Hospital was initially established in 1954 by missionaries, mainly to provide care for leprosy and related complications. Later on, the hospital started providing other medical services to the society in need. The hospital currently has 40 beds for leprosy and other dermatology cases. It has also two dermatology outpatient offices with one dermatologist, one tropical dermatology professional and one health officer with leprosy training.

Leprosy diagnosis at Boru Meda Hospital

Patients are diagnosed with leprosy based on the cardinal signs and SSS samples are sent routinely. The samples were collected and examined by trained laboratory technologists. Three skin slits are taken, one from the lesion and two additional ones from the earlobes and/or other sites. All smears are put on one slide, side to side. Grading was performed based on Ridley's scale for bacteriological index (BI), ranging from negative, to +6 (> 1,000 clumps of bacilli per field) [18-20]. Negative SSS slides were reexamined by another laboratory technologist.

Design and population

This was a cross sectional study using routine lab and clinical data from September 2018 to January 2020. All new leprosy patients identified from the case registry between September 2018 and January 2020 for whom we could find medical records were included in this study. Patients with records that did not mention the clinical classification were excluded from the analysis.

Data collection

There were three routine data sources, the routine lab register, the case registry and the patient charts. The outpatient case registry was the initial entry point to identify suspected leprosy patients. We used the specific medical record number (MRN) to retrieve patient charts to extract additional clinical data (history, physical examination, clinical classification and treatment). Clinical data were extracted from the patient chart (history and physical diagnosis) A paper-based structured data collection form was used.

The laboratory registry is an Excel database in which all patients who have a SSS performed are documented. This was the source of information for the SSS result (BI). Data from the clinical and laboratory database were merged into one electronic database by using the unique MRN. Data extraction was done by the principal investigator (PI).

Data entry and statistical analysis

Data were checked for completeness and consistency. Precoded data were entered into EpiData Entry Client version 4.6.0.2. (Epidata Association, Odense, Denmark). Statistical analysis was performed using Epidata Analysis version 2.2.3.187 (Epidata Association, Odense, Denmark). Descriptive statistics were performed to calculate frequencies and proportions.

Ethical considerations

Ethical approval was obtained from College of Medicine and Health Sciences (CMHS) ethical review committee, Dessie, Ethiopia. The protocol was also cleared from Ethics Advisory Group of the International Union against Tuberculosis and Lung Disease, Paris, France. Permission was also obtained from Amhara Public Health Institute (APHI) Dessie branch and Boru Meda Hospital. The privacy of patients was protected and ensured by keeping restricted access to the study documents and the study database using a locked cabinet and password.

Collaborative partnerships

The collaboration was between Wollo University, Boru Meda Hospital, and international partners of the SORT IT initiative, WHO/TDR, ITM and the Union.

Results

Demographic and clinical characteristics

A total of 189 records of new leprosy patients were reviewed and six were excluded for incompleteness. Therefore, 183 leprosy patients' medical records were included for final analysis. The majority of patients (132; 72.1%) were males with a median age of 41 years (interquartile range (IQR) 30-55 years). Among 136 patients with data available on the number of skin lesions, (128; 94.1%) had more than 5 lesions. Information on whether nerve enlargement was present

was available for only 34 patients, but among them, more than half (22; 64.7%) had nerve enlargement (Table 1).

Among the 183 new leprosy patients involved in the study, most (166; 90.7%) had MB, and the remaining (17; 9.3%) had PB. All clinical PB cases and almost all (150; 90.4%) of clinical MB patients had SSS done.

The overall positivity rate of SSS among clinically diagnosed leprosy patients in our study was (68; 40.7%). All PB patients had negative slit-skin smear result and only (68; 45.3%) of MB patients had a SSS positive result. The most common BI results were +1 (20, 12.0%) and +2 (25; 15.1%). Based on the SSS, no patient with a clinical classification of PB would be reclassified to MB. The detailed results are reported in Table 2.

Discussion

In several hospitals in Ethiopia, SSS for leprosy patients are routinely ordered. Although we acknowledge the use of SSS for the diagnosis of patients in which clinical suspicion is low and for whom SSS can therefore rule in disease, and for potentially reclassifying patients clinically diagnosed as PB, we question the routine use of SSS for the classification of leprosy. Potential misclassification of patients based on clinical diagnosis alone is often used as an argument to request SSS for newly diagnosed leprosy patients [21], as reclassification based on SSS could lead to more appropriate treatment regimens. However, in our patients, no one was reclassified from PB to MB based on the SSS compared to clinical diagnosis as all 17 PB leprosy patients had a negative SSS result. All MB and PB cases were treated irrespective of their SSS result. For patients with a clear clinical picture and who are classified as MB based on clinical evaluation, there is no clear added value of performing SSS. In our study, more than 90% of participants were in this group, but SSS was performed for the majority of the patients

Table 1. Socio-demographic and clinical characteristics of leprosy patients at Boru Meda hospital, Dessie, Ethiopia (September, 2018 – January, 2020).

Variable	Total, N (%)	PB, N (%)	MB, N (%)
Male gender	132 (72.1)	11 (8.3)	121 (91.7)
Age category			
< 15	4 (2.2)	0 (0)	4 (100)
15-24	20 (10.9)	1 (5)	19 (95)
25-44	73 (39.9)	5 (6.8)	68 (93.2)
45-64	61 (33.3)	8 (13.1)	53 (86.9)
≥ 65	25 (13.7)	3 (12.0)	22 (88.0)
Loss of sensation (n = 145)	127 (87.6)	9 (7.1)	118 (92.9)
Presence of anaesthetic skin lesion (n = 147)	144 (98.0)	10 (6.9)	134 (93.1)
Number of anaesthetic skin lesion > 5 (n = 136)	128 (94.1)	0 (0)	128 (100)
Peripheral nerve enlargement (n = 34)	22 (64.7)	0 (0)	22 (100)

The high proportion of MB (90.7%) is comparable to a study reported from the same hospital [22] which reported 89.5% of MB leprosy in 2017, although an old report from Addis Ababa from 1991 reported approximately 50% of cases to be MB [23]. The global trend over the past 40 years across different studies and WHO reports shows a high proportion of MB cases [24]. This may indicate high transmission levels of leprosy in our setting or may be associated with a change in the definition of the classification of leprosy across those years. Despite this high prevalence of MB in our setting there is routine usage of SSS.

All PB leprosy patients in our study had negative SSS result, meaning none were reclassified to MB. However, the reclassification rate of PB to MB based on direct microscopy is reported to be 7.2% in Brazil [25]. This may be because the proportion of PB cases could be higher in the other study.

Performing SSS routinely against the optional and rational use of the test will have physical, financial, human resource and overall efficiency impacts on diagnostic laboratories and the country at large. On top of that the patient also experienced discomfort in giving three slit-skin samples. This means we are using slit-skin smear without implications despite being done for almost all patients.

We propose a more selective or rational use of skin slit for leprosy patients. It should only be used when it can change management. For patients with PB, SSS microscopy positive result could lead to reclassification to MB, which would change the treatment. For patients with doubt of diagnosis, slit-skin could be used to rule in. For patients clinically diagnosed with MB, slit-skin should not change management, due to its poor sensitivity, as a negative test does not rule out disease. In general, we should work on having a laboratory assay with more sensitivity and specificity for routine use in the clinical setup.

The main strength of this paper is that SSS was performed for almost all patients regardless of their initial classification. Therefore, we are able to evaluate the results of the patients and its importance for their clinical care. Limitations are that this was a single center study that used retrospective data, the number of PB cases included in our study was low, and we did not have data to see the added value of SSS for diagnosis and outcome monitoring. To solve these issues, we recommend to carry out a prospective study across the different leprosy treatment centers to see the added benefit of skin slit smear for diagnosis, reclassification and treatment monitoring.

Conclusions

SSS microscopy was performed routinely for all leprosy cases without it changing the classification and management of the patients in Boru Meda Hospital. Therefore, we recommend more restricted and selective use of the SSS for PB cases in which the SSS could change management.

Table 2. Slit-skin microscopy positivity rate and bacterial index (BI) of leprosy patients stratified by clinical classification at Boru Meda hospital, Dessie, Ethiopia (September 2018 – January 2020).

Variable	Response	Clinical classification		
		PB	MB	Total
Slit-Skin result	Positive	0 (0)	68 (45.3)	68 (40.7)
	Negative	17 (100)	82 (54.7)	99 (59.3)
	Not recorded			
	Total	17 (100)	150 (100)	167 (100)
Bacterial Index	0 bacilli per 100 high power fields	17 (100)	98 (59.0)	115 (62.8)
	+1. 1 to 10 bacilli per 100 high power fields	0 (0)	20 (12.0)	20 (10.9)
	+2. 1 to 10 bacilli per 10 high power fields	0 (0)	25 (15.1)	25 (13.7)
	+3. 1 to 10 bacilli per high power field	0 (0)	16 (9.6)	16 (8.7)
	+4. 10 to 100 bacilli per high power field	0 (0)	2 (1.2)	2 (1.1)
	+5. 100 to 1,000 bacilli per high power field	0 (0)	3 (1.8)	3 (1.6)
	+6. > 1,000 bacilli per high power field	0 (0)	2 (1.2)	2 (1.1)
	Total	17 (100)	166 (100)	183 (100)

PB: Paucibacillary; MB: Multibacillary.

office in Ethiopia and the International Union Against Tuberculosis and Lung Diseases, Paris (France).

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Corresponding author
Wondmagegn Demsiss, BSc, MSc
Assistant professor in Medical Microbiology, Wollo University, Dessie, P.O.Box: 1145, Ethiopia
Phone: +251910794202
Email: wonde1980@gmail.com