DIAGNOSTIC ACCURACY OF SLIT SKIN SMEARS IN LEPROSY

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ABSTRACT

Objective: To determine the diagnostic accuracy of slit skin smears in clinically suspected patients of leprosy using histopathology as gold standard.

Study Design: Validation study

Place and Duration of Study: Study was carried out at Rawalpindi Leprosy Hospital, Dermatology Department Military Hospital (MH) and Armed Forces Institute of Pathology (AFIP), Rawalpindi from 18th August 2012 to 18 Feb 2013.

Methods: Appropriate technical and ethical approval for the study and patient consent were obtained. All suspected patients of leprosy of any age and either gender having typical hypo-aesthetic or anesthetic, erythematous or hypo-pigmented scaly skin lesions on any part of body were included in this study. All patients who have already received treatment for leprosy, patients with pure neural leprosy, patient not giving their consent for skin biopsy and patients with lepra reactions were excluded from this study. Forty eight patients fulfilling the inclusion criteria were included in the study. Sample size had been calculated by using WHO sample size calculator taking confidence level 95%, absolute precision required 14% and anticipated population proportion 40%. Non-probability consecutive sampling technique was used to collect sample.

Results: The results of the study revealed that out of 48 clinically suspected patients of leprosy skin biopsy confirmed the diagnosis in 34 patients (70.8%) and the slit skin smear had diagnostic accuracy of 68.75% with sensitivity 55.8% and specificity and positive predictive value of 100%.

Conclusion: Study suggested that although slit skin smears are rapid and inexpensive method of diagnosis but their diagnostic accuracy is low.

Keywords: Leprosy, Slit skin smear, Skin biopsy.

INTRODUCTION

Leprosy is the leading infectious cause of disability[1]. Its prevalence in Pakistan is estimated to be 0.05 / 10,000 with an incidence of 0.3 / 100,000[2]. Prevalence has fallen substantially in the past 50 years but it could not be eliminated from the world and transmission continues in some countries of the world[3]. It affects the cooler parts of body especially the skin and nerves4 Leprosy expresses in different clinico-pathological forms depending on the immune status of the host[5]. Diagnosis of leprosy is based on characteristic skin lesions and enlarged peripheral nerves[4]. Laboratory tests may be used to confirm the diagnosis. These investigations include slit-skin smears, nerve biopsy or skin biopsy showing typical histological features of leprosy. Slit skin smear is an easily performed, sensitive, specific, cost effective and rapid test that is useful in number of clinical settings encountered in dermatologist's daily practice[4]. Demonstration of acid fast bacilli (AFB) in slit skin smears by Ziehl-Neelson's staining helps in diagnosis of leprosy[4]. The density of bacilli is expressed using a logarithmic scale, extending from very few AFB to many per high-power field. The specificity of slit skin smear is 100%[6] because it directly demonstrates the presence of AFB but the sensitivity is low and varies from 10-50%[7]. Histological examination has many advantages and the yield of AFB in tissue sections are reported to be better as compared to other modalities[8]. To our knowledge, it's the first study in Pakistan which had been conducted to determine the diagnostic accuracy of slit skin smear.

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MATERIAL AND METHODS

This validation study was done over a period of 06 months (from 18th Aug 2012 to 18th Feb 2013) at three places, Leprosy Hospital Rawalpindi, Dermatology Department Military Hospital Rawalpindi and Armed Forces Institute of Pathology Rawalpindi. All suspected patients of leprosy of any age and either gender having typical hypo-anesthetic or anesthetic, erythematous, hypo-pigmented scaly skin lesions, nodules and plaques on any part of body were included in this study. All patients who have already received treatment for leprosy, patients with pure neural leprosy, patient not giving their consent for skin biopsy and patients with lepra reactions were excluded from this study. Forty eight patients fulfilling the inclusion and exclusion criteria were included through non-probability consecutive sampling.

Data collection and analysis procedure

Patients from Dermatology outpatient department (OPD) at Military Hospital Rawalpindi and Leprosy hospital Rawalpindi, fulfilling the inclusion criteria were selected after informed written consent. OPD registration number, name, age and gender were noted for each patient. Slit skin smear was prepared from the most representative lesion, stained by modified Ziehl- Neelson's stain and seen by histopathologist for the presence of AFB. The skin biopsy was obtained from the representative lesion. Tissue was then fixed in formalin and sent to Armed Forces Institute of Pathology for histopathological examination by the histopathologist. Then the number of smears positive for AFB and the number of skin biopsies consistent with the diagnosis of leprosy was calculated. Findings were recorded in a specific Performa.

Data analysis was computer based with the use of SPSS version 12. The quantitative variables like age and duration of illness were described by taking mean and standard deviation (SD). The qualitative variable like gender and presence of leprosy (true positive) were described by taking frequency and percentages. Diagnostic measures were calculated for slit-skin smears taking biopsy as gold standard.

RESULTS

The ages of patients ranged from 18-70 years with a mean age of 39.5 ± 4.67 years. Seventeen (35.5%) patients were females. The duration of illness of majority of patients was >6 months. The accuracy of slit skin smear turned out to be 68.75%, sensitivity 55.9%, specificity 100%, negative predictive value 48.2% and positive predictive value 100% (table-1). Area under ROC curve is 0.221 (*p*-value is 0.003) (Fig-1).

DISCUSSION

Most patients with leprosy infection can be readily diagnosed by characteristic clinical appearance and anaesthesia of skin lesions. However, because clinical appearance of these lesions can mimic various other skin conditions like tinea, ptyriasis alba, granuloma annulare, eczema, annular psoriasis, sarcoidosis and mycosis fungoides, so the infection can be misdiagnosed. Also the rapidity of obtaining a conclusive diagnosis is important for starting adequate anti leprosy therapy, because delay in treatment can increase the risk of complications[9].

Table-1: Diagnosis of leprosy through slit skin smear and biopsy.

Slit Skin Smear	Biopsy Diagnosis		Total
	Positive	Negative	
Positive	True positive (a) 19 (39.5%)	False positive (b) 0 (0%)	a + b 19 (39.5%)
Negative	False negative (c) 15 (31.25%)	True negative (d) 14 (29.1%)	c + d 29 (60.4%)
Total	a + c 34 (70.83%)	b + d 14 (29.17%)	48 (100%)

In clinically suspected patients of leprosy, we found 64.5% of male and 35.5% of female patients. The female to male ratio was 1:1.8. The greater ratio of male as compared to female in our study is probably due to the greater number of consultations by males as opposed to females. Similar results were found in a study conducted by Bhushan and colleagues at New Delhi, India which was carried out on 102 patients and there were 63 (72.34%) males and 39 (27.66%) females. The male: female ratio was 2.61:1[10].

In our study leprosy patients ranged from 18 to 70 years of age with mean age 39.54 ± 4.67 years. The maximum numbers of patients were in their fifth decade. This high frequency in fifth decade may be because of very long incubation period especially in case of lepromatous leprosy. Similar results were found in a study carried out in Northern Pakistan by Khan and colleagues in 2012, which was carried out on 50 patients of leprosy and majority of people belonged to old age group with mean age of 40 years[11].

The results of the study revealed that in 70.83% (n=34) patients with clinically suspected lesions of leprosy the skin biopsy showed the characteristic findings of leprosy while 29.17 % (n=14) had no findings of leprosy on skin biopsy. The specificity and positive predictive value of slit skin smear turned out to be 100% while the sensitivity is low i.e. 55.8%, negative predictive value 48.2% and accuracy rate is 68.75%. Bhushan with his collegues compared the diagnostic accuracy of slit skin smear with bacterial index of granuloma. The results of the study showed 100% specificity and positive predictive value and a negative predictive value of 66.33% for slit skin smear which was low as compared to bacterial index of granuloma on skin biopsy[10].

In another study, the importance of bacteriological examination in diagnosis and studying the prognosis of leprosy with Multi-drug therapy was emphasized. It was mentioned that the diagnosis was based mainly on the clinical assessment and was supported by the results of skin smear examination for AFB[12]. Direct and rapid confirmation of AFB is possible in patients with slit skin smear, using rapid staining techniques. The diagnostic specificity of skin smears is almost 100%, however, its sensitivity is rarely more than 50% because smear positive patients represent only 10- 50% of cases. The inherent problems of skin smears are the logistics and the reliability of the technique of taking, staining, and interpreting the slide. Skin smears identify only those with multibacillary disease who are the most infectious and also those patients who are experiencing clinical relapses. We cannots use slit skin smears to diagnose clinical case of tuberculoid leprosy i.e paucibacillary leprosy.

A study carried out in Malawi by

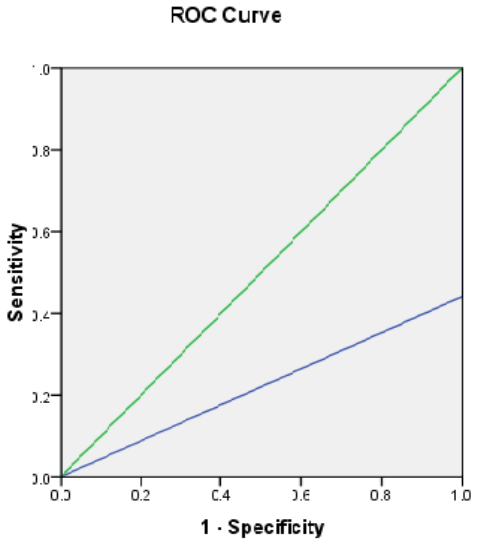

Figure-1: ROC curve of slit skin smears in leprsoy.

Ponninguas and colleagues who compared the bacterial index in slit skin smear, skin biopsy and nerve biopsy. Two hundred and twelve patients were included in the study and slit skin smear was positive in 34 (29%) out of 119 multibacillary patients and in none out of 61 paucibacillary patients. The mean bacillary index was higher in nerve biopsies than the skin biopsies[13]. Lyon and colleagues at Brazil compared the serology (antibodies against phenolic glycolipid-1) and slit skin smear in newly diagnosed patients of leprosy. Slit skin smears were positive in 35.9% of patients and 57% of patients were seropositive for Phenolic glycolipid-1 (PGL-1) antibodies[14].

The studies described above showed low sensitivity of slit skin smear when compared with various other serological tests and histopathology, which is also depicted by our study. But, despite its low sensitivity slit skin smear is currently the most important and widely used investigation to confirm the diagnosis and to classify the leprosy patients because of its increased specificity and rapid results. However, a proper laboratory infrastructure and experienced personnel, not always available in primary health care centers, are required for its execution. In Pakistan no published data was found on the diagnostic efficacy of slit skin smear in patients of leprosy. On the other hand, low sensitivity of this procedure as compared to its specificity has been documented in literature, which is in accordance with our observations in this study[6,7].

CONCLUSION

The slit skin smear has low diagnostic efficacy in leprosy. However being a rapid and cost effective method with high specificity it can be used to confirm the diagnosis in clinically suspected patients of leprosy. The skin biopsy remains the gold standard in the diagnosis of leprosy. If slit skin smear is negative, the skin biopsy should be performed in all suspected cases and a clinic-pathological correlation should be done in making the diagnosis of leprosy.

CONFLICT OF INTEREST

This study has no conflict of interest to declare by any author.

REFERENCES

1. Rodrigues LC, Lockwood DN. Leprosy now: epidemiology, progress, challenges, and research gaps. Lancet Infect Dis. 2011;11:464–70.
2. Bajaj DR, Matlani BL, Soomro FR, Iqbal PM. Knowledge, Attitude and Practices Regarding Leprosy Among General Practitioners at Hyderabad. J Coll Physicians Surg Pak. 2009; 19(4):215-8.
3. Merle CS, Cunha SS, Rodrigues LC. BCG vaccination and leprosy protection: review of current evidence and status of BCG in leprosy control. Expert Rev Vaccines 2010; 9: 209–22.
4. Naik VB, Naik UB, More S, Rao VP. Evaluation of significance of skin smears in leprosy for diagnosis, follow-up, assessment of treatment outcome and relapse. Asiatic J Biotech Res. 2011; 2 (5): 547-52.
5. Panday AN, Tailor HJ. Clinicohistopathological correlation of leprosy. Ind J Dermatol Venerol Leprol 2008; 74:174-6.
6. Diagnosis and Classification of Leprosy. In: Report of the International Leprosy Association Technical Forum 2002. Lepr Rev 2002; 73:S17-26.
7. Rao PN. Recent advances in the control programs and therapy of leprosy. Indian J Dermatol Venereol Leprol 2004; 70:269-76.
8. Rao PN, Pratap D, Ramana Reddy AV, Sujai S. Evaluation of leprosy patients with 1 to 5 skin lesions with relevance to their grouping into paucibacillary or multibacillary disease. Indian J Dermatol Venereol Leprol 2006; 72: 207-10.
9. Burki T. Old problems still mar fight against ancient disease. Lancet 2009; 373: 287–8.
10. Bhusan P, Sardana K, Korrane RV, Chaudary M, Manjul P. Diagnosing multibacillary Leprosy:A comparative evaluation of diagnostic accuracy of slit skin smear, bacterial index of granuloma and WHO operational classification.Indian J Dermatol Venereol Leprol 2008; 74: 322-6.
11. Khan I, Khan AR, Khan MS. Clinicopathological study of 50 cases of leprosy in Northern Pakistan. JPAD 2012; 22: 200-6
12. Mekhlafi GA, Al Qubati Y. Retrospective analysis of 194 leprosy cases in the Republic of Yemen'. Indian J Lepr.1996; 68: 227-34
13. Ponnighaus JM, Lienhardt C, Lucas S, Fine PE, Sterne JAI. Comparison of bacillary indexes in slit-skin smears, skin and nerve biopsies; a study from Malawi. Int J Lepr, 1997; 65(2): 211-6
14. Lyon S, Lyon AC, Castorina-Silva R, Grossi MAF, Lyon SH, Bührer-Sékula S et al. A comparison of ML Flow serology and slit skin smears to assess the bacterial load in newly diagnosed leprosy patients in Brazil. Lepr Rev 2008; 79: 162-70.