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Original Article

Validity of FNAC for the Diagnosis of Leprosy

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Early and accurate diagnosis of leprosy is crucial because delay may lead to some permanent disability and sequelae. Histopathological examination of skin and nerves and slit skin smears examination to assess the bacillary load, have been the major tools used in the laboratory diagnosis, classification and follow up of patients with leprosy. Histopathological evaluation is not feasible in many leprosy endemic areas. Fine needle aspiration cytology (FNAC) is a simpler tool compared to histopathology for the evaluation of the cytomorphology of skin lesions. Aims of this study are to evaluate the role of cytology in diagnosing leprosy patients, to study the cytomorphology of leprosy lesions in fine needle aspirates and to compare the diagnostic value of FNAC with that of standard histopathological diagnosis. Sensitivity of FNAC in diagnosing leprosy was observed to be BT (87.5%); LL (80%); TT and BL (66.67%) in the descending order. Specificity of FNAC in diagnosing leprosy was in the following order BL (96.29%); followed by LL (96%); TT (92.59%) and BT (85.71%). Positive predictive value was observed to be BT-87.5%; LL-80%; BL-66.67% and TT-50%. Fairly good correlation was observed between clinical, histological and cytomorphological features in the aspirates taken from the skin lesions. A reasonably good sensitivity, specificity, positive and negative predictive values were obtained in all types of leprosy except for mid borderline spectrum. FNAC is a simple, easy, cost effective, relatively non invasive procedure which provides faster results than biopsy. Drawbacks include- "dry taps", bloody smears and lack of cellular infiltrate in case of macular lesions.

Keywords : Leprosy, Biopsy, Acid fast bacilli, FNAC

Introduction

Leprosy is a chronic infectious disease caused by *Mycobacterium leprae* which manifests itself in different clinicopathological forms, depending on the immune status of the host (Abulafia and Vignale 1999). Laboratory diagnosis of leprosy is conventionally done by slit skin smear and skin biopsy (Nigam et al 2007). These techniques are

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simple but both these methods have their own limitations. The examination of slit skin smears after staining for acid fast bacilli (AFB) is a simple field technique for the diagnosis of leprosy. However, slit skin smear is negative in paucibacillary cases and its use has been restricted to the determination of the presence or absence of AFB and evaluation of the bacteriological index and morphological index. (Prasad et al 2008, Gulati et al 2012). Histopathological biopsy is an invasive procedure and leads to a biopsy scar which may not be cosmetically acceptable (Prasad et al 2008). As the disease burden is greater in communities and countries with limited resources, a cheaper and less demanding diagnostic test needs to be used, so that it can be applicable in the field conditions.

Cytology is a widely accepted diagnostic procedure for a large variety of malignant and inflammatory lesions (Singh et al 1996). Fine needle aspiration cytology (FNAC) is the study of cells obtained through a small gauge needle, under vacuum system, provided by an airtight syringe. It has been proved to be a simple, acceptable, inexpensive, rapid and effective minimally invasive diagnostic tool with minimal trauma and low complication rate, which can be used for definitive diagnosis and classification in an office as well as field setting. (Gulati et al 2012) Also, FNAC smears, unlike slit skin smears are free of confounding epidermal squamous cells and therefore is better suited for evaluating cell morphology. (Nigam et al 2007). The technique of obtaining aspirates is simple and can be applied in the field (Prasad et al 2008).

In the present study for determining the diagnostic accuracy and utility of FNAC in patients of leprosy, it has been compared with histopathology from the same patient in patients, presenting to the outpatient Department of Dermatology at KIMS, Narketpally, Telangana, 508254, India.

Materials and Methods

The study was undertaken over a period of two years from September 2012 to October 2014. A total of 50 untreated clinically diagnosed new leprosy patients of all age groups presenting with the clinical symptoms and signs of leprosy who attended the outpatient Department of Dermatology, Venereology and Leprosy at KIMS, Hyderabad were included in the study. Patients presenting with pure neuritic lesions, and old cases i.e patients already on treatment were excluded from the study. A written informed consent was taken at the time of enrollment of the patients.

The distribution, number, size, shape, margin, colour (erythematous or macular), borders, infiltration, dryness, loss of hair etc of the skin lesions were noted and recorded. Presence of pain, touch sensation in and around the lesions and limbs was tested and recorded. Similarly, all peripheral and cutaneous nerves were palpated for their size, nodularity, abscess formation, and tenderness and recorded. These findings were entered in a body chart for each patient.

Patients were classified clinically into tuberculoid, borderline tuberculoid, mid-borderline, borderline lepromatous and lepromatous disease based on lesional count and characteristics (Prasad et al 2008). After obtaining informed consent from the subjects for diagnostic procedures, slit skin smears were done from 5 sites (both earlobes, forehead above both eyebrows and one from lesion) for all patients, stained by Ziehl Neilson Method and examined. Acid Fast bacilli (AFB) were identified and smears graded using Ridley's logarithmic scale.

Skin biopsy was performed in all patients and findings recorded. After local infiltration of 1cc to 2cc of xylocaine at the edge of the lesions, a piece of skin was taken from the lesion over the involved area for histopathological study. The

tissue was preserved in 10% formalin before it was processed for histopathological examination. The sections were stained routinely with the haematoxylin and eosin. Histological diagnosis and grading was done as defined by Ridley (Prasad et al 2008). This involved the evaluation of cell types composing the granulomas, i.e., epithelioid cell or macrophage, cellular infiltrate surrounding the granuloma as well as in the dermis and AFB bacterial load in Fite's stain.

FNAC aspiration was done from the same skin lesion from where the skin biopsy was taken. The site was cleaned with alcohol and an assistant pinched the skin for 30 seconds to blanch the skin. A 10 ml syringe was fitted with a 21-gauge needle and negative pressure was created by holding back the piston with the forefinger and index finger. The aspirated material was transferred onto 4 glass slides. The flat surface of another slide was used to spread the aspirated material. Two smears were made from the aspirates. One of the smears was air-dried and stained with May-Grunwald-Giemsa (MGG) stain (Prasad et al 2008). The other was also air dried and stained by modified Ziehl Neilson stain (Bhake et al 2001). Cytological smears were coded and assessed by the pathologist. Cytological specimens were considered adequate if there was a heavy cellular yield of inflammatory cells (Singh et al 1996), The criteria as laid down by Prasad et al (2008), was followed.

Validity of a test refers to what extent the test accurately measures which it purports to measure. Validity of the test can be determined by measuring its sensitivity (no. of true positives) and specificity (no. of true negatives). Predictive values reflect the diagnostic power of the test (Fletcher and Fletcher 2005). In this study, cytological specimens were compared with histological diagnosis to obtain the validity of FNAC in diagnosing leprosy.

Results

A total of 50 untreated clinically diagnosed new leprosy patients of all age groups presenting with the clinical symptoms and signs of leprosy were taken up for the study out of a total of 63 leprosy cases who attended the outpatient Department of KIMS. Out of 50 cases included in the study, majority of the cases (34%) were in 21- 30 yrs age group. Age of the patients ranged from 7-75 years with a mean age of 33.16 years. Males 29(58%)

Table 1 : Age wise distribution of study population

age group in years	Males & percentage	Females & percentage	Total no. of cases and percentage
0-10	1(2%)	0	1(2%)
11-20	5(10%)	4(8%)	9(18%)
21-30	8(16%)	9(18%)	17(34%)
31-40	7(14%)	3(6%)	10(20%)
41-50	5(10%)	2(4%)	7(14%)
51-60	2(4%)	2(4%)	4(8%)
61-70	0	1(2%)	1(2%)
71-80	1(2%)	0	1(2%)
TOTAL	29(58%)	21(42%)	50(100%)

were more commonly affected than females 21(42%) (Table 1). Clinical pattern of leprosy was as follows: TT- 3 cases (6%), BT- 21 cases (42%), BB- 3 cases (6%), BL- 13 cases (26%), LL- 10 cases (20%) Majority of the patients belong to clinical type Borderline Tuberculoid i.e; 21 cases (42%). Borderline Tuberculoid leprosy was the most common type in both males (34.48%) and females (52.38%). Majority (52%) of the cases belonged to multibacillary group. Majority (54%) of the cases were smear negative (Table 2).

Correlation of clinical and histopathological features

On correlating clinical cases with the histopathology of skin lesions, we found that out of three patients who had been clinically diagnosed as TT, only one showed epithelioid cell granuloma. The other two patients showed a few lymphocytes. Among 21 BT patients, 11 cases showed lymphocytes and epithelioid cell transformation. 3 cases showed well defined epithelioid granulomas. The remaining showed a nonspecific infiltrate. Out of 3 mid borderline cases, one case showed histological correlation with lymphocytic infiltrate and loosely arranged granulomas. Out of 13 BL patients, five patients showed a mixture of foamy macrophages, histiocytes, and lymphocytes. Five out of eight LL patients showed foamy macrophages with a few lymphocytes. Both of the cases which were

Table 2 : Distribution of study population according to pattern of leprosy and gender

Type of leprosy	Males	Females	Number of cases and percentage
Tuberculoid	2(6.9%)	1(4.76%)	3(6%)
Borderline tuberculoid	10(34.48%)	11(52.38%)	21(42%)
Mid borderline	2(6.9%)	1(4.76%)	3(6%)
Borderline lepromatous	8(27.6%)	5(23.8%)	13(26%)
Lepromatous	7(24.14%)	3(14.28%)	10(20%)
Total	29(58%)	21(42%)	50(100%)

Table 3 : Clinical and histological correlation of the leprosy patients

clinical diagnosis	no.of cases	Histopathology						
		TT	BT	BB	BL	LL	histoid	chronic nonspecific inflammation
TT	3	1	2					
BT	21	3	11					7
BB	3		2	1				
BL	13		1	1	5	3		3
LL	8				2	5		1
Histoid	2						2	

*TT - Tuberculoid leprosy, BT-Borderline tuberculoid leprosy, BB- Mid borderline leprosy, BL- Borderline lepromatous leprosy, LL- Lepromatous leprosy.

Table 4 : Cytohistological correlation of the study population

Cytology		Histopathology					
	No. of cases	Chronic inflammation	TT	BT	BB	BL	LL	Histoid
Unsatisfactory smears	20	9	1	3	1	3	3	
Satisfactory smears	30							
TT	4		2	2				
BT	16	1	1	14				
BB	0							
BL	3					2	1	
LL	5					1	4	
Histoid	2							2
TOTAL	**50**							

*TT- Tuberculoid leprosy, BT-Borderline tuberculoid leprosy, BB- Mid borderline leprosy, BL- Borderline lepromatous leprosy, LL-Lepromatous leprosy.

Table 5 : Clinico-cytological correlation of satisfactory FNAC smears

Spectrum	Clinical Diagnosis	FNAC Diagnosis	Correlation %
TT	1	1	100
BT	14	11	78.57
BB	3	0	0
BL	3	1	33.33
LL	7	5	71.43
Histoid	2	2	100
Total	30	20	

*TT- Tuberculoid leprosy, BT-Borderline tuberculoid leprosy, BB- Mid borderline leprosy, BL- Borderline lepromatous leprosy, LL-Lepromatous leprosy.

clinically diagnosed as histoid leprosy showed elongated spindle cells surrounded by a pseudo-capsule. The clinicohistopathological correlation was found to be the highest for Histoid leprosy (100%), followed by LL(62.5%), BT (52.38%), BL(38.46%), TT(33.33%) and BB (33.33%) (Table 3).

Correlation of cytology with histological diagnosis

Of the 50 cases which were aspirated, 30 aspirates were satisfactory. The remaining 20 showed blood, skin appendage cells with or without chronic inflammatory cells. The cytohistological correlation is maximum for histoid leprosy (100%)

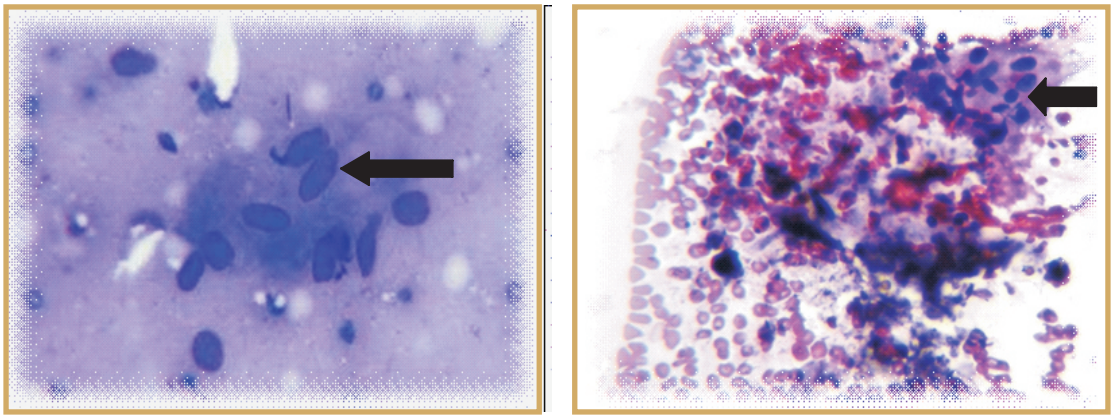

Fig 1 : FNAC smears showing epithelioid cell clusters in TT leprosy (MGG stain, 100X)

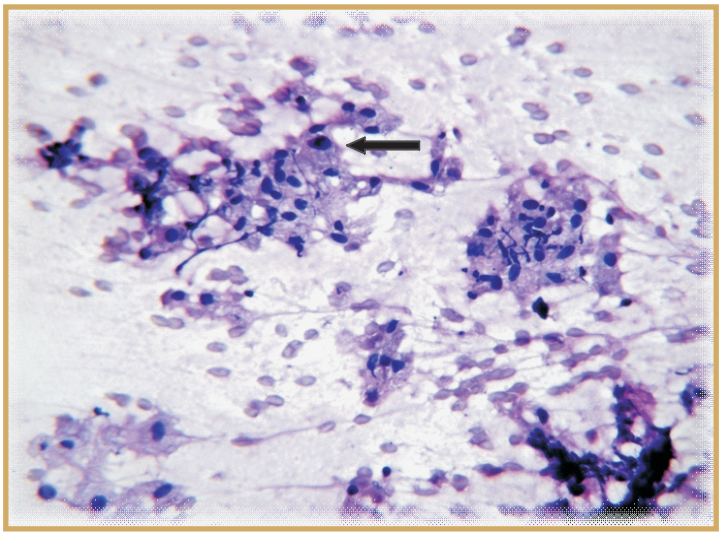

Fig 2 : FNAC smear from lesion of Borderline tuberculoid type of leprosy showing clusters of histiocytes admixed with lymphocytes (MGG stain, 100X)

followed by BT (87.5%), LL (80%), BL (66.66%), TT (50%) (Table 4).

Correlation of clinical diagnosis with features of FNAC

The clinic-cytological correlation is maximum for histoid leprosy and TT (100%) followed by BT (78.57%), LL (71.43%) and BL (33.33%) (Table 5).

Comparing clinical and FNAC features, in TT leprosy epithelioid cell clusters were seen (Fig. 1). Among 14 cases of BT leprosy, 11 cases showed epithelioid cells in addition to lymphocytes (Fig. 2). Three cases showed predominantly epithelioid cells. Of the 3 cases of BL leprosy, one case showed predominantly foamy macrophages

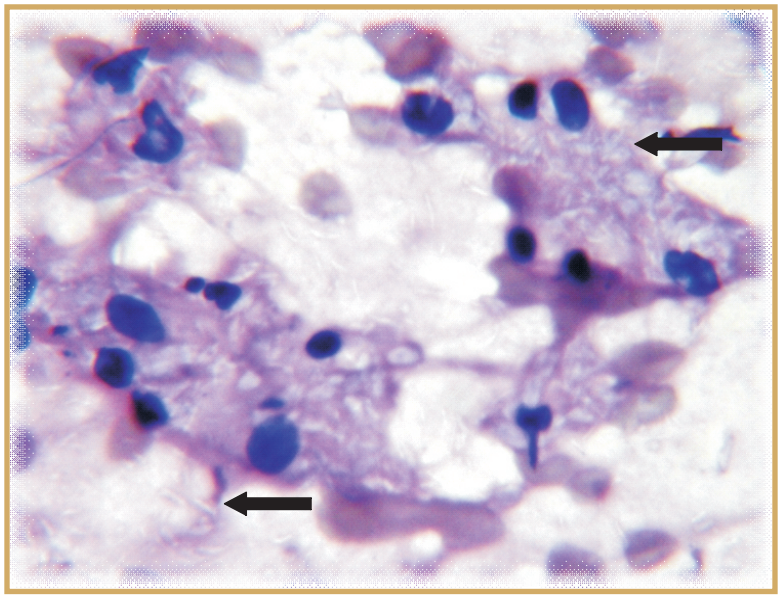

Fig 3 : FNAC Aspirate from a skin nodule in a lepromatous leprosy, showing histiocytes with negative images (corresponding to the unstained lepra bacilli) in the background (May-Grunwald-Giemsa stain, 100x).

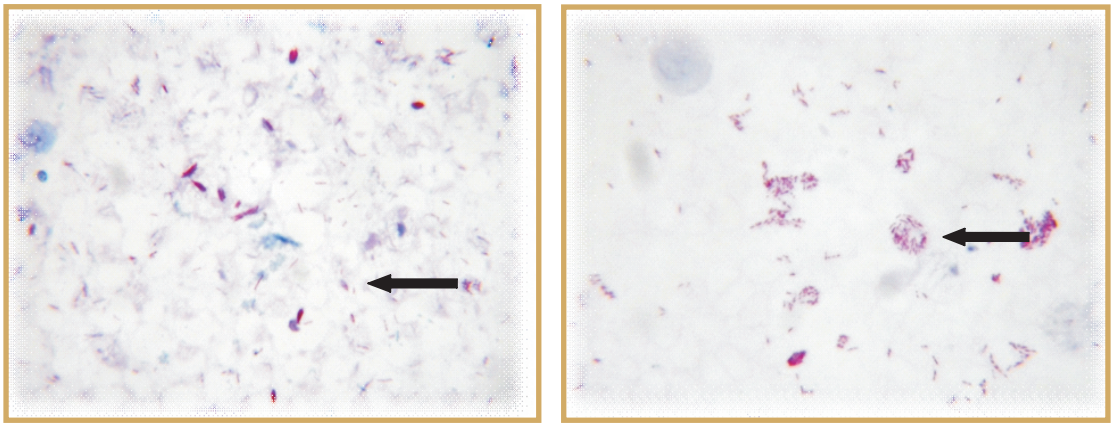

Fig 4 : FNAC smears showing *lepra bacilli* arranged individually and in clusters (Haematoxillin and eosin stain, 100x)

with a few lymphocytes. Out of seven cases of LL, five cases showed foamy macrophages and a few lymphocytes and the remaining showed only a few lymphocytes. In lepromatous leprosy, skin aspirate from nodule showed histiocytes and multiple unstained bacilli in the form of negative

images (Fig. 3). *M. leprae* in FNAC were present as dispersed or in clusters (Fig. 4). This finding was also reported by Rao et al (2001). Both the histoid leprosy patients showed elongated, spindle-shaped cells along with scattered lymphocytes; Fite's stain was strongly positive. Sensitivity of FNAC in diagnosing leprosy was in the following order BT (87.5%) >LL (80%) >TT=BL (66.67%).

Discussion

Histopathological means of diagnosis in leprosy is considered to be gold standard for diagnosis and classification. However, clinical and histology may not correlate in a section of cases (Jha and Karki 2010). Further , there is a growing need for a simpler, quicker and a non/ less invasive modality which provides an equally accurate diagnosis. Fine needle aspiration cytology fulfils all these needs and is a relatively non invasive method. We have attempted to use this method in diagnosing leprosy and determine its validity.

In the present study, various types of skin lesions such as macules, infiltrated papules, plaques, and nodules were observed. Out of 50 cases subjected to FNAC, 30 aspirates were satisfactory and in the rest of the cases (20), the smears showed blood elements only and adequate diagnostic material for cytological interpretation was not obtained. These 20 cases, were diagnosed clinically as follows: 2(TT), 5(BT), 2(BB), 5(BL), 6(LL). On histopathology, 9 cases showed nonspecific inflammation, 1 (TT), 3 (BT), 1 (BB), 3 (BL), 3 (LL).

Leprosy cases can occur at any age. In our study commonly affected age group was 21-30 years. Similar trend was seen in other studies by (Jindal et al 2009, Bijjaragi et al 2012). Leprosy affects both sexes, however males are affected more often than females, generally in the proportion of 2:1 (Thorat and Pankaj 2010). In the present study there was preponderance of male cases (58%, Table 2). This is also as reported earlier by others (Jindal et al 2009, Bijjaragi et al 2012, Sharma et al 2004).

This preponderance in males may be due to social, economic, health seeking behavior, biological reasons and also attitude of society, methods of case detection, due to operational factors like preponderance of male health staff etc. Other factors attributed to finding more cases among males are industrialization, urbanization, greater mobility and increased opportunity for contact. Males are also active in reporting to health facility for seeking treatment (Thorat and Pankaj 2010, Richard et al 2010). In our study we found more male cases because of greater health seeking behavior, social and economic factors and a few of them were detected by male health staff.

In the present study majority of patients (74%) belonged to borderline group (BT, BB, BL) which is similar to and as reported by Giridhar et al (2012).

As the borderline lesions are more apparent on the skin, patients tend to self report. Decreased proportion of cases in early polar form of disease may be due to increase of herd immunity in the community or perhaps patients still tend to hide their unapparent/early lesions due to fear and stigma (Arora et al 2008).

Correlation of clinical with histopathological diagnoses was 33.33 - 100% in various types of leprosy in the present study. Maximum positive and correct correlation was observed in histoid leprosy (100%) which is similar to as reported by Prasad et al (2008).

The cytohistological correlation in tuberculoid pole of the disease is similar to that of lepromatous pole in the present study which is slightly lesser than the findings in Rao et al (2001). Clinico cytological correlation with FNAC samples in the present study varies from 33.33-100%. Maximum correlation was observed in histoid leprosy (100%) which is similar to study by Prasad

et al (2008). Other spectra of the disease showed lesser correlation. This may be due to larger number of patients having macular lesions which are common in India which are usually characterized by scanty inflammatory infiltrate (Singh et al 1996). We observed macular lesions in 46% (23 patients) of our cases which is higher than 42.5% found in Prasad et al. study. FNAC was quiet difficult to obtain in these cases and we could obtain adequate aspirates in only 9 cases. This difficulty in obtaining aspirates from macular leprosy was also reported in earlier study by Singh et al (1996). We found highest sensitivity in BT leprosy. Specificity was almost equally high in BL and LL leprosy. Positive predictive value was highest for BT whereas negative predictive value was highest for BL leprosy. This result of our study practically means that the number of detecting truly positive cases and excluding truly negative cases by using FNAC technique are highest in BT and lepromatous patients respectively.

In the present study, there is a fairly good correlation between clinical, histological and cytomorphological features in the aspirates taken from the skin lesions. A reasonably good sensitivity, specificity, positive and negative predictive values was obtained in all types of leprosy in the Ridley-Jopling system of classification except for mid borderline spectrum.

FNAC is a simple, easy, cost effective, relatively non invasive procedure which provides quicker results than conventional biopsy technique. FNAC can be used to supplement histopathological diagnosis. In endemic areas where histopathological services are not available, cytological assessment of the lesions can be used for quick definitive diagnosis of leprosy. It can also be a useful method in patients who refuse biopsy especially when the lesions involve the face due to fear of scarring. Drawbacks of this procedure include- "dry taps" and bloody smears with lack of cellular infiltrate in macular leprosy lesions.

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