

## Fine needle aspiration cytology in leprosy

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### ABSTRACT

**Background:** Laboratory diagnosis of leprosy by slit skin smear and skin biopsy is simple but both techniques have their own limitations. Slit skin smear is negative in paucibacillary cases whereas skin biopsy is an invasive technique. Fine needle aspiration cytology (FNAC) from skin lesions in leprosy with subsequent staining with May-Grunwald-Giemsa (MGG) stain has been found useful. **Aim:** To evaluate the possible role of cytology in classifying leprosy patients. **Methods:** Seventy-five untreated cases of leprosy attending the outpatient department were evaluated. Smears were taken from their skin lesions and stained using the MGG technique. Skin biopsy was also done from the lesions, which was compared with cytology smears. **Results:** A correlation of clinical features with FNAC was noticed in 87.5% of TT, 92.1% of BT, 81% of BL, and 66% of LL cases. Correlation of clinical with histopathological diagnoses revealed 12.5% specificity in TT leprosy, 55.3% in BT, 52.4% in BL and 50% in LL, and 100% in neuritic and histoid leprosy cases. Both correlations were found to be statistically significant by paired t test analysis. Thus, it was possible to distinguish the tuberculoid types by the presence of epithelioid cells and the lepromatous types by the presence of lymphocytes and foamy macrophages. **Conclusion:** FNAC may be used to categorize the patients into paucibacillary and multibacillary types, but is not a very sensitive tool to classify the patients across the Ridley-Jopling spectrum.

**Key Words:** Fine needle aspiration cytology, Hansen's disease, Leprosy, *Mycobacterium leprae*, Skin biopsy

### INTRODUCTION

The Ridley-Jopling (RJ) classification currently in use for classifying leprosy is based on widely acknowledged clinical, bacteriological, immunological, and histological parameters.<sup>[1-4]</sup> It divides the leprosy spectrum into 'five' clinically and histologically recognizable groups. Application of the RJ scale in the classification of leprosy helps in understanding the immunology of the patient to predict prognosis and possible complications. But, histopathological investigation is an invasive procedure and leads to a biopsy scar, which may not be cosmetically acceptable. Slit skin smear technique stained with Ziehl-Neelsen (ZN) is considered as a simple field procedure for the diagnosis of leprosy. However, its application is limited to the determination of the presence or absence of acid fast bacilli (AFB).

Fine needle aspiration cytology (FNAC) is a safe and noninvasive procedure. The first recorded utilization of this technique is available from St. Bartholomew's hospital in the year 1833. Later, Sir James Paget widely used this technique.<sup>[5]</sup> In 1989, Ridley used ZN stain to study the nature of the exudates and to assess their cytology.<sup>[6]</sup> In 1994, Singh *et al*, used FNAC to diagnose a case of nodular lepromatous leprosy<sup>[7]</sup> and later used the same technique in 30 leprosy cases.<sup>[8]</sup> We undertook the present study to evaluate the possible utility of cytology in classifying lesions of leprosy on the R-J scale.

### METHODS

This study included all new leprosy patients attending the outpatient department of the Rajah Muthiah Medical College Hospital during a one-year period. Two independent

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assessors, including the investigator, confirmed the clinical diagnosis. Skin lesions were examined for their number, size, shape, margin, erythema, border, infiltration, dryness, and loss of hair and sensation. Similarly, all peripheral and cutaneous nerves were palpated for their number, size, nodularity, abscess formation, and tenderness. These findings were entered in a chart. Patients were classified according to RJ criteria into tuberculoid (TT), borderline tuberculoid (BT), mid-borderline (BB), borderline lepromatous (BL), and lepromatous (LL) types. Slit skin smears were done for all patients followed by staining using the Z-N technique by a microbiologist. Skin biopsy was performed in all patients and two pathologists reported them independently. Histological classification of leprosy was based on standard histopathological criteria defined by Ridley.<sup>[9]</sup> This involved the evaluation of cell types composing the granulomas, *i.e.*, epithelioid cell or macrophage, cellular infiltrate containing lymphocytes, and bacterial load in Fite's stain.

FNAC was done from skin lesions after obtaining informed consent. The site was cleaned with alcohol and an assistant pinched the skin for 30 seconds to blanch the skin, as done in the slit skin smear technique. A 20 mL syringe was fitted with a 21-gauge needle and the assistant created negative pressure by holding back the piston with the forefinger and index finger of the right hand. The aspirated material was transferred onto glass slides. The flat surface of another slide was used to smear the material. The smears were air-dried and stained with May-Grunwald-Giemsa (MGG) stain. Cytological smears were coded and independently assessed by two pathologists who were not informed about the classification of the patients according to the R-J scale. Cytological specimens were considered adequate if there was a cellular yield of inflammatory cells. We followed the

criteria laid down by Singh *et al.*, in reporting the cytology smears after adopting a few modifications<sup>[8]</sup> given in Table 1. The results were analyzed using t test.

## RESULTS

One hundred five cases out of a total of 21,850 patients who attended the outpatient department during the study period were new leprosy cases. Out of these, 75 patients, who were willing to undergo the study procedures, were selected for the study. The total number of leprosy cases comprised 0.48% of the dermatology outpatient population. Their ages were 4-72 years with a mean age of 31.2 years. Most patients belonged to the 21-40 years' age group. There were 53 males including three male children and 22 females including three female children; the male: female ratio was 2.4:1. The shortest duration of the disease was one month in fourteen patients whereas the longest duration was ten years in a tuberculoid leprosy patient. The correlation of classification of the patients using clinical features with the FNAC findings and histopathological findings is shown in Tables 2-5.

### Correlation of clinical diagnosis with features of FNAC

Comparing clinical and FNAC features revealed that out of eight cases of TT leprosy, seven cases (87.5%) fulfilled the criteria while epithelioid cells were also seen in one case. Among 38 cases of BT leprosy, 32 cases (92.1%) showed epithelioid cells in addition to lymphocytes. Three cases showed predominantly epithelioid cells while the rest showed nonspecific changes.

Of the 21 cases of BL leprosy, 13 cases (81%) showed many lymphocytes and a few foamy macrophages without

**Table 1: Fine needle aspiration cytology diagnostic criteria by Singh *et al.*,<sup>[8]</sup> compared with modified criteria by the authors**

TT	TL including BT: cellular smears, cohesive epithelioid cell granulomas, numerous lymphocytes not infiltrating the granuloma, no stainable AFB	Cellular material with predominantly lymphocyte population and histiocytes without epithelioid transformation. No stainable AFB
BT	Same as TT	Cellular material with lymphocytes, histiocytes and epithelioid cells. Foamy macrophages are not a feature. No stainable AFB
BL	Moderate cellularity, singly dispersed macrophages with no epithelioid cells. Numerous lymphocytes diffusely scattered along with macrophages. BI 3-4+	Moderate cellularity, singly dispersed macrophages with no epithelioid cells. Numerous lymphocytes diffusely scattered along with macrophages. BI 3-4+
LL	Heavy cellularity, numerous foamy macrophages in fatty background with a few lymphocytes. BI 5-6+	Heavy cellularity, numerous foamy macrophages in fatty background with a few lymphocytes. BI 5-6+
Histioid leprosy	-	Cellular yield, elongated spindle cells, scattered lymphocytes. BI 6+
Neuritic leprosy	-	Variable cellular material, presence of lymphocytes, and occasional histiocytes, neural tissue and histiocytes surrounding adipose tissue. Variable BI

TT: Tuberculoid, BT: Borderline tuberculoid, BL: Borderline lepromatous, L: Lepromatous

**Table 2: Percentage of clinical diagnosis**

Type of leprosy	n	%
Tuberculoid	8	10.66
Borderline tuberculoid	38	50.66
Borderline lepromatous	21	28.0
Lepromatous	6	8.0
Neuritic	1	1.33
Histoid	1	1.33
Total	75	100

**Table 3: Percentage of histological diagnosis**

Type of leprosy	n	%
Tuberculoid	3	4.0
Borderline tuberculoid	30	40.0
Borderline lepromatous	12	16.0
Lepromatous	7	9.33
Indeterminate	18	24
Neuritic	1	1.33
Histoid	1	1.33
No evidence	3	4.0

**Table 4: Clinical vs fine needle aspiration cytology diagnosis**

	Clinical diagnosis	FNAC diagnosis	Percentage correlation	Comments
TT	8	7	87.5	One case showed epithelioid cell
BT	38	35	92.1	Three patients showed nonspecific changes
BL	21	17	81	Four cases showed mixture of all cells
LL	6	4	66	Two cases showed only a few lymphocytes
Neuritic	1	1	100	-
Histoid	1	1	100	-

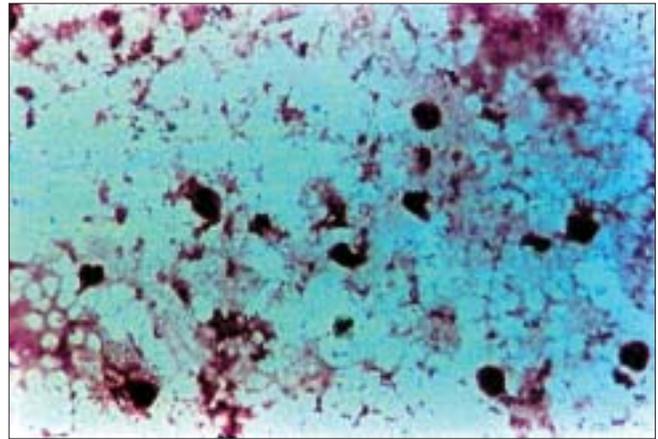
TT: Tuberculoid, BT: Borderline tuberculoid, BL: Borderline lepromatous, L: Lepromatous

epithelioid cells, whereas there were predominantly foamy macrophages with a few lymphocytes in four cases. In four other patients, FNAC revealed a mixture of all cells including a few epithelioid cells. Out of six cases of LL, four cases (66%) showed foamy macrophages and a few lymphocytes [Figure 1] and the remaining showed only a few lymphocytes.

A histoid leprosy patient showed elongated, spindle-shaped cells along with scattered lymphocytes; Fite's stain was strongly positive. A purely neuritic leprosy patient showed scattered lymphocytes along with benign, spindle-shaped cells and adipose tissue in the FNAC from the nerve.

**Correlation of clinical and histopathological features**

On correlating FNAC with the histopathology of skin lesions, we found that out of three patients who had been histopathologically confirmed as TT, only one showed epithelioid cells but no cohesive epithelioid cell granuloma. The second patient showed a few lymphocytes and the third many lymphocytes and histiocytes. Hence, this correlation does not have any statistical significance. Among 30 BT



**Figure 1: FNAC in LL showing foamy macrophages and a few lymphocytes in a fatty background**

**Table 5: Clinical vs histopathological diagnosis**

Clinical spectrum	Number of patients	HPE diagnosis No.	%	Comments
Tuberculoid	8	1	12.5	Five cases were BT, indeterminate in one and normal in one patient
Borderline tuberculoid	38	21	55.3	13 cases were indeterminate, TT in two, BL in one and normal in one patient
Borderline lepromatous	21	11	52.4	BT in three, LL in three, Indeterminate in three and normal in one patient
Lepromatous	6	3	50	BT in one, indeterminate in one and BL in one patient
Neuritic	1	1	100	-
Histoid	1	1	100	-

patients, 50% showed lymphocytes and epithelioid cell transformation. The remaining showed a mixed picture. Out of 12 BL patients, ten patients showed a mixture of foamy macrophages, histiocytes, and lymphocytes. Five out of seven LL patients showed foamy macrophages with a few lymphocytes [Figure 1].

Among the 18 patients diagnosed as having indeterminate leprosy by histopathological examination, 14 patients showed a few lymphocytes with epithelioid cells and histiocytes while a few lymphocytes and macrophages were seen in the remaining cases.

Correlations among clinical and histological diagnoses and FNAC are shown in Table 6, which shows a variation from 12.5-92%.

## DISCUSSION

In our study, various types of skin lesions such as macules, infiltrated papules, plaques, and nodules were observed. Out of 75 biopsies including one nerve biopsy, histological evidence of leprosy was absent in only three cases, which could be due to inadequate representation of the biopsy site.

Correlation of clinical diagnoses with FNAC examination revealed varying results contrary to expectations. We could not observe organized granulomas reported by Singh *et al.*<sup>[8]</sup> We did however, notice a very high correlation between clinical diagnoses and FNAC in all types of leprosy. Singh *et al.*<sup>[8]</sup> did not differentiate between TT and BT leprosy while we noticed organized collections of epithelioid cells in one case, which was highly consistent with BT. Correlation was also high in BL and LL types where there were scanty cellular infiltrates and more foamy macrophages. Macular lesions are common in leprosy cases in India. We also observed macules in 42.5% of our study cases, which could be responsible for the poor cellularity observed in cytology. This was also observed in the earlier study by Singh *et al.*<sup>[8]</sup>

**Table 6: Clinical vs fine needle aspiration cytology vs histopathology**

Type	n	FNAC	%	HPE	%
TT	8	7	87.5	1	12.5
BT	38	35	92.1	21	55.3
BL	21	17	80.9	11	52.4
LL	6	4	66.7	3	50
Neuritic	1	1	100	1	100
Histoid	1	1	100	1	100

TT: Tuberculoid, BT: Borderline tuberculoid, BL: Borderline lepromatous, L: Lepromatous, HPE: Histopathology

Thus, it was possible to distinguish tuberculoid types by the presence of epithelioid cells and lepromatous types by the presence of lymphocytes and foamy macrophages.

Ridley<sup>[6]</sup> examined cellular exudates in slit skin smears by the Z-N technique for AFB and observed that this generated more information on the immune status of the patient than the estimation of BI and MI alone. However, Z-N staining does not provide morphological details comparable to MGG.<sup>[8]</sup>

Singh *et al.* opined that cytological features of LL showed negative images of *M. leprae* on MGG-stained smears, which were later confirmed by AFB staining,<sup>[8]</sup> but not observed in the present study. On FNAC, we could not appreciate cohesive epithelioid cell granulomas with lymphocytes in tuberculoid spectrum. Lymphocytes form a major part of smears in the borderline types of the disease. In this study also, we found the largest number of lymphocytes in BL leprosy. Fite's stain from FNAC was also positive in one case of 'histioid' leprosy, which should stimulate interest for future studies. Application of histological criteria to cytological smears did not give us the expected results observed by others,<sup>[8]</sup> hence, we could not agree that the diagnosis and classification of leprosy were simplified by cytology.

Correlation of clinical with histopathological diagnoses was 12.5-50% in various types of leprosy in our study but 60-80% in earlier studies.<sup>[9,10]</sup> Niranjana Murthy *et al.*, observed a maximum correlation in BL and LL cases,<sup>[10]</sup> whereas this was only true in BT, BL, and LL cases in our study. Eighteen cases (24%) were histologically diagnosed as indeterminate and were clinically classified into various types depending on the number and type of skin lesions, which could explain the difference in the results in our study. Recently, in a comparative evaluation of skin and nerve histopathology in single skin lesion leprosy in 27 patients, the clinicopathological correlation was found to be positive in 51.85% of skin biopsy specimens.<sup>[11]</sup>

As we have only adopted the R-J scale for classification, indeterminate cases did not form a part of our clinical classification. However, indeterminate cases were diagnosed histologically in 18 cases, out of which 14 patients showed evidence of a tuberculoid spectrum and four cases showed a lepromatous spectrum by FNAC. This finding correlated with clinical diagnoses.

From this study, we conclude that FNAC only supplements

histopathological diagnosis in some cases and can not be taken as a sensitive investigation for the diagnosis of leprosy. However, it has excellent value in patients who are not willing to undergo a biopsy and also serves to classify the patients quickly into pauci- or multibacillary types while awaiting the final pathology results. FNAC is a simple, cost-effective method of investigation that is more useful in TT and LL patients.

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