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Lesional fine needle aspiration (FNA) cytology in reactional and non-reactional leprosy patients

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Abstract
Fine needle aspiration cytology (FNAC) is a relatively simpler tool as compared to histopathology for the evaluation of the cytomorphology of skin lesions. Fifty consecutive leprosy patients diagnosed and classified according to Ridley Jopling scale1 were taken to study the cytomorphology of leprosy lesions in fine needle aspirates and correlate it with their histopathology by a skin biopsy from the same site for histopathological examination after H/E staining. Borderline leprosy patients with Type I reaction showed significantly more numbers of giant cells, collagen and elastin in their smears as compared to those without reaction. The smears were more heavily cellular with fragmented collagen and elastin along with significant increase in neutrophils in patients with Type II reaction while foamy macrophages with fatty background were common in non-reactional lepromatous leprosy patients. A complete correlation between histopathological and cytomorphological findings was observed in 79.4% of cases.

Keywords: Fine needle aspiration cytology, cytomorphology, histopathology, leprosy, reactions

Introduction
Cytology is a widely accepted diagnostic procedure for a large variety of inflammatory, benign and malignant conditions. Skin-slit smears stained for acid-fast bacilli (AFB) have been conventionally used for the assessment of leprosy lesions but their use has been restricted to the evaluation of the bacterial index and morphological index. Ridley emphasized the interpretation of cellular exudate in conventionally stained Ziehl-Neelsen smears [2]. Singh *et al.* [3] studied the cytomorphology of the cellular exudates in fine needle aspirates (FNA) of leprosy lesions and could sub-classify them on the same position on the Ridley Jopling (R-J) scale as seen in histology. It was also observed that FNA smears, in contrast to slit-skin smears, were free of confounding epidermal squamous cells and therefore better suited for evaluating cell morphology. The current study was undertaken to evaluate the utility of FNA in reactional and non-reactional leprosy lesions.

Materials and methods
Fifty patients attending the skin and leprosy clinic were studied. Their diagnosis and classification in the R-J scale [1] were based on the clinical examination using standard criteria. Fine needle aspirations were done on the representative lesions followed by a skin biopsy from the same site for histopathological examination. Biopsies were evaluated and classified according to the histological criteria established by Lucas and Ridley [4] and the World Health Organization [5]. Fine needle aspiration smears were specially evaluated for cellularity, morphological details of macrophages, accompanying inflammatory cells and bacterial index. All smears were air-dried and stained with May-Grunwald-Giemsa (MGG) stain and modified Ziehl-Neelsen's stain [5]. Skin tissue for histopathology was obtained in 34 patients and stained with H and E stain. Cytological criteria for sub-classification of leprosy were applied as defined by Singh *et al.* [2] these are as follows:

1. Tuberculoid leprosy (including TT and BT)
a. Cellular smears
b. Cohesive epithelioid cell granulomas
c. Numerous lymphocytes not infiltrating the granuloma
d. No stainable AFB (BI=0)

2. Mid-borderline leprosy (BB)
a. Fair cellular yield
b. Poorly cohesive granulomas composed of an admixture of epithelioid cells and macrophages
c. Few lymphocytes infiltrating the granulomas
d. BI = 1+ to 2+

3. Borderline lepromatous leprosy (BL)
a. Moderate cellularity
b. Singly dispersed macrophages with 'negative images'; no epithelioid cells
c. Numerous lymphocytes diffusely admixed with macrophages
d. BI = 3+ to 4+

4. Lepromatous leprosy (LL)
a. Heavy cellularity
b. Numerous foamy macrophages in a fatty background with intracellular and extracellular negative images
c. Few lymphocytes
d. BI = 5+ to 6+ (globi)

5. Reaction
Numerous fragmented AFB (MI< 1) and neutrophils suggest a Type II reaction in LL (Erythema nodosum leprosum).

Results
A total of 50 patients (34 males and 16 females) were studied. Their age ranged from seven years to 54 years (mean 31.7 ± 3.23 years). The duration of leprosy ranged from one month totwo years (mean 1.4± 0.8 years). Three patients had indeterminate leprosy (I), eight patients had tuberculoid leprosy (TT), 13 patients had borderline tuberculoid leprosy (BT), 6 patients had mid-borderline leprosy (BB), 12 patients had borderline lepromatous leprosy (BL) and 8 patients had lepromatous leprosy (LL). Of these, 11 patients presented with a lepra reaction (seven with a Type 1 lepra reaction and four patients with a Type 2 lepra reaction). The histopathological study of the tissue confirmed the clinical diagnosis in all the 31 patients except in three patients in whom the histopathological picture was nonspecific.
Patients with tuberculoid and borderline tuberculoid leprosy showed moderate to heavily cellular smears, cohesive epithelioid cell granulomas, numerous lymphocytes not infiltrating the granulomas and no stainable AFB. Skin smears of mid-borderline leprosy patients showed fairly cellular smears, poorly cohesive epithelioid cell granulomas composed of an admixture of epithelioid cells and macrophages and a few lymphocytes infiltrating the granuloma. Borderline leprosy patients were characterized by moderately cellular smears, singly dispersed macrophages with 'negative' images, no epithelioid cells and numerous lymphocytes diffusely admixed with macrophages. FNA in lepromatous leprosy patients showed heavy cellularity, foamy macrophages, fatty background, neutrophils, lymphocytes, collagen and elastin and stainable AFB in all the smears.
The smears of patients with reactional leprosy were highly cellular with foamy macrophages and 'negative' images, neutrophilic infiltrate, degenerating collagen and elastin, a few lymphocytes and a fatty background (Table – 1, 2). Among seven patients with a Type 1 lepra reaction (BT, four; BB, two; and BL, one), neuritis was present in four; none of them had a nerve abscess. All the patients presented with exacerbation of erythema and inflammation over existing lesions and appearance of a few new lesions. The cytomorphological features in five of the seven clinically diagnosed patients with type 1 reaction were confirmed by FNAC of skin aspirates using the standard cytological criteria described by Ridley and Radia [7]. The remaining two patients showed nonspecific cytological features. All the borderline group patients (BT, BB and BL) had heavily cellular smears, lymphocytes, neutrophils and edematous background. However, loose epithelioid cell granulomas and giant cells, collagen, elastin and proliferating fibroblasts were present in a significantly large number in smears of borderline leprosy patients with a Type 1 lepra reaction. The cytological features of Type 2 lepra reaction were present in three of the four patients with ENL. The histopathological examination of the skin biopsy showed evidence of ENL in all of them except one. The smears were more heavily cellular with fragmented collagen and elastin and a significant increase in neutrophils in patients with a Type 2 lepra reaction, while foamy macrophages with fatty background were relatively more commonly seen in non-reactional lepromatous leprosy patients.

Table 1: Cytomorphological features on skin aspirates of borderline leprosy patients with and without Type I reaction

Cytomorphology	Borderline leprosy patients (BT,BB,BL)	
	Without Reaction (n = 31)	With Reaction (n = 7)
Heavy cellularity	15	05
Loose granuloma	03	06
Compact granuloma	19	01
Plenty lymphocytes	03	04
Giant cells	02	05*
Collagen & elastin	04	06*
Edema	02	03
Neutrophils	02	02
Fibroblasts	01	02
Foamy macrophages	03	04
Acid fast bacilli positivity	01	02

Table 2: Cytomorphological features on skin aspirates of lepromatous leprosy patients with and without Type II reaction

Cytomorphology	Lepromatous leprosy patients (LL)	
	Without Reaction (n = 4)	With Reaction (n = 4)
Heavy cellularity	4	3
Foamy macrophages	4	4
Neutrophils	1	2
Lymphocytes	2	2
Blood vessels	-	1
Collagen& elastin	1	2
Fatty ground tissue	2	3
Acid fast bacilli	3	3

Discussion

Ridley and Jopling [1] proposed a histological classification of leprosy reflecting the immunological spectrum of the disease. It divides leprosy into five clinically and histologically recognizable groups based on the distribution of epithelioid cells, Langhans giant cells, foam cells, globi, lymphocytes, plasma cells, fibroblasts, a clear sub-epidermal zone, cellular cuffing and infiltration of nerves. The histopathological criteria for sub-classification of leprosy as defined by Ridley and Jopling have been applied to cytological smears by Singh *et al*. [4] they correlated and succeded to place most of their patients in the same group as on histopathology.

The material collected by FNA is in general highly cellular, which in most cases allows the diagnosis of diseases without the need for a biopsy [9]. FNA also allows the performance of histochemical, immunohistochemical, and molecular techniques for the identification of infectious agents and the searching for oncogenic proteins and specific molecular changes causing disease [9]. The results of FNA reported in the literature are comparable to the results of biopsy [10, 11, 12]. Out of the 34 patients of leprosy in whom histopathology was done, 27 (79.4%) showed correlation of cytomorphological features with clinico-pathological diagnosis. In the remaining 7patients (20.6%) there was poor correlation, possibly because of incorrect site selection. Moreover, this number might have been less if aspirates had been taken from multiple sites, as was observed by Singh *et al*. [2, 8] Similar findings were also observed by Nigam *et al*. [12]

Conclusion/Key Message

It may be concluded that there is a fairly good correlation between the clinical, histological and cytomorphological features of skin aspirates and that it is possible to classify leprosy along the R-J scale based on cytological smears. Thus, in endemic areas, especially where histopathological services are not available, cytomorphological assessment of leprosy lesions may be advocated as a simple tool for evaluation of the primary diagnosis and natural course of the disease.

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