Original Article

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Diagnostic Utility of Fine Needle Aspiration Cytology of Sensory Cutaneous Nerve in Leprosy

Naik Reena[1], Sa Dilip Kumar[2], Panda Kishori M[1]*

[1]Dept. of Pathology, Govt Medical College (LSLAMMC), Raigarh, Chhattisgarh. India
[2]Dept of Skin &VD , Govt Medical College(LSLAMMC), Raigarh, Chhattisgarh, India

ABSTRACT

Background: Peripheral neuropathy is a central feature of leprosy. Intraneural inflammation caused by M. leprae is the morphological hallmark of this disease, FNAC of sensory cutaneous nerve has proved to be a valuable diagnostic tool.

Methods: The data of patients with sensory cutaneous nerve involvement were retrieved from our record for the period from Nov 2014 to Sept 2015. The hematoxylin and eosin (H and E)- and May-Grünwald-Giemsa (MGG) stained slides were screened for Schwann cells, granuloma, and necrosis. Modified Ziehl-Neelsen (ZN) stained smears were searched for single lepra bacilli and globi.

Results: Twenty-five sensory cutaneous nerves were aspirated. Out of which 19 yielded diagnostic aspirate. Five cytologic pictures were seen - epithelioid cell granulomas (6), epithelioid cell granulomas with necrosis (3);necrosis + lepra bacilli (4); only lepra bacilli (2); and lymphocyte & macrophage infiltrate (4).Following the Ridley-Jopling classification, in our study there were 9 cases of TT, 4 of BT-TT, 1 of BB, 2 of BL, and 3 of LL.

Conclusion: FNAC of sensory cutaneous nerve is useful in diagnosis and classification of leprosy on the R-J scale.

Keywords: *Peripheral Neuropathy, Fine-Needle Aspiration Cytology, Hansen's Disease, Lepra Bacilli, Sensory Cutaneous Nerve.*

Introduction

Leprosy is a chronic granulomatous infection caused by Mycobacterium leprae, affecting the skin and peripheral nerves and resulting in disabling deformities.[1] Pure neuritic leprosy (PNL) is a type of leprosy, which is clinically limited to peripheral nerves and constitutes 4-8% of all leprosy. [2].The clinical features of leprosy include anesthetic skin lesions, nerve enlargement, tenderness, pain and sensory motor impairment. These are not specific and may not always be present. So diagnosis remains difficult in early stages of leprosy including PNL cases. It is recommended that sensory cutaneous nerve fine needle aspiration cytology (FNAC) is a feasible, viable, effective, and relatively "nerve sparing" procedure, which can be done routinely as an outdoor procedure in the evaluation of leprosy patients.[3] Only a few studies have evaluated the role of fine needle aspiration cytology (FNAC) in the diagnosis of leprosy, especially in PNL cases.[2] This study was undertaken to evaluate the diagnostic role of nerve FNAC in leprosy and to evaluate the possible utility of cytology in classifying lesions of leprosy on the R-J scale.

Materials and methods

This retrospective study included leprosy patients attending the outpatient department of our medical college hospital during one-year period from Nov 2014 to Sept 2015. FNAC was done from the enlarged thickened nerve in 25 cases, where diagnosis of leprosy was suspected clinically including relapse cases. All peripheral and cutaneous nerves were palpated for their number, size, nodularity and tenderness. These findings were entered in a chart. The cases were examined for most prominent site of thickened nerve. The area was cleaned with an alcohol swab. The prominent part of nerve was fixed by index finger and thumb of left hand and FNAC was done using 22 G needle fitted in 10 mL disposable plastic syringe. The suction was applied and aspiration was performed using a single-puncture. The direction of the needle was always kept parallel to the length of the nerve so as to cause minimal damage to the nerve. The material aspirated was smeared on glass slides. Minimum three smears were made for each case. The wet smear was fixed in 95% ethanol and stained by Papanicolaou stain after 30 minutes of fixation. One of the dried smear was stained by Giemsa stain and the other dried smear was stained by modified acid fast bacilli (AFB for Lepra) stain demonstrate AFB. Both Papanicolaou and Giemsa stained smears were examined for cytological details. Smear stained by modified AFB (Lepra) stain was examined for the presence or absence of AFB. If the AFB was seen, it was quantified according to the presence of their number per high-power field. It was denoted as present (+), if occasional bacilli was seen after searching many high-

power fields, and many (++) if many bacilli per high-power field were seen. Negative finding was denoted as absent (−). Cytological criteria for sub-classification of leprosy were applied as defined by Singh et al (table 1).[4] Patients were classified according to RJ criteria into tuberculoid (TT), borderline tuberculoid (BT), mid-borderline (BB), borderline lepromatous (BL), and lepromatous (LL) types.

Results

Out of total 25 cases of FNAC done from sensory cutaneous nerves 19 cases (76%) yielded diagnostic aspirates. The sensory cutaneous nerves aspirated are detailed in [Table-2]. Most common nerve affected in the present study was right ulnar nerve. But the most common nerve aspirated was right posterior tibial nerve. Mononeuropathy was seen in 6 cases and polyneuropathy was seen in 13 cases. Affected age group was 20 to 61 years [Table 3].

In cytology smears, in order to ascertain if the aspiration was done from a nerve, a search was made for schwann cells. Schwann cells may be present either singly or in fascicles along with other features like granuloma, necrosis, lymphocytic infiltrate and lepra bacilli [Figure1& 2].Five cytomorphologic patterns were observed in smears of nerve aspirates[Table 4].These are inflammation composed of epithelioid cell granulomas (6), epithelioid cell granuloma with necrosis (3); necrosis + lepra bacilli (4); only lepra bacilli (2); and lymphocyte & macrophage infiltrate (4). Following the Ridley-Jopling classification, in our study there were 9 cases of TT, 4 of BT-TT, 1 of BB, 2 of BL, and 3 of LL.

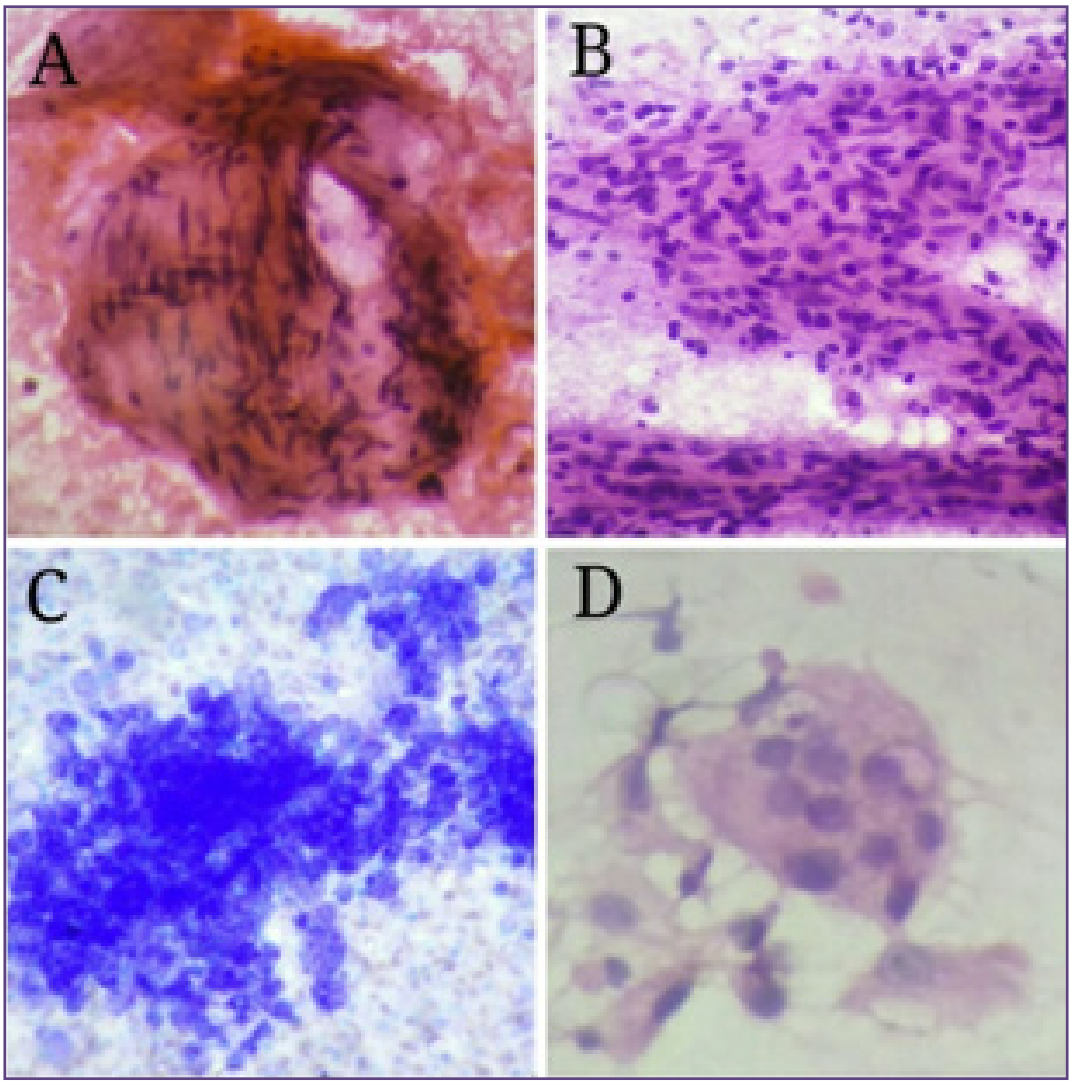

Fig. 1: (A) Showing Schwann cell in fascicle. (B) Epitheloid granuloma. (C) Dense lymphocytic infiltrate. (D) Epitheloid cell with giant cell.

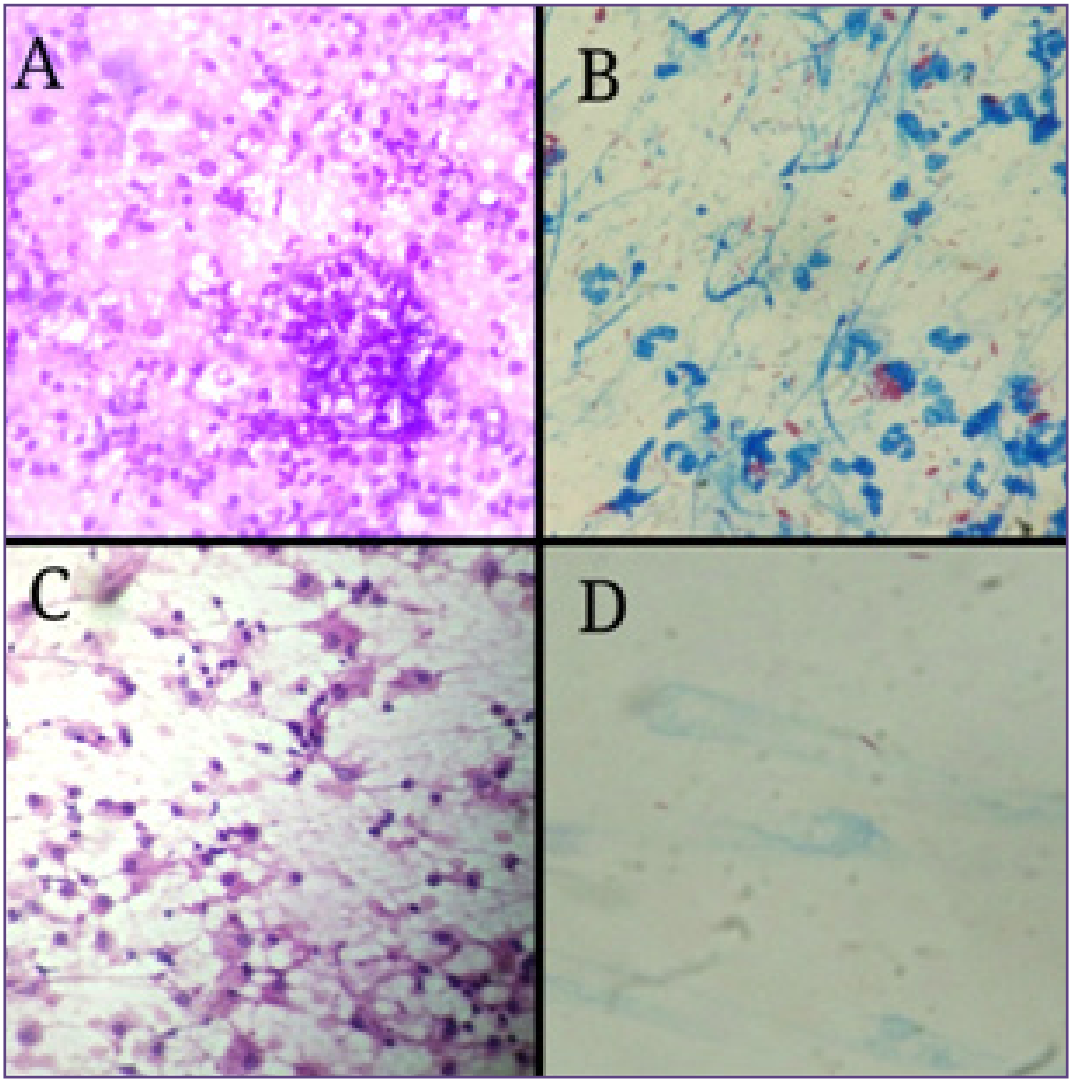

Fig. 2: Microphotographs showing (A) Necrosis. (B) Lepra bacilli in globi. (C) Foamy macrophages. and (D) Singly present lepra bacilli.

Table 1: Cytological criteria (R-J) for sub-classification of leprosy.

Diagnosis	Cellularity	Macrophages	Epitheloid cell granuloma	Lymphocytes	BI
Tuberculoid- Borderline tuberculoid	good	absent	well formed	numerous	0
Mid borderline	moderate	few	diffuse	few	1+,2+
Borderline lepromatous	fair	fair number	absent	numerous	3+,4+
Lepromatous	heavy	foamy	absent	few	5+,6+
Lepra reaction	-	Neutrophil +	-	Neutrophils +	Fragmented

Table 2:Showing details of sensory cutaneous nerve aspirated(n=19)

Sensory Cutaneous nerve aspirated	n
Right posterior tibial nerve	9
Left posterior tibial nerve	1
Right radial nerve	2

Sensory Cutaneous nerve aspirated	n
Left radial nerve	3
Left ulnar nerve	2
Right ulnar nerve	1
Left common peroneal nerve	1
TOTAL	**19**

Table 3: showing clinical details and cytological diagnosis (n=19)

S.No.	Age/Sex	Involment		Site of FNAC	Mono	Poly	Cytological diagnosis
		N	S+N				
1	40/F	+	-	Rt Post tibial	-	+	BT-TT
2	34/F	+	+	Rt Post tibial	-	+	LL
3	33/F	+	-	Lt Post tibial	-	+	BT-TT
4	22/M	+	-	Rt Radial	+	-	BB
5	38/M	+	+	Rt Post Radial	-	+	TT
6	61/M	+	+	Rt Post tibial	-	+	LL
7	20/F	+	+	Rt Post tibial	-	+	TT
8	31/M	+	-	Lt ulnar N	+	-	TT
9	27/M	+	+	Rt ulnar	-	+	LL
10	38/M	+	+	Lt ulnar	-	+	BL
11	42/M	+	+	Rt Post tibial	-	+	TT
12	42/F	+	-	Lt commopero	+	-	TT
13	42/F	+	-	Rt Post tibial	-	+	TT
14	48/M	+	+	Lt radial	+	-	TT-BT
15	25/M	+	+	Rt Post tibial	+	-	TT-BT
16	30/M	+	+	Lt radial	-	+	TT
17	40/M	+	-	Rt post tibial	+	-	TT
18	29/M	+	-	Rt Post tibial	-	+	TT
19	22m	+	+	Left radial	-	+	BL

Table 4: Showing five cytologic patterns.

Cytomorphological picture	n=19
Epithelioid cell granuloma only	6
Granuloma +Necrosis	3
Necrosis+ lepra bacilli	4
Only lepra bacilli	2
Lymphocyte, macrophage infiltration	4

Table 5: Cytomorphological classification of leprosy according to Ridley-Jopling spectrum.

Class	Singh et al. [4] (skin smear)	Prasad PV et al. [19] (skin smear)	Jaswal et al. [20] (skin smear)	Vijaikumar et al. [15] (nerve aspirate)
TT	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	Cellular smears, cohesive epithelioid cell granulomas, numerous lymphocytes not infiltrating the granuloma. BI 0–3+	Good cellular aspirate · Cohesive epithelioid cell granuloma or lymphocytic cell collection · Predominantly epithelioid cells with predominant to moderate number of lymphocytes. Occasional giant cells and neutrophils · BI 0-1+.
BT	Same as TT	Cellular material with lymphocytes, histiocytes and epithelioid cells, foamy macrophages are not a feature, no stainable AFB.	Same as TT	Same as TT

Class	Singh et al. [4] (skin smear)	Prasad PV et al. [19] (skin smear)	Jaswal et al. [20] (skin smear)	Vijaikumar et al. [15] (nerve aspirate)
BB			Fair cellular yields, poorly cohesive granuloma composed of an admixture of epithelioid cells and macrophages, few lymphocytes infiltrating the granulomas. BI 1-2+	Fair cellular aspirate · Mixed cellularity of predominantly nonfoamy macrophages, moderate number of epithelioid cells and lymphocytes. Macrophage granuloma · BI 2-3+.
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+	Moderate cellularity, singly dispersed macrophages with negative images, no epithelioid cells, numerous lymphocytes diffusely admixed with macrophages. BI 3-4+	Fair cellular aspirate · Predominantly lymphocytes and moderate number of foamy macrophages. BI. 4-5+.
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+	Heavy cellularity, numerous foamy macrophages in fatty background with intracellular and extracellular negative images, few lymphocytes. BI 4–6+	Fair to poor cellular aspirate ·Predominantly foamy macrophages and few lymphocytes · BI 6+

TT, tuberculoid; BT, borderline tuberculoid; BB, borderline borderline; BL, borderline lepromatous; LL, lepromatous leprosy; BI, Bacillary index.

Discussion

The Ridley-Jopling (RJ) classification is used currently for classifying leprosy, which is based on clinical, bacteriological, immunological, and histological parameters [5-8] It divides the leprosy spectrum into 'five' clinical and histological groups. Use of the RJ scale in the classification of leprosy helps in understanding the immunology of the patient to know the prognosis and possible complications. Ridley used ZN stain in 1989, to study the cytological material in leprosy cases [9]. In 1994, Singh et al used FNAC to diagnose 30 leprosy cases including a case of nodular lepromatous leprosy[10] using same technique.[4]

It is proved in many studies that FNAC of sensory cutaneous nerve helps in detection of leprotic inflammation especially granulomas and lepra bacilli.[11-17] Schwann cells arranged in fascicles could be seen along with granulomas. [13] These cytological features of nerve aspirates also helps in the categorization of leprous neuritis along the Ridley-Jopling scale.[11,14,15] Vijaikumar et al. studied cases with nerve involvement in leprosy and classified leprous neuritis into paucibacillary (PB), borderline borderline (BB), borderline lepromatous (BL), and polar lepromatous leprosy (LL) types.[15] (Table-5) describes the Ridley-Jopling classification as given by different authors.

The accuracy of cytological classification along the Ridley-Jopling spectrum in nerve aspirate was found in 92% cases. [15] However, a negative aspirate does not entirely rule out leprosy.[15] A strong concordance in tuberculoid (90%) and in lepromatous (93.7%) cases has been documented. Mid-borderline cases of leprosy show a problem in proper diagnosis.[17] Correlation of clinical diagnoses with FNAC examination has revealed varying results in different studies. In the present study, we were able to classify all nerve aspirates in 19 cases according to R-J criteria. We could observe organized granulomas as reported by Singh et al .[4] We did however, notice a very high correlation between clinical diagnoses and FNAC in all types of leprosy. We could not differentiate between TT and BT leprosy in four cases as reported by Singh et al .[4]. Correlation was also high in BL and LL types where there were scanty cellular infiltrates and more foamy macrophages. 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. 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,[4] and we could find the same in one case in the present study. Predominant lymphocytes are seen in cytology smears in the borderline types of the disease. In this study also, we found the largest number of lymphocytes in BL cases.

Singh et al. [4] in 1995 attempted the cytological diagnosis and classification of leprosy and found 100% cytohistological concordance. Rao et al. [18] also evaluated the utility of

FNAC in the classification of leprosy and found 90% concordance in cases of tuberculoid leprosy and 93.75% concordance was observed in lepromatous leprosy. They, however, observed difficulty in differentiating tuberculoid leprosy (TT) from borderline tuberculoid leprosy (BT) and borderline lepromatous leprosy (BL) from lepromatous leprosy (LL) on cytology. In this study, similar problem was seen in differentiating TT and BT cases.

Conclusion

Sensory cutaneous nerve FNAC is a quick & safe procedure, which can be done routinely as an outdoor procedure in the evaluation of leprosy patients. Not only it is useful in diagnosis of PNL, but also in patients having concomitant skin & nerve involvement. It is a sensitive tool for classifying the nerve lesions as per the Ridley-Jopling classification. It is also useful in patients with relapse.

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***Corresponding author:**
Dr Kishori M. Panda, Prof. of Pathology, Dept of Pathology, Govt Medical College, Raigarh, Chhattisgarh, India
Phone: +91 8763874020
Email: drkishoripanda@gmail.com

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