

## NERVE FNAC IN DIAGNOSING HANSEN'S DISEASE: A PROSPECTIVE STUDY

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### ABSTRACT

Leprosy is a progressive disease causing permanent damage to the skin, peripheral nerves, multiple organs, limbs and eyes and produces disabling deformity. Peripheral nerves involvement with thickening of one or more nerves is a common finding in Hansen's disease due to neurotrophism of these bacilli. In few cases of Hansen's disease, nerve thickening may be the only presentation (Pure neuritic type). In such cases, nerve biopsy is done for confirmation of the diagnosis. Nerve biopsy is an invasive procedure with complications of nerve deficit. Fine needle aspiration as in this study proved to be a simple technique to demonstrate inflammation, granulomas and AFB from these involved nerves. Hence, aim of the study is to analyse sensitivity, specificity of nerve FNAC in cases of Hansen's disease and its utility towards diagnosing and classifying the disease.

### METHODOLOGY

This study was carried out in the Department of Pathology, Victoria and Bowring Hospital, BMCRI, Bengaluru, on 100 patients presenting to skin OPD with hypoesthetic, hypopigmented patches, nerve thickening suspicious of Hansen's disease patients from November 2012 to October 2014. These patients were subjected to nerve FNAC and skin biopsy. FNAC smears were studied for cytological details and ZN stain for acid fast bacilli. Skin biopsy was subjected to histopathological examination and slides stained with H and E, Fite-Ferraco stains.

### RESULTS

Nerve FNAC 92% cases yielded diagnostic aspirates and 08% cases, the material was inadequate for opinion; 51% cases were showed AFB positivity and 49% cases were AFB negative. All the six cases diagnosed as pure neuritic leprosy diagnostic nerve aspiration was obtained and all were classified as PB type of leprosy based on cytology with all cases showing AFB positivity. Nerve FNAC was found to be sensitive in 86% cases.

### CONCLUSIONS

FNAC of nerve yields diagnostic aspirates in Hansen's disease comparable with skin biopsy and cytologically can be classified into paucibacillary and multibacillary types. The nerve FNAC and subsequent ZN staining of smears were diagnostic in all 6 cases with 100% accuracy in all PNL type of Hansen's disease. Hence FNAC was a safe, easy, quicker, time saving, cost effective, less invasive technique and alternative technique to nerve biopsy which is reserved only inconclusive cases, particularly useful in PNL cases.

### KEYWORDS

Hansen's Disease, Peripheral Nerve, FNAC.

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### INTRODUCTION

Leprosy, also known as Hansen's Disease (HD), is one of chronic infective skin diseases caused by the bacteria *Mycobacterium leprae*.<sup>1</sup> *Mycobacterium leprae* was discovered by Gerhald Henrik Armauer Hansen in 1873.<sup>2</sup>

Leprosy is a progressive disease causing permanent damage to the skin, peripheral nerves, multiple organs, limbs and eyes and produces disabling deformity.<sup>3</sup> Hansen's disease or Leprosy with neural involvement in the absence of dermal lesions have been named as the Pure Neuritic Type (PNL) of Hansen's disease.<sup>4</sup> These cases develop dermal lesions sometime later, therefore have been redesignated as primary neuritic Hansen's disease.<sup>1</sup> About 4–8% of all leprosy is clinically limited to the peripheral nerve.<sup>2</sup>

Only a few studies have evaluated the role of Fine Needle Aspiration Cytology (FNAC) of the nerve in the diagnosis of PNL. Pure neuritic form of this disease manifests by involvement of the nerve in the absence of skin lesion and can sometimes create a diagnostic problem. Diagnostic nerve biopsy is often required, which is an invasive procedure and may lead to complications.<sup>5</sup> Slit Skin Smears (SSS) and FNAC smears of skin lesions and Ziehl Neelson (ZN) staining have been conventionally used for diagnosing Leprosy.<sup>5</sup> Primary neuritic Hansen's disease is traditionally diagnosed by nerve biopsy, which is subjected to histopathological examination. This is a procedure which is done under anaesthesia, requires trained surgeons and complications are common. Nerve biopsy is done only on sural nerve, which is a sensory nerve.

Biopsy of other nerve leads to motor deficit. Therefore biopsy cannot be done on all the nerves, whereas nerve FNAC can be done on all the nerves due to minimal tissue damage.

The diagnosis of Primary Neuritic Leprosy (PNL).<sup>6</sup> and its differentiation from other causes of peripheral neuropathy is difficult since slit skin smears for Acid-Fast Bacilli (AFB) smears and skin biopsy are negative from anaesthetic areas.

Nerve FNAC is an easy, cost effective outpatient procedure and can be done with minimal expertise,

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subsequently followed by ZN staining. It can be done on any nerve as compared to nerve biopsy, which can be done only on sural nerve. However, fine needle aspiration has in this study proved to be a simple technique to demonstrate inflammation, granulomas and AFB from these involved nerves.<sup>7</sup> Hence, this study was undertaken to know the sensitivity, specificity of nerve, FNAC in cases of Hansen's disease and its utility towards diagnosing and classifying the disease.<sup>8</sup>

#### AIMS AND OBJECTIVES

1. To study the utility of nerve FNAC in diagnosing and classifying Hansen's disease.
2. To correlate the cytology findings of nerve FNAC with histopathology of skin biopsy.

#### SUBJECTS AND METHODS

A fine needle aspiration of thickened peripheral nerves and their histopathological study of skin biopsy of 100 Hansen's diseases cases obtained from the Department of Dermatology and Venereology, Victoria Hospital and Bowring and Lady Curzon Hospital, Bangalore, between November 2012 and October 2014 was done.

Materials for the study consisted of skin biopsies obtained in the Department of Pathology. An informed written consent was obtained from these patients prior to FNAC procedure and skin biopsy. The clinical findings including history, general examination, local examination of nerves and skin, clinical diagnosis and site of biopsy of the patients were noted.

The cases were examined for the most prominent site of thickened nerve, which was fixed by index finger and thumb of left hand and the 22-G needle fitted in 10mL disposable plastic syringe was inserted along the length of the nerve. The suction was applied and aspiration was performed using a single puncture, multidirectional technique.

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 aspirates were hemorrhagic in most of the cases and scanty in a few cases. 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 Haematoxylin and Eosin (H and E) after 30 minutes of fixation.

One of the dried smear was stained by May-Grünwald-Giemsa (MGG) stain and the other dried smear was stained by ZN stain with 5% sulphuric acid to demonstrate Acid Fast Bacilli (AFB). The H and E and MGG stained smears demonstrated scanty cellularity with few benign spindle cells which were not conclusive of diagnosis. The ZN stained smears showed presence of numerous acid fast bacilli arranged singly and in bundles, both uniformly stained and with beaded appearance which confirmed the diagnosis of Hansen's disease in both the cases.

Skin biopsies for the study were obtained by punch/incisional biopsy, which was performed by the Dermatologist. These biopsies were taken in 10% formalin. After adequate fixation for about 8-12 hours, the biopsies were submitted in Toto for routine processing, following which the paraffin embedded sections of 3-4 $\mu$  thickness were stained with H and E for morphological analysis and Fite-Faraco staining for identifying the bacilli.

Following was the scale used to calculate the bacteriological index (BI).<sup>9</sup>

1+ = 1 - 10 bacilli in 100 OIF (Oil immersion field) - Examine 100 OIF

2+ = 1 - 10 bacilli in 10 OIF - Examine 100 OIF

3+ = 1 - 10 bacilli in 1 OIF - Examine 25 OIF

4+ = 10 - 100 bacilli in 1 OIF - Examine 25 OIF

5+ = 100 - 1000 bacilli in 1 OIF - Examine 25 OIF

6+ = > 1000 bacilli in 1 OIF - Examine 25 OIF

The sections were examined for epidermal atrophy, granulomas and infiltrates of lymphocytes, histiocytes, foam cells, infiltration of nerves, blood vessels and adnexa and the presence of Grenz zone. They were grouped histopathologically as per the criteria formulated by Ridley and Jopling. Subsequently, a correlation was made between the cytological and histopathologic classification.

#### OBSERVATION

The total number of cases of present study were 100. This includes all Hansen's cases with thickened nerves and also skin biopsy from hypoaesthetic patches obtained. Out of the 100 cases, 96 cases yielded diagnostic aspirates, non-diagnostic aspirates were 04 cases. The aspirates were haemorrhagic in most of the cases. In the present study patient's age ranged from 12 years to 70 years. Among them 44 (44%) of the patients were in 2<sup>nd</sup> to 3<sup>rd</sup> decade, 35 (35%) patient were in 4<sup>th</sup> to 5<sup>th</sup> decade, 16 (16%) patients were in 1<sup>st</sup> to 2<sup>nd</sup> decade, 05 (5%) patients were in 6<sup>th</sup> to 7<sup>th</sup> decade. There were 67 (67%) male patients and 33 (33%) female patients with male-to-female ratio (M:F) of 2:1.

23 (23%) cases were presented with upper extremities as site of the lesion followed by trunk and back 21 (21%), Multiple sites -20 (20%), head-neck 18 (18%) and lower extremities 12 (12%) and others presenting as decreased sensation of extremities with no skin lesions (pure neuritic)-06 cases (6%). There were 65 (65%) cases presented with hypopigmented lesion and 35 (35%) cases presented with erythematous lesion. Out of 100 available patient's clinical data 76 (76%) patient presented with hypoesthesia, 15 (15%) patients with anaesthesia, 05 (5%) patients with tenderness and 4 (4%) patients with intact sensation status.

According to clinical type of cases 8(8%) cases were diagnosed as TT, 31 (31%) cases of BT, 10 (10%) cases of BL, 22 (22%) cases of LL, 12 (12%) cases of IL and 02 (02%) cases of BB type, 04 (04%) cases of pure neuritic type and undiagnosed cases are 11 (11%) cases. [Table 1] and [Table 2].

In the present study, it was observed that good aspirate was seen in IL, BT, TT. Poor aspirate was seen in BL, LL and 4 cases of PNL type. Also it was observed that plenty of lymphocytes are seen in IL, BT and TT cases and few lymphocytes were seen in BL, LL, PNL type cases. Plenty of epithelioid cells were seen in TT and BT cases. [Fig. 1] Few epithelioid cells were seen in IL, BB, BL, LL. Macrophages were seen only in BL and LL cases. On cell cytology, positivity for bacilli were seen in 51 cases (BL, PNL and LL) [Fig. 2] with negativity seen in 49 cases (IL, TT, BT). Only few bacilli were seen in IL, TT, BT cases.

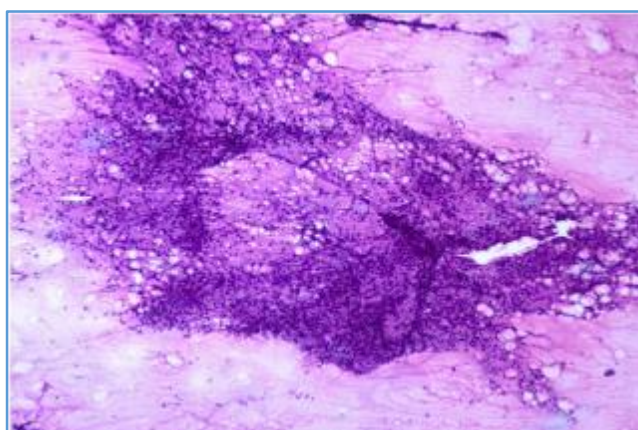
The 100 cases of nerve aspirates were classified into paucibacillary and multibacillary types according to bacillary index based on a study done by Vijaykumar et al. 2001.

Histopathological Type of Leprosy	No. of Cases	Fite Faraco Positive Cases (MB)	
		Number	Percentage
TT	11	00	00
BT	28	01	3.57%
BB	04	00	00
BL	13	13	100%
LL	21	21	100%
IL	17	1	5.88%
<b>TOTAL</b>	<b>94</b>		

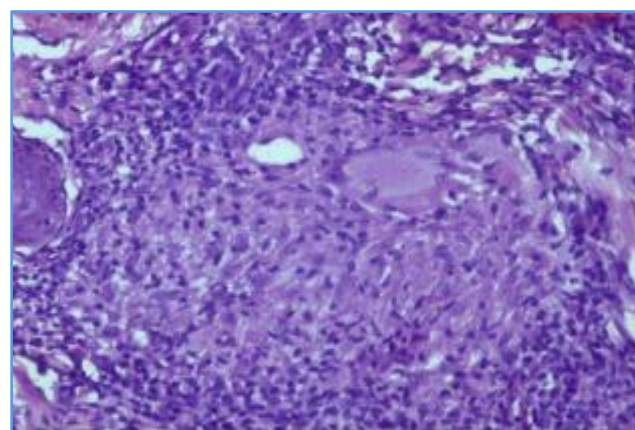
**Table 1: Histopathological Type of Leprosy and Modified Fite-Faraco stain Positivity (MB) for M. Leprae**

Skin Biopsy	Nerve cytology							AFB
	Aspirate		Lymphocyte		Epithelioid cell		Macrophages	
	Good	Poor	Plenty	Few	Plenty	Occasional		
TT	10	01	10	1	08	03	-	00
BT	24	04	24	04	18	10	-	02
BB	03	01	03	01	01	03	-	00
BL	04	09	09	04	-	07	04	13
LL	06	15	05	16	-	03	18	21
IL	09	06	10	05	-	02	-	01
Pure neuritic	02	04	-	06	-	-	-	06

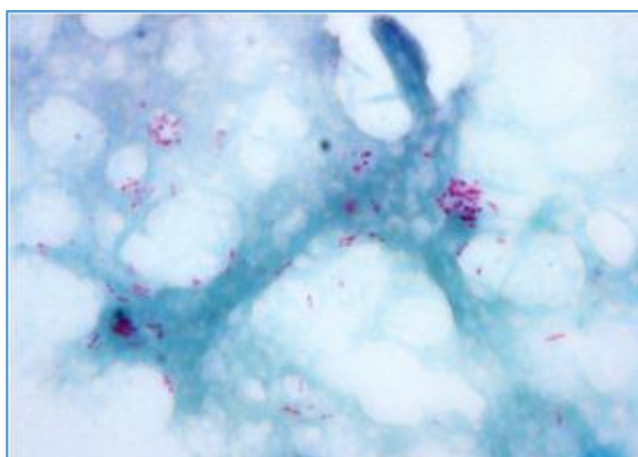
**Table 2: Correlation of nerve cytology and skin biopsy**



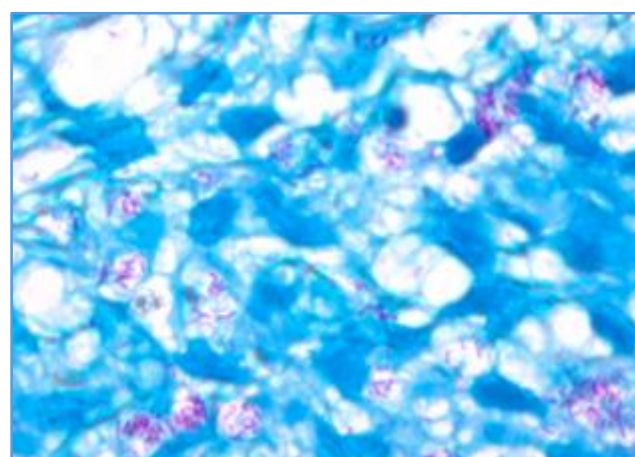
**Fig. 1: FNAC Nerve Smear showing granulomatous inflammation with numerous lymphocytes, histiocytes, epithelioid cells (H & E stain x20)**



**Fig. 3: Skin biopsy histopathology in a case of Tuberculoid leprosy showing Langhans giant cells amidst epithelioid cells and lymphocytic infiltrates (H & E stain x20)**



**Fig. 2: FNAC Nerve smear showing foamy macrophages containing plenty of Acid fast bacilli in Globi multibacillary type (ZN stain x100)**



**Fig. 4: Skin biopsy histopathology in a case of Lepromatous leprosy. Plenty of acid fast bacilli in clusters (Fite-Faraco stain 100X)**

### Characteristics of Paucibacillary (PB) pole (Consisting of indeterminate, TT, BT and BB)

- Good cellular aspirate.
- Cohesive epithelioid cell granuloma or lymphocytic cell collection.
- Predominantly epithelioid cells with predominant to moderate number of lymphocytes.
- Occasional giant cells.
- BI- zero to 1+.

### Characteristics of Multibacillary (MB) pole (Consisting of BL, LL and Histoid leprosy)

- Fair-to-poor cellular aspirate.
- Mixed cellularity of predominantly foamy macrophages, few number of.
- Epithelioid cells and lymphocytes.
- Macrophage granulomas.
- BI - >2+.

In the present study, out of 100 cases 66 were paucibacillary and 34 cases were multibacillary. Differentiation between indeterminate, TT, BT and BB forms were difficult in cytological smears and were grouped under the paucibacillary group.

It was observed that the cellular characteristics were most useful in paucibacillary pole and BB type and the Bacillary Index was more useful in the lepromatous pole. The nerve aspirate was negative for AFB in two BB cases.

Cell cytological study was done in all cases to find out presence of lymphocytes, epithelioid cells, granulomas, giant cells, macrophages and acid fast bacilli.

Out of 100 cases of nerve aspirate were correlated with skin biopsy and histopathological classification was done for type of Hansen's disease [Fig. 3&4], 86 cases (86%) showed correlation with histopathological findings for presence of Hansen's disease; 14 cases (14%) nerve FNAC did not show correlation with histopathology. These cases showed scanty cells, adipose tissue, few lymphocytes and were inadequate for opinion.

## DISCUSSION

Leprosy, also known as Hansen's Disease (HD) is one of chronic infective skin diseases caused by the bacteria *Mycobacterium leprae*. Leprosy is a progressive disease causing permanent damage to the skin, peripheral nerves, multiple organs, limbs and eyes and produces disabling deformity.<sup>1</sup> It is an important public health problem in our country with Uttar Pradesh, Chhattisgarh, West Bengal, Bihar and Maharashtra being the states with the highest number of cases. Timely and accurate diagnosis is the corner stone of leprosy control as it is helpful in case management, prevention of deformity and transmission of disease. Hence, it is imperative to have in-depth knowledge and clarity regarding the diagnosis and classification of leprosy cases at the field level.

Peripheral nerve involvement with thickening of one or more nerves is a common finding in Hansen's disease due to neurotrophism of these bacilli. In few cases of Hansen's disease, nerve thickening may be the only presentation (Pure neuritic type). In such cases, nerve biopsy is done for confirmation of diagnosis. Nerve biopsy is an invasive procedure with complications of nerve deficit.

However, fine needle aspiration has in this study proved to be a simple technique to demonstrate inflammation, granulomas and AFB from these involved nerves.<sup>6</sup>

### CYTOMORPHOLOGICAL FEATURES OF NERVE ASPIRATE

FNAC is widely accepted as a diagnostic procedure for several malignant and inflammatory lesions.<sup>10,11</sup> The procedure is simple and can be repeated a second or third time if needed. The efficacy of this procedure in Hansen's disease has, however, not been exploited fully, though slit skin smears have been conventionally used in leprosy to assess the bacteriological and morphological indices. Also, Ziehl-Neelsen staining does not provide adequate morphological details of the cell types.<sup>8</sup>

It is a general concept that leprosy affects males more than females. In concurrence, the majority of the patients in our study were males (67%) with females at (33%). The same observation is confirmed by various other authors.<sup>3</sup> Male to female ratio observed in this study was 2:1, which is similar to that observed in the other studies.<sup>12</sup>

Noorden SK.<sup>13</sup> observed that the dominance in males is because of their lifestyle, which is associated with greater risks of acquiring the infection and also the social taboos and customs which account for underdetection of leprosy among females as lesser numbers report to the hospital for treatment. The pattern of leprosy in the present study showed a predominance of BT cases (28%) followed by LL (21%) and the least common type was the BB type (04%).

Borderline group constituted the major spectrum (45%), which included BT, BB, BL and these were similar to the findings in the other studies.<sup>12,14</sup> An immunological instability is seen in the borderline cases, which with treatment moves towards the tuberculoid pole and without treatment towards the lepromatous pole. If the disease is recognized at an early stage, the biopsies taken will have features of the BT stage and if recognized late they have the features of BL stage.

An increased awareness towards leprosy due to the increasing efficacy of the public health system as well as to the patient's health seeking behaviour makes them present at an early stage to the leprosy clinics, which may contribute to the increased number of cases seen in the borderline group.

PB - Paucibacillary - BI = 0

MB - Multibacillary - BI =  $\geq 1+$

In the present study, majority of the patients were of paucibacillary type 56 (61.5%) and the rest were of multibacillary type 35 (38.5%). All the biopsies of TT were of paucibacillary type (100%) similar observation was made by Moorthy NB et al. and Veena S et al.<sup>14</sup>

Majority of the biopsies of BT were of paucibacillary 26 (92.9%) type and the rest were of multibacillary type (7.1%). Similar observations were made by other authors.<sup>14</sup> All the 21 (100%) LL type were of multibacillary type similar to observations of other authors.<sup>14</sup> All the 16 (94.1%) biopsies of IL type were of paucibacillary type similar to other authors.<sup>15</sup> but only one case (5.9%) shows multibacillary type in our study. In the present study it was observed that out of 100 cases, 67 cases (67%) were showed paucibacillary pole and 33 cases (33%) showed multibacillary pole. Such good correlation is noted by other authors as seen in Table No. 20 M. Vijaikumar et al.<sup>16</sup> has studied on 25 patients with clinically active leprosy and at least one thickened peripheral sensory nerve.

Nerve aspirates were evaluated by May-Grunwald-Giemsa and Fite's staining and showed that 20 cases were (80% paucibacillary pole and 03 cases (12%) were multibacillary pole, other 02 cases were non-diagnostic and concluded that FNAC of the nerve yields diagnostic aspirates in leprosy comparable with nerve pathology and the proposed cytological criteria may be useful in classification of nerve aspirates.

The present study shows ZN staining/Rapid AFB methods are more useful in diagnosing more number of acid fast bacilli in FNAC smears as compared to slit skin smear study and skin histopathology. Such good correlation was also seen in the study of different authors.

This is the large series in which the cytological findings of nerve aspirates have been compared with the histopathology of the skin and by analysing these, a set of criteria was devised to classify the cytological smears. Differentiation between indeterminate, TT, BT and BB forms was difficult in cytological smears, which were grouped together as PB leprosy.

In the PB pole, cohesive epithelioid cells and lymphocytes were observed. Towards the lepromatous pole, no cohesion of cells was observed. It was observed that the cellular characteristics were more useful in PB pole and BB type and that the BI was more useful in the lepromatous pole. The nerve aspirate was negative for AFB in BB cases.

In the present study, all the six cases diagnosed as pure neuritic leprosy yielded diagnostic nerve aspirates and were classified as PB type of leprosy based on cytology with all cases showing AFB positivity. In the study by Jayaseelan et al.<sup>7</sup> 18 of 27 cases were diagnosed as pure neuritic leprosy based on cytology of nerve aspirate, but only three showed AFB positivity. Hence, we recommend the use of both cytological and AFB evaluation of the nerve aspirates.

In the study by Theuvenet, et al. 7 of the 11 cases of suspected pure neuritic leprosy showed multiple AFB in the nerve aspirate. No cytological evaluation in that study but in our study few bacilli seen in all 6 cases of PNL.

In both the previous studies published on FNAC of the nerve, aspiration of motor nerves was also done safely. Though no iatrogenic sensory loss was noticed in the superficial sensory nerves, the use of FNAC on motor nerves requires caution and further standardization of the technique. A nerve aspirate with cytology as described in the devised criteria or AFB positivity is diagnostic as seen by the 61% positive aspirates in this study. However, a negative aspirate does not entirely rule out leprosy.

Hence FNAC, being a simpler, quicker and less invasive technique, can be attempted on the nerve before deciding on a nerve biopsy. This would be particularly useful when pure neuritic leprosy is suspected.

#### SUMMARY AND CONCLUSION

Nerve FNACs has in this study proved that it is simple technique to demonstrate inflammation granulomas and AFB from these involved nerves and its utility towards diagnosing and classifying the disease.

When nerve cytology is compared with the skin biopsy, an equal concordance is observed. FNAC has limited usefulness in classifying the patients according to Riedly and Jopling classification with an accuracy of 60-80%.

ZN staining of these aspirates can be useful in differentiating paucibacillary and multibacillary types.

Some patients have been found to be multibacillary in nerve and paucibacillary in skin and nerve biopsy has been useful in these cases for typing the patients into multibacillary and paucibacillary types.<sup>6</sup> Nerve FNAC can be a substitute to nerve biopsy in such cases.

Hence FNAC, being a simpler, quicker and less invasive technique can be attempted on the nerve before deciding on a nerve biopsy. This would be particularly useful when pure neuritic leprosy is suspected and FNAC may be used as an alternative tool to assess leprosy lesions in areas, where histopathological services are not readily available.

The nerve FNACs and subsequent ZN staining of the smears were diagnostic in all cases of pure neuritic type of Hansen's disease. Hence nerve FNAC can be used as an alternative diagnostic modality to nerve biopsy due to its advantages over nerve biopsies in pure neuritic type of Hansen's disease and nerve biopsy should be reserved only for inconclusive cases.

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