Sensory cutaneous nerve fine-needle aspiration in Hansen’s disease: A retrospective analysis of our experience

ABSTRACT
Background: Leprosy affects peripheral nerves. As Mycobacterium leprae has unique tropism for Schwann cells, thickened sensory cutaneous nerves provide an easy target for the detection of lepra bacilli and other changes associated with the disease.

Materials and Methods: The data of patients with sensory cutaneous nerve involvement were retrieved from our record for the period January 2006 to December 2014. 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 lepra bacilli and globi. Morphological index was calculated in multibacillary lesions.

Result: Twenty-nine sensory cutaneous nerves were aspirated in 23 patients. While 15 cases showed skin and nerve involvement, 8 cases showed only nerve involvement. Terminal cutaneous branch of the radial nerve was most often aspirated. No motor loss was observed after aspiration. Five cytologic pictures were seen — epithelioid cell granuloma only in 6 cases, epithelioid cell granuloma with necrosis in 1 case, epithelioid cell granuloma with lepra bacilli in 3 cases, necrosis with lepra bacilli in 1 case, and only lepra bacilli in 12 cases. Morphological index ranged from 20% to 80%.

Conclusion: Sensory cutaneous nerve fine-needle aspiration (FNA) is a feasible, viable, effective, and safe procedure. It adds to diagnostic FNA yield in patients with concomitant skin involvement and offers a way to evaluate patients with only nerve involvement. Calculation of morphological index allows prognostication and may have a role in assessing response to therapy and/or relapse.

Key words: Fine-needle aspiration (FNA) cytology; Hansen’s disease; lepra bacilli; sensory cutaneous nerve

Introduction
Leprosy is a slowly progressive infection caused by Mycobacterium leprae, affecting the skin and peripheral nerves and resulting in disabling deformities.[1] The outcome of leprosy depends largely on the extent of nerve damage in the patient. Thus, thickened peripheral nerves, anesthetic areas in the skin, weakness or paralysis of the muscles in the hands, legs, or face are important components of leprosy as is the presence of M. leprae in nerves and/or presence of granuloma in and around a nerve.

M. leprae is the only bacterium to invade peripheral nerves. It has a peculiar tropism for peripheral nerves, from large nerve trunks to microscopic dermal nerves. The unique
bacilli. Thickening of nerves in leprosy is localized to portions that are most superficial. Thus, thickened nerves provide a logical target for the aspiration and detection of lepra bacilli and other changes associated with the disease.

The thickened nerve may either be a sensory nerve or a motor nerve or a mixed sensory and motor nerve, depending on whether there is an associated sensory and/or motor dysfunction in the area innervated by that nerve. Fine-needle aspiration (FNA) should be undertaken only in sensory nerves, supplying areas of lesser functional importance, to avoid loss of motor function after the procedure. Some of the sensory nerves that can be targeted for aspiration are the supraorbital branch of the fifth cranial nerve, the great auricular nerve in the neck, supraclavicular nerves as they cross the clavicles, the antebibrachial cutaneous nerves in the forearms, the terminal cutaneous branch of radial and median nerves at the wrists, the femoral cutaneous nerves in the thighs, the saphenous nerves, the sural nerves at the back of the legs, and the superficial peroneal nerves in front of the ankles and on the dorsum of feet.

Each peripheral nerve is composed of one or more fascicles of nerve fibers (axons are commonly referred to as nerve fibers). Within the fascicle, each individual nerve fiber with its investing Schwann cells is surrounded by a delicate packing of loose vascular supporting tissue called the endoneurium. The cell bodies of the nerve fibers are located either in the central nervous system or in the ganglia at peripheral sites. Thus, the nuclei seen within the fascicle are either of the more numerous Schwann cells or of the sparse fibroblasts of the endoneurium, the nuclei of latter being more slender and condensed. Thus, in aspirates from peripheral nerves a majority of the nuclei seen belong to Schwann cells with few interspersed nuclei belonging to fibroblasts. Consequently, Schwann cells with intracellular and extracellular lepra bacilli can be readily detected in sensory cutaneous nerve FNA smears.

In cytology, a few papers have been published on this subject but most are case reports. A comprehensive view of the issues involved has not been detailed in any of them or in other publications on this subject. This retrospective study was undertaken to analyze the feasibility, efficacy, and role of FNA cytology of sensory cutaneous nerves in Hansen’s disease.

Materials and Methods

Data were retrieved from our cytology record for the period January 2006 to December 2014. Patients presenting with sensory cutaneous nerve involvement, with or without skin lesions, and those who had not received any prior therapy were included in this study. Only those cases who were clinically and therapeutically proven to have leprosy were included in this study. The sample size was determined by the period of study and the inclusion criteria detailed above. Data regarding the age, sex, and duration of disease at presentation were documented. A thorough clinical examination was done before aspiration was performed. Details of nerve(s) involved and the presence of any concomitant cutaneous lesion were documented.

Nonsuction method was mostly used for collecting the sample. No local anesthesia was given before the procedure. Nodular and thickened areas of the nerve were targeted. If required, multiple sites from a given nerve were aspirated. Wherever required, aspiration was done using a 22-gauge needle and 10 ml syringe. Informed consent of the patient was taken for clinical photographs. The patient was assessed for loss of motor function after the procedure and during subsequent follow-up with the leprologist.

Three smears were made for each nerve aspirated. The first slide was stained with hematoxylin and eosin (H and E) stain, the second slide was stained with May-Grünwald-Giemsa (MGG) stain, and the third slide was stained with modified Ziehl-Neelsen (ZN) stain using 5% sulfuric acid. If ZN stain was unsatisfactory or if a need for more screening was felt, the slide with MGG stain was decolorized with 1% acid alcohol and restained with modified ZN stain.

The H and E and MGG-stained slides were screened for the presence of Schwann cells, granuloma, and necrosis. In modified ZN stained smears, the search was made for lepra bacilli and globi. If at least 200 discrete lepra bacilli were found in the modified ZN-stained smears, the morphological index was also calculated.

Results

During the study period, 23 cases were retrieved from our records where aspiration was done from sensory cutaneous nerve. Out of these, 15 cases showed skin and nerve involvement while 8 cases showed only nerve involvement. The age of the patients ranged from 12 years to 60 years. The male:female ratio was 3.6:1. The duration of disease at presentation ranged from 25 days to 7 years (Table 1).

The sensory cutaneous nerves aspirated are detailed in Table 2. A total of 29 sensory cutaneous nerves were aspirated in 23 patients. In 6 patients, two nerves each were aspirated.
The terminal cutaneous branch of the radial nerve was most often aspirated (19 out of 29).

Aspiration of the sensory cutaneous nerve was well-tolerated by all the patients. Barring some tingling sensation in the innervated area of the aspirated nerve, no complication was encountered in the course of performing the aspiration. No motor loss was observed in the innervated area of the nerve after the procedure and on subsequent follow-up with the leprologist.

In cytology smears, 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 [Figure 1]. They constitute the predominant cell population in a nerve aspirate and are differentiated from fibroblasts of the endoneurium, the nuclei of latter being more slender and condensed. However, in the presence of necrosis it may be difficult to identify Schwann cells. As the aspiration was targeted at the nodular areas, which are often the site of necrosis, the presence of granuloma, lepra bacilli, and correlation with clinical data are used to make a final diagnosis in such cases.

Five cytologic pictures were seen [Table 3]. Epithelioid cell granuloma was only seen in six cases (26.1%). Epithelioid cell granuloma with necrosis was seen in one case (4.3%) while epithelioid cell granuloma with lepra bacilli was seen in three cases (13.1%). Necrosis with lepra bacilli was seen in one case (4.3%) while only lepra bacilli was encountered most often in 12 cases (52.2%). No case was encountered where epithelioid cell granuloma was seen, along with both necrosis and lepra bacilli.

![Figure 1: Sensory cutaneous nerve aspirate showing a nerve fascicle with numerous Schwann cells surrounded by many lymphocytes (H and E, ×100)](image)

| Age and Sex | Duration of disease at presentation | Involvement | ECG | Necrosis | Leprabacilli | Globi | MI | Subtype |
|-------------|-------------------------------------|-------------|-----|----------|--------------|-------|----|---------|
| 22M         | 2-3 years                           | +           | −   | −        | +            | −     | −  | BL      |
| 45F         | 1 year                              | −           | +   | −        | +            | −     | −  | LL      |
| 18F         | 2.5 years                           | −           | +   | −        | +            | −     | 60| BT      |
| 21M         | 1 year                              | −           | +   | −        | +            | +     | 24| BL      |
| 48F         | 2 years                             | +           | −   | −        | +            | −     | −  | BT      |
| 22M         | 5-6 months                          | −           | +   | +        | −            | −     | −  | TT      |
| 30M         | 2 months                            | +           | −   | −        | +            | −     | −  | BL      |
| 20M         | 3 years                             | −           | +   | +        | −            | −     | −  | BT      |
| 40M         | 7 months                            | −           | +   | −        | +            | +     | 38| BL      |
| 52M         | 25 days                             | −           | +   | −        | +            | +     | 20| BB      |
| 60M         | 2.5 months                          | −           | +   | −        | +            | +     | 60| BL      |
| 17M         | 6 months                            | +           | −   | −        | +            | +     | −  | BT      |
| 30M         | 6 months                            | −           | +   | +        | −            | −     | −  | BT      |
| 15M         | 1 month                             | +           | −   | −        | +            | −     | −  | TT      |
| 35M         | 3 years                             | +           | −   | −        | +            | −     | −  | BT      |
| 12M         | 1 year                              | −           | +   | +        | −            | −     | −  | BT      |
| 65F         | 1 year                              | −           | +   | +        | +            | +     | 80| BL      |
| 30M         | 4 years                             | +           | −   | −        | +            | −     | −  | BT      |
| 18F         | 7 years                             | −           | +   | −        | +            | −     | −  | BT      |
| 21M         | 4 months                            | +           | −   | −        | +            | −     | −  | TT      |
| 32M         | 2.5 months                          | −           | +   | −        | +            | −     | −  | BL      |
| 14M         | 6 months                            | −           | +   | +        | −            | −     | −  | TT      |
| 40M         | 2 months                            | −           | +   | −        | +            | −     | −  | BT      |

N: Nerve only, S + N: Skin with nerve involvement, ECG: Epithelioid cell granuloma, MI: Morphological index, TT: Polar tuberculoid leprosy, BT: Borderline tuberculoid leprosy, BB: Mid-borderline leprosy, BL: Borderline lepromatous leprosy, LL: Polar lepromatous leprosy
In patients showing lepra bacilli, globi were seen in five cases. In multibacillary lesions where morphological index was calculated, it ranged from 20% to 80% [Table 1].

Discussion

Leprosy presents as a spectrum of clinical manifestations that have bacteriologic, pathologic, and immunologic counterparts, ranging from polar tuberculoid (TT) to borderline tuberculoid (BT) to mid-borderline (BB) to borderline lepromatous (BL) to polar lepromatous forms [lepromatous leprosy (LL) — Which includes LLs and LLp]. However, it must be remembered that borderline patients are unstable. BT patients may upgrade to TT though advanced cases are more likely to downgrade. BB patients always downgrade to LLs without treatment. LLp patients probably always originate as such. However, although the static nature of some groups and the range of movement of other groups appear to be distinctive, histological distinctions between adjacent groups are not asserted to be absolute. The spectrum is an infinitely graded continuum.

Nerve involvement in leprosy is varied across the Ridley-Jopling spectrum.

In tuberculoid leprosy, patients have asymmetric enlargement of one or few peripheral nerves, which occurs early in the course of the disease. Owing to a type 1 cytokine pattern, strong T cell and macrophage activation result in a localized infection. T cells breach the perineurium and destruction of Schwann cells and axons may be evident, resulting in fibrosis of the epineurium, replacement of the endoneurium with epithelioid granulomas, and occasionally caseous necrosis. Such invasion and destruction and actual epithelioid granuloma formation within the dermal nerves is pathognomonic of leprosy. Bacilli are either not found or are few in number. In our study, six cases showed only epithelioid granuloma [Figure 2], one case showed epithelioid granuloma with necrosis, three cases showed epithelioid granuloma with lepra bacilli, and one case showed necrosis with lepra bacilli.

In tuberculoid leprosy, if the individual’s immunity is capable of containing the infection within one or more nerves without evidence of skin involvement, this is referred to as pure neural tuberculoid leprosy [Figure 3]. But if bacilli or their antigens escape from the nerve into the surrounding or neighboring

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**Table 2: Showing details of sensory cutaneous nerve aspirated**

| Sensory cutaneous nerve aspirated | n  |
|----------------------------------|----|
| Terminal cutaneous branch of right radial nerve | 11 |
| Terminal cutaneous branch of left radial nerve | 8  |
| Terminal cutaneous branch of right ulnar nerve | 1  |
| Right great auricular nerve | 1  |
| Left great auricular nerve | 1  |
| Right post auricular nerve | 1  |
| Right lateral antebrachial cutaneous nerve | 3  |
| Left medial antebrachial cutaneous nerve | 1  |
| Right supraorbital nerve | 1  |
| Left superficial peroneal nerve | 1  |
| Total | 29 |

**Table 3: Showing detail of cytologic picture**

| Cytologic picture | n | %     |
|-------------------|---|-------|
| Epithelioid cell granuloma only | 6 | 26.10% |
| Epithelioid cell granuloma + necrosis | 1 | 4.30% |
| Epithelioid cell granuloma + lepra bacilli | 3 | 13.10% |
| Necrosis + lepra bacilli | 1 | 4.30% |
| Lepra bacilli only | 12 | 52.20% |
| Total              | 23 | 100.00% |

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Figures:

**Figure 2:** Sensory cutaneous nerve aspirate showing an epithelioid cell granuloma surrounded by many lymphocytes (H and E, ×200)

**Figure 3:** Clinical photograph of patient showing a thickened left great auricular nerve (marked by arrow)
Prasoon, et al.: FNA of sensory cutaneous nerves in Hansen’s disease

In LL, nerve enlargement and damage result from actual bacillary invasion. Nerve damage is more insidious, occurs late in the disease, is symmetric but ultimately more extensive than tuberculoid leprosy. Thus, skin manifestations are always present when neurological signs and symptoms occur. They have a tendency toward symmetric nerve trunk enlargement. The lepra bacilli initially invade Schwann cells, resulting in foamy degenerative myelination and axonal degeneration. Numerous lepra bacilli are present in Schwann cells and macrophages that infiltrate the nerves. At this end of the spectrum, numerous bacilli are seen either singly or in small groups or as globi [Figure 5]. Our study showed 12 cases where only lepra bacilli were detected without any granuloma or necrosis. Globi were seen in five cases.

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In borderline leprosy, neural involvement is common. The findings vary with the success of the T cell reaction against M. leprae. If the response is good, there are well-formed epithelioid granuloma with a lymphocytic exudate around them. The destruction of dermal nerves is less complete than in tuberculoid leprosy. If the immunologic response is weaker, the granuloma are less well-formed, the lymphocytic exudate is less marked, and the dermal nerves are largely spared, showing only proliferation of their Schwann cells. If the immunologic response is still weaker, there is a diffuse infiltration of foamy macrophages and more marked proliferation of Schwann cells. M. leprae is present in less numbers if the lesion is granulomatous but is present in more numbers in macrophages and Schwann cells as it approaches the lepromatous pattern. Nerves are frequently damaged in borderline leprosy. It is not uncommon for a prolonged polyneuritic phase to precede the appearance of skin lesions. In such cases, there is evidence of thickened nerves with or without muscle paralysis or skin anesthesia by the time skin lesions appear. Nerve abscesses are most common in LL.

In lepra reaction, nerve involvement is common. Type 1 lepra reaction is seen in borderline patients and may either be a reversal reaction or a downgrading reaction. Here, nerve involvement takes the form of rapid swelling of one or more nerves with pain and tenderness at the site of nerve swelling, usually where the nerve is most superficial. Sensory and/or motor deficit may occur depending on the nerve involved. Rarely, a nerve abscess may form. In reversal reaction there is edema, reduced bacilli, and increase in defensive cells such as lymphocytes, epithelioid cells, and giant cells. In downgrading reaction, there is an increase in the number of bacilli and replacement of defensive cells by macrophages. Type 2 lepra reaction is seen in LL only — LLp, LLs, and occasionally BL. Patients may develop neuritis. In the lesions there is edema, neutrophil infiltration, and sometimes vasculitis. Bacilli are fragmented and granular. In our study, we did not encounter any patient with lepra reaction.

For classification, one biopsy is normally sufficient as lesions show a fairly uniform picture not only in the skin but also in other involved tissues except during reversal reactions, which often affect some lesions more than the others. Following the Ridley-Jopling classification, in our study there were 4 cases of TT, 10 of BT, 1 of mid-borderline, 7 of BL, and 1 of polar LL.

In our study, terminal cutaneous branch of the radial nerve was most often aspirated (19 out of 29). Pedley et al. in their series of biopsy of peripheral nerves in 119 leprosy cases also...
chose only sensory nerves, the most common ones being the great auricular nerve and terminal radial and terminal ulnar nerves, and less common ones being the superficial peroneal and sural nerves.\(^{[13]}\) Meanwhile, Margery et al. performed a biopsy of the terminal cutaneous branch of the radial nerve and superficial peroneal nerves.\(^{[14]}\)

The morphological index is the percentage of solid stained bacilli calculated after examining 200 bacilli lying singly. A decrease in morphological index indicates worsening of the patient’s condition or relapse if not under treatment.\(^{[10]}\) We were able to calculate the morphological index in sensory cutaneous nerve aspirates in six cases. It may be difficult to calculate the morphological index if the bacillary load is low. However, effort should be made to do the same, as far as possible, for prognostic purposes.

Biopsy is considered as the gold standard investigation for diagnosing leprosy. If a concomitant skin lesion is present, nerve biopsy is usually not required. In pure neural forms, it is mandatory but is rarely performed.\(^{[14]}\) For biopsy, local anesthesia is infiltrated around the nerve before the procedure and one or two fascicles are stripped from the nerve bundle for about 1 cm by a careful, sharp dissection. If the nerve fascicles are damaged and obliterated by disease, a small longitudinal wedge of nerve is removed for biopsy.\(^{[8]}\) In FNAC, neither is infiltration by local anesthesia required and nor are the fascicles stripped from the nerve bundle; there is also no need for removal of a wedge from a nerve. The procedure is as easy to perform as for any other organ; it is well-tolerated by the patients and there is relatively much less trauma to the nerves. In addition, the cellular yield is good. On the contrary, as the Schwann cells harbor \textit{M. leprae}, thickened and/or nodular areas of the nerve provide a small area that is rich in lepra bacilli and thus, if targeted they readily yield the bacilli. In our experience, we feel that in patients presenting with skin lesions with concomitant nerve involvement, sensory cutaneous nerve FNA should be done routinely whenever possible. They add to the diagnostic FNA yield. Moreover, FNA can be repeated at the same site for prognostic purposes.

Conclusion

As of now, we feel that the following conclusions can be drawn from the above study:

1. Sensory cutaneous nerve FNA is a feasible, viable, effective, and safe procedure, which can be done routinely as an outdoor procedure in the evaluation of leprosy patients.

2. It adds to the diagnostic FNA yield in patients having concomitant skin and nerve involvement.

3. It offers an easy way to evaluate patients presenting with only nerve involvement.

4. It is an effective method for assessing the pathology of the affected nerve in leprosy, allowing classification of lesion as per the Ridley-Jopling classification.

5. It allows prognostication as morphological index can be calculated in the nerve aspirates.

6. It may have a role in assessing response to therapy and/or relapse in patients under follow-up.

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Conflicts of interest
There are no conflicts of interest.

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