Peripheral neuropathy, though a common neurological problem, continues to be a diagnostic challenge. Its heterogeneity in the etiology, diversity in pathology, and degree of severity make it difficult to diagnose in many cases. The initial workup of the patients with peripheral neuropathy includes complete blood count, erythrocyte sedimentation rate, fasting blood glucose, vitamin B12, and thyroid-stimulating hormone level estimations. Almost a quarter of the patients remain undiagnosed despite exhaustive initial diagnostic workup.

In a series from Italy, of the selected 100 patients with chronic neuropathies, one-fifth (19%) remained idiopathic. The common etiologies were diabetes, immune-mediated neuropathies, and vitamin B12 deficiencies. The electrophysiological assessment (nerve conduction studies and electromyography) helped in the differentiation of axonal variety from a demyelinating or mixed variety of neuropathies.

Nerve biopsy is a common diagnostic method that may establish a diagnosis in many peripheral neuropathies of unexplained etiology. In a prospective study that included 50 patients with nerve biopsies in patients with peripheral neuropathy:

**Introduction**

Peripheral neuropathy, though a common neurological problem, continues to be a diagnostic challenge. Its heterogeneity in the etiology, diversity in pathology, and degree of severity make it difficult to diagnose in many cases. The initial workup of the patients with peripheral neuropathy includes complete blood count, erythrocyte sedimentation rate, fasting blood glucose, vitamin B12, and thyroid-stimulating hormone level estimations. Almost a quarter of the patients remain undiagnosed despite exhaustive initial diagnostic workup. In a series from Italy, of the selected 100 patients with chronic neuropathies, one-fifth (19%) remained idiopathic. The common etiologies were diabetes, immune-mediated neuropathies, and vitamin B12 deficiencies.

The electrophysiological assessment (nerve conduction studies and electromyography) helped in the differentiation of axonal variety from a demyelinating or mixed variety of neuropathies.

**Keywords:** Amyloidosis, leprosy, nerve biopsy, sural nerve
In 14% of the cases, the nerve biopsy changed the diagnosis. In this prospective follow-up study, we evaluated the role of nerve biopsies in the patients of peripheral neuropathies, who remain undiagnosed after the initial workup.

Materials and Methods

The study was conducted in the Department of Neurology in collaboration with the Department of Pathology, Dr. Ram Manohar Lohia Institute of Medical Sciences, Lucknow. Ethical approval was taken from the Institutional Ethics Committee. Informed consent was taken from all included patients.

All patients with peripheral neuropathy attending the indoor/outdoor of the Department of Neurology were included. A detailed clinical evaluation, electrophysiological assessment, and laboratory workup consisting of complete blood count, erythrocyte sedimentation rate, fasting blood glucose, vitamin B12, and thyroid-stimulating hormone level estimations were performed.

Inclusion criteria

We included the patients for nerve biopsy if the initial workup failed to diagnose the cause of peripheral neuropathy.

Nerve conduction study

Nicolet 5 channel EP and EMG system (Middleton, WI, USA) were used for nerve conduction studies. All nerve conduction studies followed standard procedures.

Nerve biopsy

Sural nerve biopsy was done in all the cases. At least 3.0 cm in length of sural nerve biopsies were taken and dispatched for histopathological examination in 2.5% of glutaraldehyde solution; 5-μm thickness sections were cut and stained with the Wade Fite stain for lepra bacilli and hematoxylin and eosin stain for routine analysis. The histopathological alterations like endoneurial infiltration, perineurial thickening, and endoneurial fibrosis were carefully analyzed. Kulchitsky Pal stain was done to assess myelinated fiber loss and demyelination.

Statistical analysis

The Chi-square test was applied for categorical variables. For numerical variables, the Mann–Whitney U test and Kruskal–Wallis H were applied as they did not meet the criteria for normalcy after applying the Kolmogorov–Smirnov test and Shapiro–Wilk's test. For paired variables, Wilcoxon's rank-sum test was used. Binary logistic regression analysis was used to evaluate the histopathological findings on nerve biopsy predicting leprosy cases. SPSS statistics 24 was utilized for the data analysis.

Results

In this study, we evaluated nerve biopsies of 55 patients. In 20 patients, leprosy was the cause of neuropathies. In 26 cases, the diagnosis remained obscure despite nerve biopsies. In the remaining nine patients, four patients had features of a chronic demyelinating neuropathy, one patient each had amyloidosis. Hereditary neuropathy and possible vasculitis were diagnosed in two patients each [Table 1, Figure 1].

We compared the clinical and histopathological features of the leprosy-associated neuropathy with that of a variety of non-leprous neuropathies. We noted that thickened nerves were more frequently observed in the patients with leprosy. We also noted that perineurial, epineurial, and endoneurial inflammatory cell infiltration was significantly more frequent in leprosy-associated neuropathy (P = 0.001). Epineurial thickening and perineurial thickening were more frequent in leprosy-associated neuropathy (P = 0.001). Demyelinating patterns and regenerating clusters were much more frequent in the non-leprous neuropathies (P = 0.001) [Tables 2 and 3].

Discussion

Our most significant finding was that in 36% (20/55) of the patients, leprosy was found as a cause of peripheral neuropathy. Pant and colleagues noted that 4 (5.4%) of the 74 cases had leprosy. In western countries, the spectrum of diseases causing peripheral neuropathy is different. The common causes

| Table 1: Diagnostic etiology based on nerve biopsy (n=55) |
|-----------------------------------------------|
| **Diagnosis based on nerve biopsy** | **Values n (%)** |
| Leprosy | 20 (36.4%) |
| Chronic inflammatory demyelinating polyneuropathy | 4 (7.3%) |
| Hereditary | 2 (3.6%) |
| Vasculitis | 2 (1.8%) |
| Amyloidosis | 1 (1.8%) |
| Non-specific | 26 (47.3%) |

| Table 2: Comparison of the clinical, electrophysiological, and histopathological characteristics between patients with leprosy-associated neuropathy and neuropathies of other causes (n=55; leprosy=20 and other neuropathies=35) |
|-----------------------------------------------|
| **Variables** | **Leprosy n (%)** | **Other neuropathies n (%)** | **P** |
| Age (mean±SD) | 34.90±15.221 | 34.20±14.754 | 0.707 |
| Sex | | | |
| Male n (%) | 19 (43.2%) | 25 (56.8%) | 0.042 |
| Female n (%) | 1 (9.1%) | 10 (90.9%) | |
| Type of neuropathy | | | |
| Mononeuropathy multiplex n (%) | 15 (41.7%) | 21 (58.3%) | 0.378 |
| Symmetrical polyneuropathy n (%) | 6 (26.3%) | 14 (73.7%) | |
| Type of neuropathy | | | |
| Axonal n (%) | 20 (40%) | 30 (60%) | 0.208 |
| Demyelinating n (%) | 0 (0%) | 1 (100%) | |
| Demyelinating with secondary axonal degeneration n (%) | 0 (0%) | 4 (100%) | |
of peripheral neuropathy include diabetes mellitus, nerve injuries, alcohol, toxin exposure, genetic disorders, nutritional deficiencies, vasculitis, and immune-mediated disorders.\[1,5\] Diabetes mellitus, worldwide, is considered the most common cause of peripheral neuropathy. Infective diseases, in addition to leprosy that can cause peripheral neuropathy include Lyme disease, human immunodeficiency virus, and hepatitis.\[6\] The nerve biopsy data from Italy demonstrated a different spectrum of diseases causing peripheral neuropathy. Out of the 717 nerve biopsies, approximately in 50% of the cases, the pathological diagnosis was non-specific axonal polyneuropathy. In this study, the dominant pathological diagnoses were vasculitis, acquired demyelinating neuropathy, and hereditary neuropathies.\[7\]

In our study, leprosy, in approximately one-third of the patients with unexplained peripheral neuropathy, was the cause of nerve disorder. Pure neuritic leprosy is a distinct variety of leprosy that is characterized by the absence of characteristic skin lesions. It has dominantly been described in India but has also been reported from other parts of the world. Pure neuritic leprosy constitutes approximately 10% of all kinds of leprosy. An early diagnosis is crucial as a delay in the diagnosis and treatment can lead to severe disability. A distal symmetrical pattern of nerve involvement is often seen in patients with a lepromatous form of leprosy. In the lepromatous form of leprosy, Mycobacterium leprae harbors in the Schwann cells, endothelial cells, and macrophages.\[8\]

We noted that out of 20 confirmed cases, 80% had demonstrable lepra bacilli in the nerve tissues. The demonstration of lepra bacilli is not always possible on nerve biopsy. A study, from Brazil, noted that out of the 104 biopsied patients, leprosy was confirmed in 89.4% (93/104) of the cases. The presence of lepra bacilli was possible only in four cases. The quantitative polymerase chain reaction was positive in 46 cases (50%).\[9\] Other histopathological characteristics become important to correctly diagnose leprosy on nerve biopsy. We noted that perineurial, epineurial, and endoneurial inflammatory cell infiltration was significantly more frequent in leprosy-associated neuropathy. Hui and colleagues also found that endoneurial inflammation, dense fibrosis, and reduction in the number of myelinated nerve fibers were strongly in favor of leprosy.\[10\]

In isolated instances, we made a histopathological diagnosis of vasculitis, amyloid, hereditary, and inflammatory demyelinating neuropathies. This is important because all these disorders cannot reliably be differentiated from leprosy on clinical grounds alone.\[11\]

In our study, we were not able to confirm a definitive diagnosis in approximately half of the patients despite nerve biopsies.
A much more extensive pre-biopsy workup would have increased the proportion of patients with a confirmed diagnosis of neuropathies. The addition of serum protein electrophoresis, immunofixation, and antinuclear antibody testing would have increased the yield of the pre-biopsy workup. Smith and Singleton in their series of 138 patients noted that a 2-h oral glucose tolerance test was the most rewarding test that provided a diagnostic yield of 61%. Other useful tests were vitamin B12 estimation, serum protein electrophoresis, immunofixation, and antinuclear antibody testing. In this series, the authors were able to confirm an etiological diagnosis in approximately 69% of the cases.[12]

**Conclusion**

Nerve biopsy did help in making the diagnosis of unexplained neuropathy in approximately 50% of the cases. Leprosy was the most common diagnosis among these undiagnosed cases.

### Table 3: Comparison histopathological characteristics between patients with leprosy-associated neuropathy and neuropathies of other causes (n=55; leprosy=20 and other neuropathies=35)

| Histopathological features                      | Leprosy n (%) | Other neuropathies n (%) | P     |
|-------------------------------------------------|---------------|--------------------------|-------|
| Perineurial infiltration                         | 6 (66.7)      | 3 (33.3)                 | 0.059 |
| Epineurial infiltration                          | 10 (83.3)     | 2 (16.7)                 | 0.00  |
| Endoneurial infiltration                         | 20 (69)       | 9 (31)                   | 0.00  |
| Degree of inflammation (moderate to dense)      | 17 (60.7)     | 11 (39.3)                | 0.00  |
| Foamy macrophages                                | 16 (100)      | 0 (0)                    | 0.00  |
| Langerhans giant cells                           | 2 (100)       | 0 (0)                    | 0.128 |
| Granuloma                                        | 4 (100)       | 0 (0)                    | 0.014 |
| Epineurial thickening                            | 14 (87.5)     | 2 (12.5)                 | 0.00  |
| Perineurial thickening                           | 17 (77.3)     | 5 (22.7)                 | 0.00  |
| Endoneurial edema                                | 0 (0)         | 2 (100)                  | 0.529 |
| Reduction in number of myelinated fibers         | 14 (37.8)     | 23 (62.2)                | 0.745 |
| Demyelinating pattern                            | 0 (0)         | 8 (100)                  | 0.041 |
| Perineurial fibrosis                             | 3 (50)        | 3 (50)                   | 0.657 |
| Epineurial fibrosis                              | 2 (50)        | 2 (50)                   | 0.616 |
| Endoneurial fibrosis                             | 3 (50)        | 3 (50)                   | 0.657 |
| Perivascular infiltration                        | 3 (60)        | 2 (40)                   | 0.342 |
| Epineurial vessel wall infiltration              | 1 (100)       | 0 (0)                    | 0.364 |
| Regenerating clusters                            | 0 (0)         | 8 (100)                  | 0.041 |
| Perineurial edema                                | 0 (0)         | 5 (100)                  | 0.147 |
| Onion Bulbs                                      | 0 (0)         | 1 (100)                  | 1.00  |
| Perineurial and Epineurial vessel wall thickening| 0 (0)         | 2 (100)                  | 0.529 |
| Lepra bacilli positivity (%)                     | 16 (100%)     | 0 (0%)                   | 0.00  |

### Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

### Financial support and sponsorship

Nil.

### Conflicts of interest

There are no conflicts of interest.

### References

1. Castelli G, Desai KM, Cantone RE. Peripheral neuropathy: Evaluation and differential diagnosis. Am Fam Physician 2020;102:732-9.
2. Ricci I, Luigetti M, Florio L, Capone F, Di Lazzaro V. Causes of chronic neuropathies: A single-center experience. Neurol Sci 2019;40:1611-7.
3. Gabriel CM, Howard R, Kim SH, Shin HY, Kim SM, Kwon KH, Minn YK. Leprotic neuropathy misdiagnosed as chronic inflammatory demyelinating polyneuropathy. Lepr Rev 2012;83:93-7.
4. Smith AG, Singleton JR. The diagnostic yield of a standardized approach to idiopathic sensory-predominant neuropathy. Arch Intern Med 2004;164:1021-5.