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Histological spectrum of pure neuritic leprosy with atypical clinical presentation at a tertiary care centre

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Summary

Introduction: Pure neuritic leprosy (PNL) is a rare disease and is characterised by isolated involvement of one or more peripheral nerves by Hansen's disease (HD) in the absence of skin involvement. The aim of this study was to assess the histological spectrum of PNL cases with atypical clinical presentation.

Material and Methods: A retrospective analysis of all biopsy proven PNL cases (total 23) over the past 16 years was done. Detailed histopathological examination of the nerve was performed. Myelin and axonal status were evaluated using luxol fast blue/ Periodic acid Schiff (LFB/PAS) stain and immunohistochemistry (IHC) for neurofilament protein (NFP) respectively.

Results: Clinically a diagnosis of HD was suspected in 70%, mononeuritis multiplex in 12%, vasculitis and demyelination in 9% cases each. Moderate to severe epineurial and endoneurial inflammation were seen in 91% and 96% of cases respectively. Granulomatous inflammation was seen both in the endoneurial (70%) and epineurial (17%) location. Foam cell infiltration was more common in the endoneurial (60%) than the epineurial (22%) location. Occasional cases showed necrosis (4%) and vasculitis (13%). Severe myelin and axonal loss was seen in 74%. Leprosy bacilli were identified in 12 (52%) cases. One case showed normal morphology although leprosy bacilli were present.

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Conclusion: Although PNL is known to cause endoneurial inflammation, epineurial inflammation or even granulomas can be seen. Necrosis and vasculitis are rarely seen in PNL. Myelin and axonal loss are almost universal. Even if morphologically the biopsy is normal, staining for leprosy bacilli should be performed on all suspected PNL cases.

Keywords: Hansen's disease, neuritis, granuloma, foam cells, acid fast bacilli, pure neuritic leprosy

Introduction

Leprosy, also called Hansen's disease (HD), is an important public health problem in developing countries including India, despite the fact that its worldwide prevalence has decreased from 5·4 million in the 1980s to a few hundred thousand now.[1] India accounted for 60% of new cases of leprosy detected worldwide in 2015 despite the fact that leprosy has been declared eliminated from India since December 2005.[1]

Skin involvement is almost universal in HD, while some cases show isolated nerve involvement. Pure neuritic leprosy (PNL), defined as neural involvement by HD in the absence of skin involvement, accounts for 4–8% of all HD cases.[2–4] Other names used in the literature to describe this entity include purely neural or poly-neuritic, pure neural, primary neural and primary neuritic leprosy.[4] PNL may be associated with considerable deformity and disability.

Histopathological examination of a peripheral nerve biopsy is the gold standard for diagnosis of PNL, though most of the cases are diagnosed on clinical findings and electroneuromyographical (ENMG) findings.[5] Clinically, peripheral nerves may be thickened in PNL. Nerve conduction studies may show sensory or sensorimotor, axonal, demyelinating, or mixed axonal and demyelinating multiple mononeuropathy or symmetrical confluent peripheral neuropathy.[6] Thus a nerve biopsy is required only in clinically doubtful or challenging cases. The underlying mechanism of neural involvement in HD is an inflammatory process involving the nerve due to *Mycobacterium leprae*. These affected nerves can show well- to ill-formed epithelioid cell granulomas, neural fibrosis, lymphocytic inflammation or foam cells depending on the type of leprosy.[7] As several other infectious and noninfectious granulomatous conditions may mimic HD on histopathology, it is important for a histopathologist to be aware of the histological spectrum of PNL, more so as treatment protocols differ for different conditions. This study was performed to evaluate the histological spectrum of PNL with atypical clinical and electrophysiological findings and to compare various histological parameters between leprosy bacilli positive and negative patients.

Materials and methods

This retrospective study included all histologically proven cases of PNL at the Department of Histopathology in the Post Graduate Institute of Medical Education and Research (PGIMER), Chandigarh between January 2000 and December 2016. Cases which were clinically diagnosed as PNL were not biopsied. As a protocol, all the cases with symptoms suggestive of

peripheral neuropathy are evaluated in detail including routine tests (complete haemogram, detailed biochemistry including renal, liver and thyroid function tests, erythrocyte sedimentation rate, C reactive protein and complement levels, autoantibody profile, Sjogren's work up, serum and urine electrophoresis) and special investigations (nerve conductions studies, paraneoplastic work up including antibody screen and whole body positron emission tomography and nerve biopsy), which are guided by the clinical scenario.

The criteria for diagnosing PNL were as follows- (1) Clinically, the patient presents with peripheral neuropathy. (2) The patient is from endemic area or has a family history of HD. (3) Peripheral nerve thickening on palpation. (4) Typical ENMG abnormalities. (5) Absence of other co-morbidities including diabetes mellitus, autoimmune diseases, paraproteinemia or other systemic disorders. (6) Absence of any skin manifestation. Cases fulfilling all these criteria were clinically diagnosed as PNL and were not biopsied. Cases not meeting all the criteria, but where PNL was clinically suspected, were subjected to nerve biopsy.

All the biopsies were retrieved from the archive and reviewed independently by three histopathologists. A final decision on histopathological findings was reached through consensus. Detailed demographic profile, clinical and electrophysiological findings were recorded from histopathology requisition forms. Histopathological features were noted in each case in detail. Histochemical stains including modified Ziehl-Neelsen stain for detection of leprosy bacilli/AFB (acid fast bacilli) and Luxol Fast Blue/periodic acid schiff (LFB/PAS) stain for myelin were performed in all cases. Axonal damage was assessed using immunohistochemistry for neurofilament protein (NFP). The histological parameters which were assessed included:

1. Epineurial and endoneurial fibrosis
2. Epineurial and endoneurial lymphomononuclear inflammation
3. Epineurial and endoneurial granuloma
4. Epineurial and endoneurial foam cells
5. Perivascular inflammation
6. Vasculitis
7. Necrosis
8. Leprosy bacilli positivity
9. Myelin and axonal loss

All these histological parameters were further graded on a scale from 0 to 3 i.e. (0 - absent, 1 - mild, 2 - moderate and 3 - marked).

Results

Within this study period of 16 years, a total of 76 patients were suspected to have PNL. Among them, 46 showed classical clinical and ENMG findings and met the diagnostic criteria of PNL. Nerve biopsy was not carried out in these cases. Out of the remaining 30 cases suspected as PNL clinically, nerve biopsy showed features of HD in 16 patients, while it was either normal or showed alternate pathology in 14 patients. In addition during this period seven nerve biopsies taken from patients clinically diagnosed with illnesses other than HD, showed histological features of HD. Thus there were 23 (16 suspected and 7 unsuspected) biopsy-proven patients with PNL who were included in the study.

The mean age of the patients was 41 years (range: 22–82 years). Men ($n = 20$) outnumbered women ($n = 3$) by 6·7:1. In 16 (70%) patients, HD was the primary clinical diagnosis, while it was mononeurtitis multiplex of undetermined etiology in 3 (12%), vasculitis in 2 (9%) and chronic inflammatory demyelinating polyneuroradiculopathy (CIDP) in 2 (9%) patients. The sural nerve was the most common nerve biopsied in 16 cases (70%) followed by the dorsal cutaneous branch of the ulnar ($n = 5$; 17%), dorsal cutaneous branch of the radial ($n = 1$) and lateral cutaneous nerve of the forearm ($n = 1$). None of these patients had any cutaneous involvement by HD. A skin biopsy was performed in seven patients who did not reveal any evidence of HD. Slit skin smears were negative in all patients.

Both longitudinal and transverse sections of the nerve were present in 12 cases (52%); transverse section only in 7 cases (30%) and longitudinal section only in four cases (17%). The average number of fascicles noted was five (range: 3–13). Epineurial thickening and fibrosis were present in 22 (96%) cases, while endoneurial fibrosis was noted in 12 (52%) cases (Figure 1A).

Inflammation was noted both in endo and epi-neurial regions. The inflammatory infiltrate was composed of variable proportions of lymphomononuclear cells, foamy macrophages and epithelioid cells, with or without the formation of granulomas. Endoneurial lymphomononuclear inflammation was noted in 22 (96%) patients whereas epineurial lymphomononuclear inflammation was seen in 21 (91%) patients (Table 1).

Evidence of epithelioid cell granulomas was present in 16 (70%) patients. All of them showed granulomas in the endoneurial location (Figure 1B), while four (17.4%) patients

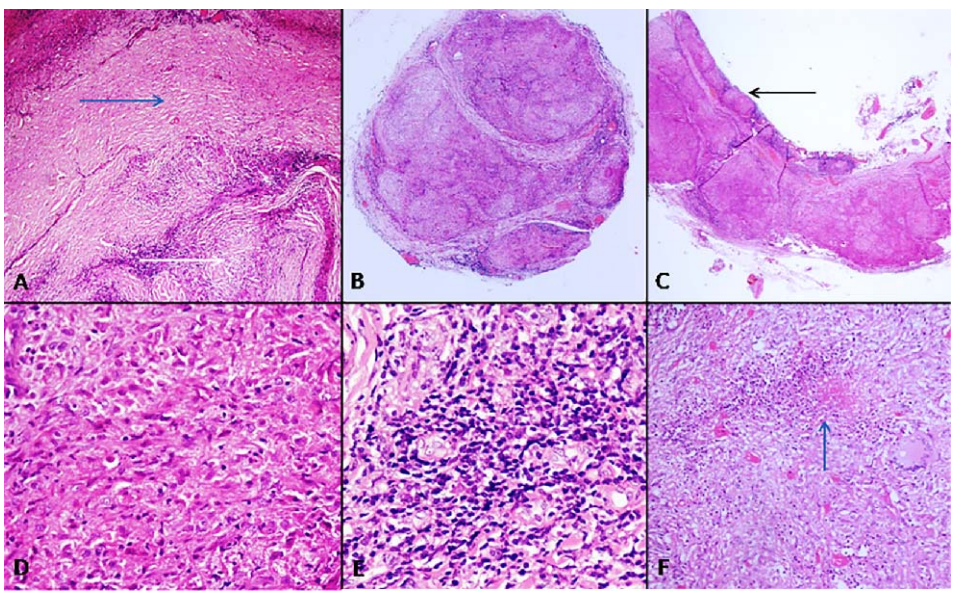

Figure 1. (A) Photomicrograph of nerve biopsy showing marked epineurial (blue arrow) and endoneurial (white arrow) fibrosis (HE, ×100). (B) Transverse section of nerve biopsy showing multiple endoneurial epithelioid cell granulomas (HE, 40). (C) Longitudinal section of nerve biopsy showing multiple endoneurial and few epineurial (black arrow) epithelioid cell granulomas (HE, ×40). (D) Endoneurial foamy macrophage infiltrate in a nerve biopsy (HE, ×400). (E) Nerve biopsy showing moderately dense perivascular infiltrate in the epineurial compartment (HE, ×400). (F) Focal endoneurial fibrinoid necrosis (blue arrow) surrounded by granulomas and Langhans giant cell (HE, ×200).

Table 1. Spectrum of morphological changes in pure neuritic leprosy

Feature	No. of cases	Percentage
Epineurial fibrosis	22	95·6
Endoneurial fibrosis	12	52·2
Epineurial lymphomononuclear infiltrate	21	91·3
Mild	3	13
Moderate	10	43·5
Severe	8	34·8
Endoneurial lymphomononuclear infiltrate	22	95·6
Mild	0	0
Moderate	14	60·9
Severe	8	34·8
Endoneurial granuloma	16	69·6
Epineurial granuloma	4	17·4
Endoneurial foam cells	14	60·9
Epineurial foam cells	5	21·7
Myelin loss	22	95·6
Mild	0	0
Moderate	5	21·7
Severe	17	73·9
Axonal degeneration	22	95·6
Mild	0	0
Moderate	5	21·7
Severe	17	73·9

showed granulomas in the epineurial space as well (Figure 1C). Out of 16 patients having granulomas, 10 showed hyalinised granulomas due to extensive endoneurial fibrosis and hence were difficult to recognise. No case featured isolated epineurial granulomas. Granulomas were seen better by performing serial sections and the examination of multiple sections. None of these cases had any evidence of sarcoidosis elsewhere in the body either clinically or on laboratory evaluation. Foam cell formation was observed in 14 cases (61%). These were predominately endoneurial in all (Figure 1D). Five (22%) patients also showed foam cells in the epineurial space. Only granulomas (in the absence of foam cells) were seen in six (26%) and only foam cells (in the absence of granulomas) were seen in four (17%) patients. Three (12%) patients did not show either of these but showed AFB positivity along with a dense endoneurial and epineurial lymphomononuclear cell infiltrate with variable endoneurial fibrosis. Based on histological features, 16 (70%) patients were diagnosed as borderline tuberculoid (BT) leprosy, four (18%) as borderline lepromatous (BL) leprosy and three (12%) as indeterminate.

Perivascular inflammation was present in 22 (96%) patients (Figure 1E). This involved both epineurial as well as endoneurial vessels. In addition, some cases showed atypical findings such as necrosis (Figure 1F), vasculitis (Figure 2A) and a plasma cell rich infiltrate (Figure 2B).

Vasculitis was seen in three (12%) patients. It involved epineurial arterioles in all, with the presence of fibrinoid necrosis, nuclear debris in the vessel wall, endothelial swelling and extravasation of erythrocytes. All these patients also had endoneurial granulomas and foam cell infiltration, while two patients showed leprosy bacilli. All these cases presented with mononeurtitis multiplex with sensorimotor axonal and demyelinating neuropathy on nerve conduction studies. Detailed work up for systemic vasculitis was negative in all these

patients. One (4%) patient each showed endoneurial necrosis and endoneurial plasma cell rich infiltrate. One (4%) patient who was positive for leprosy bacilli showed normal morphology. Staining for acid fast bacilli was positive in 12 (52%) patients. We compared all the above mentioned histological parameters between leprosy bacilli positive and negative groups. There was no significant histological difference between positive and negative cases (Table 2).

Myelin and axonal losses ($n = 22$; 95·6%) were a universal finding [moderate myelin and axonal loss in five (22%); severe myelin and axonal loss in 17 (74%)] (Figure 2C and D).

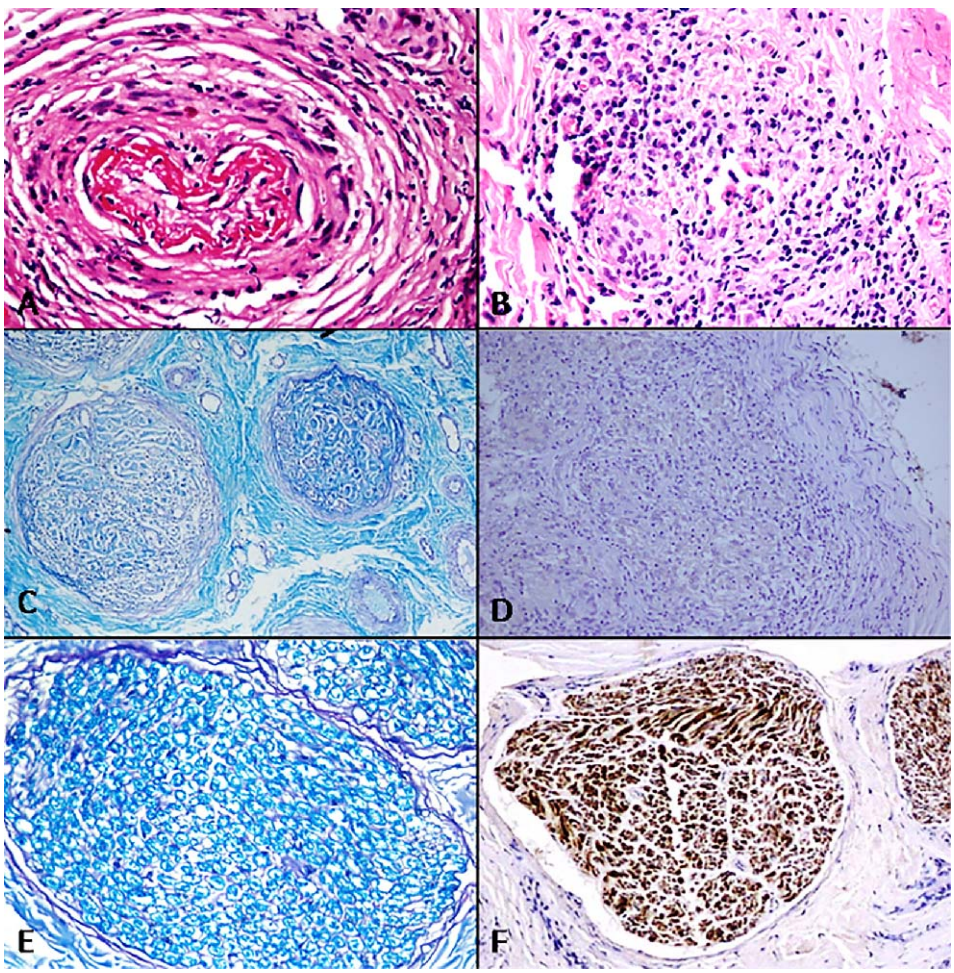

Figure 2. (A) Photomicrograph of nerve biopsy showing the presence of small vessel vasculitis involving an arteriole in the epineurial space (HE, ×400). (B) Presence of plasma cell rich endoneurial inflammation in a case of pure neural leprosy (HE, ×400). (C) Luxol fast blue/periodic acid schiff (LFB/PAS) stain showing extensive loss of myelin (×100). (D) Neurofilamant protein (NFP) immunostain showing complete loss of the axon in pure neural leprosy (immunohistochemistry, ×200). (E) Well preserved myelin in a normal nerve biopsy highlighted by Luxol fast blue/periodic acid schiff (LFB/PAS) stain (×400). (F) NFP immunostain showing closely packed axons in a normal nerve biopsy (immunohistochemistry, ×400).

Table 2. Comparison of AFB positive and negative cases

Parameters	AFB positive cases (12)	AFB negative cases (11)
Epineurial thickening	11 (91·6%)	11 (100%)
Endoneurial thickening	5 (41·6%)	6 (54·5%)
Epineurial lymohomononuclear infiltrate	11 (91·6%)	11 (100%)
Endoneurial lymphomononuclear infiltrate	11 (91·6%)	11 (100%)
Epineurial granuloma	3 (25%)	1 (9·1%)
Endoneurial granuloma	10 (83·3%)	8 (72·7%)
Epineurial foam cells	2 (16·6%)	3 (27·2%)
Endoneurial Foam cells	8 (66·6%)	7 (63·6%)
Perivascular inflammation	11 (91·6%)	11 (100%)
Vasculitis	2 (16·6%)	1 (9·1%)
Necrosis	0	1 (9·1%)
Myelin loss	11 (91·6%)	11 (100%)
Axonal loss	11 (91·6%)	11 (100%)

Discussion

HD continues to be an important public health problem in tropical countries including India, with profound social and economic consequences. Nerve involvement can occur in 60% of HD patients if adequate and prompt therapy is not started. Diagnosis of neural involvement in HD relies on clinical features supplemented by histopathological examination of skin and nerve. In the absence of skin involvement, the diagnosis of PNL remains challenging. Usually these patients present with nerve thickening and tenderness as well as sensory and motor impairment. However, these changes are non-specific and may not be present in all cases. Thus peripheral nerve biopsy remains the gold standard for the diagnosis of PNL. Histopathologists must be aware of the morphological spectrum of PNL. In the current study, HD was suspected clinically in 16 (70%) patients. This stresses the need for a high index of clinical suspicion for HD even in the absence of nerve thickening or other classic clinical features. Some centers prefer cytological examination of the nerve by fine needle aspiration cytology (FNAC) for the diagnosis of PNL. However, FNAC is not recommended as it may miss other conditions involving nerves and FNAC specimens may not give a clear indication as to which compartment of the nerve (epineurium, perineurium and endoneurium) is affected, a finding which is of utmost importance for the diagnosis of peripheral nerve pathology. Thus histopathology continues to be the most important diagnostic tool in these clinically ambiguous cases.

Our study highlights the wide histological spectrum observed in PNL. It also emphasises the involvement of neural compartments with specific histological features, and the importance of special stains and immunohistochemistry (IHC) as an adjunct to routine hematoxylin and eosin (HE) staining for the diagnosis of PNL. The male preponderance observed in our study was in accordance with various other studies, though the reason for male preponderance is unknown.[8–11]

Job CK,[12] Antia *et al.*[13] and Dastur *et al.*[14] studied the histopathology of nerve biopsies from suspected HD patients and reported findings similar to the current study. Jardim *et al.*[15] ($n = 19$) observed epineurial and endoneurial fibrosis to be present in 79% and 74% of PNL patients, respectively. Chimelli *et al.*[16] ($n = 53$) found inflammation, fibrosis and granulomas

in 75%, 66% and 13% of PNL patients, respectively. However they did not comment on specific neural compartment involvement. The current study revealed epineurial and endoneurial fibrosis in 96% and 52% of PNL patients, respectively, which is similar to previously published studies.[15] The current study revealed epithelioid cell granulomas in 70% of patients, mainly in the endoneurium. In addition, four (17%) patients also showed epineurial granulomas. Thus the histological findings of clinically atypical PNL do not differ from PNL with a classical clinical presentation. Classically, the presence of endoneurial granulomas has been described in neural leprosy though there are descriptions of epineurial granulomas also. The presence of granulomas predominantly in the endoneurial compartment is highly suggestive of leprosy and this feature helps in differentiating PNL form other granulomatous inflammatory illnesses like sarcoidosis, which usually produce predominantly epineurial granulomas, although endoneurial involvement can be seen.[17–18] Out of 16 patients with granulomas, 10 showed extensive endoneurial fibrosis producing hyalinized granulomas, even masking them. Thus a careful search for granulomas should be made in such situations, with multiple sections. Chimelli *et al.*[16] found evidence of vasculitis and myelin loss in 3% and 17% of patients, respectively, which is much less than our study.[16] Vasculitis, though rare in HD, has been documented previously.[19–20] It probably represents Type 2 lepra reaction, which may or may not be associated with systemic manifestations. Vasculitis may be focal and patchy, so it may be missed unless the biopsy is examined at multiple serial sections. In the presence of histological features suggestive of vasculitis, the presence of significant endoneurial and epineurial inflammation in addition to vasculitis should raise the suspicion of HD with secondary vasculitis. In this study, we found almost universal loss of myelin and axons in almost all cases. This may be due to the use of sensitive histochemical stains like LFB/PAS for the evaluation of myelin and IHC for NFP for axonal loss. The myelin loss occurs due to damage of Schwann cells caused by the bacilli per se and axonal damage results from the destructive inflammatory process which occurs in these patients.[21] These changes must be incorporated into the final histopathological report to assess the degree of nerve damage by the neurologists/dermatologists. Antunes *et al.*[21] and Hui *et al.*[5] have also emphasised the utility of recognition of various histological parameters in AFB negative cases, similar to our study.

In the current study, we did not observe any significant difference in histology between AFB positive and AFB negative cases. Our results contrasted from those of Antunes *et al.*[21] who reported epineurial infiltrate, endoneurial infiltrate, endoneurial fibrosis and myelin loss to be more significant in the AFB-negative PNL group.[21] This discrepancy may be due to selection bias as we selected only cases of PNL with atypical presentation and the study population does not truly represent the entire PNL cohort.

Conclusion

Endoneurial lymphomononuclear inflammation, granulomas and foam cell infiltrate are histological features which are commonly seen in PNL. However all the features can involve the epineurial space as well. There may be variable epineurial and endoneurial fibrosis, depending on the duration of the disease. Necrosis, vasculitis and plasma cell rich infiltrate, though rare in PNL, may be observed occasionally. Myelin and axonal loss are almost universal, thus myelin staining and immunohistochemistry for Neurofilament Protein should

be performed to assess both parameters. Even in a morphologically normal biopsy, AFB staining should be performed on all clinically suspected PNL cases.

Contribution

DC and GK analysed the data and prepared the manuscript. UN conceived the study idea, examined the skin biopsies, and reviewed the manuscript. DC and BDR analysed the nerve histology. TN, SD and MG provided the clinical information and critically reviewed the manuscript.

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