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Original Article

Study of Correlation of High-Resolution Ultrasonography and Ultrasonography Guided Fine Needle Aspiration Cytology in Diagnosis of Pure Neuritic Leprosy in a Tertiary Care Hospital

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This study assesses the features of high-resolution ultrasonographic and ultrasound-guided FNAC of peripheral nerves and correlates the findings in clinically suspected cases of pure neuritic leprosy (PNL). As per the study protocol, clinically screened pure neuritic leprosy cases from January 2017 to June 2018 were subjected to high resonance ultrasonography and ultrasonography-guided FNAC. The aspirated material was stained with modified ZN stain for AFB. Nerves showed hypoechogenicity, loss or distorted echogenic rim, and fibrillary echotextures in ultrasonography. Epithelioid cells, epithelioid cell granuloma was found in the histopathology sections of FNAC specimens with the presence of AFB in some cases. From these findings it may be concluded that HRUS and ultrasound-guided FNAC could be incorporated as rapid and reliable diagnostic tools for PNL. It may enlighten the future path as an early indicator of neural damage and be critical and useful to prevent the disabilities.

Keywords : Pure Neuritic Leprosy, High Resonance Ultrasonography (HRUS), Ultrasonography Guided FNAC, Newer Investigative Modalities

Introduction

Peripheral nerve involvement is a hallmark of leprosy. The concept of pure neuritic leprosy is well established in India and addressed in the Indian classification (1955) with the revised new IAL classification (1982) as a separate entity, i.e. polyneuritis leprosy.

In developing countries like India, leprosy remains a major health problem, and early diagnosis of PNL is a challenge to the clinician. Though nerve thickening is one of the three cardinal signs for the clinical diagnosis of leprosy by WHO, a more remarkable skill is required to assess nerve thickening, which either may be missed or misdiagnosed sometimes. The invasive nerve biopsy is a risk for further compromising the nerve function. The diagnosis method of PNL by Jardim et al (2003) by nerve biopsy and detection of Anti-PGL-1 detection remains unpopular. To improve the provision of the

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diagnostic procedure, we have done HRUS, and the ultrasonography-guided FNAC, which are safe and sensitive. The HRUS and Colour Doppler are new emerging noninvasive diagnostic tools to confirm the thickening, morphological alteration, exact location of enlargement in the course of the nerve and haemodynamic changes in the nerve, which may be an early prediction of the development of the reaction. Akita et al (2021) observed the usefulness of HRUS and CD, similar to NCS, as a tool to diagnose leprosy reactions. This information also brings a new dimension to the diagnosis of PNL (Kumar & Kar 2015).

Another newer diagnostic modality is USG-guided FNAC, which has the potential to improve the diagnosis without compromising the nerve function, and it is the best alternative to tru cut biopsy (Kumar & Pradhan 2011). Limitations of tru cut biopsy are sampling error, low sensitivity, and permanent nerve deficit. So a more straightforward method is required to evaluate the nerve involvement, especially in pure neuritic leprosy (PNL) (Wilder-Smith 2002). Few studies have evaluated FNAC as a diagnostic tool in PNL (De et al 2017, Reja et al 2003). We could not find any literature showing USG-guided FNAC for the diagnosis of PNL. USG-guided FNAC gives a better picture of nerve involvement than simple FNAC and prevents unnecessary FNAC of the clinically suspected nerve. Thus our study assists in the early detection of nerve function impairment with the help of these newer and safer modalities of diagnosis so that before time intervention can be done.

Methodology

This study was a hospital-based cross-sectional study conducted in the department of dermatology from January 2017 to June 2018 and approved by the institution's ethics committee. All the study subjects signed the written informed consent before enrolment. 32 clinically suspected new cases of pure neuritic leprosy [PNL], i.e. patients with peripheral nerve involvement, either thickening or tenderness and with clinical signs and symptoms of neural deficit, were selected and enrolled in the study. Patients with comorbidity like metabolic disorders, diabetes, uremia, hypercholesterolemia, porphyria, other vasculitic conditions like PAN (Poly arteritis nodosa) and allergic vasculitis, trauma, chronic compressive neuropathy, familial neuropathy, and the patient who were skin smear positive and who had taken MDT earlier were excluded for this study. Detailed clinical history like pain, trauma, duration of disease, mode of onset and progression of symptoms was noted. Examination of sensory, motor & autonomic nervous systems like the sensory deficit, thickness, consistency and regularity of peripheral nerves were done and was procured in a data proforma. Mapping of sensory deficit, neuropathic pain, thickness and tenderness were graded. Slit skin smear as the routine investigation was done from five different sites.

Sonographic examination (High-Resolution Ultrasonography (HRUS) with Colour Doppler of peripheral nerves was done, and the sonographic findings were analysed simultaneously by two radiologists using a linear array transducer with a frequency of 14 to 18 MHz probe with Colour Doppler (Samsung HS70A by SAMSUNG MEDISON CO.LTD). they were blinded from the clinical findings. The following parameters were noted by examining nerves both transversely and longitudinally by changing the direction of the linear array transducer.

1. Cross-sectional area (CSA) - in cm^2 was determined from the site within the inner margin of the hyperechoic rim on transverse sections at a point where the

value is maximum. The measurements thus determined were compared with the set of reference values (Boehm et al 2014).

2. Change in fibrillary echotexture was estimated at multiple sites in the transverse plane. To standardise the measurements, a computerised grey-scale analysis was implemented.
3. Peripheral echogenic rim - maintained/lost was noted.
4. The echogenicity of the nerve- normal/ hypoechoic/hyperechoic was observed. (Figures 1 to 3)
5. Blood flow in colour doppler- epineural or Intra fascicular blood flow - present/absent was detected. (Figure 4)

Two radiologists did ultrasound-guided FNAC of nerves. Smears were examined for cellular structure and AFB after staining with modified Ziehl Neelsen (ZN stain) and Hematoxylin & eosin stain (H & E Stain).

The obtained data were tabulated in a Microsoft Excel worksheet. Descriptive statistics with mean, frequency and percentage were calculated. Statistical analysis was done by IBM-SPSS statistics Version-21 2012. The chi-square value & kappa value were calculated.

Results

Clinical observation of nerves is tabulated in Table 1. The most common nerve involvements were ulnar and common peroneal, with ulnar nerve involvement being more common.

Table 1 : Clinical Observation of nerves

Nerve	Side	Thickened	%age	Total thickened	Tender	%age	Total tender	Painful	%age	Total painful
Ulnar nerve	Right	17	53.1%	32 (50%)	9	28.1%	19 (29.7%)	3	9.4%	10 (15.6%)
	Left	15	46.9%		10	31.3%		7	21.9%	
Median nerve	Right	3	9.4%	4 (6.2%)	1	3.1%	1 (1.6%)	0	0	0
	Left	1	3.1%		0	0%		0	0%	
Common peroneal nerve	Right	10	31.3%	20 (31.2%)	5	15.6%	7 (10.9%)	0	0%	0
	Left	10	31.3%		2	6.3%		0	0%	
Posterior tibial nerve	Right	2	6.3%	5 (7.8%)	0	0%	1 (1.6%)	0	0%	1 (1.6%)
	Left	3	9.4%		1	3.1%		1	3.1%	
Great auricular nerve	Right	8	25%	12 (18.7%)	2	6.3%	2 (3.1%)	1	3.1%	1 (1.6%)
	Left	4	12.5%		0	0		0	0	
Total		73	22.8%	73	30	9.4%	30	12	3.7%	12

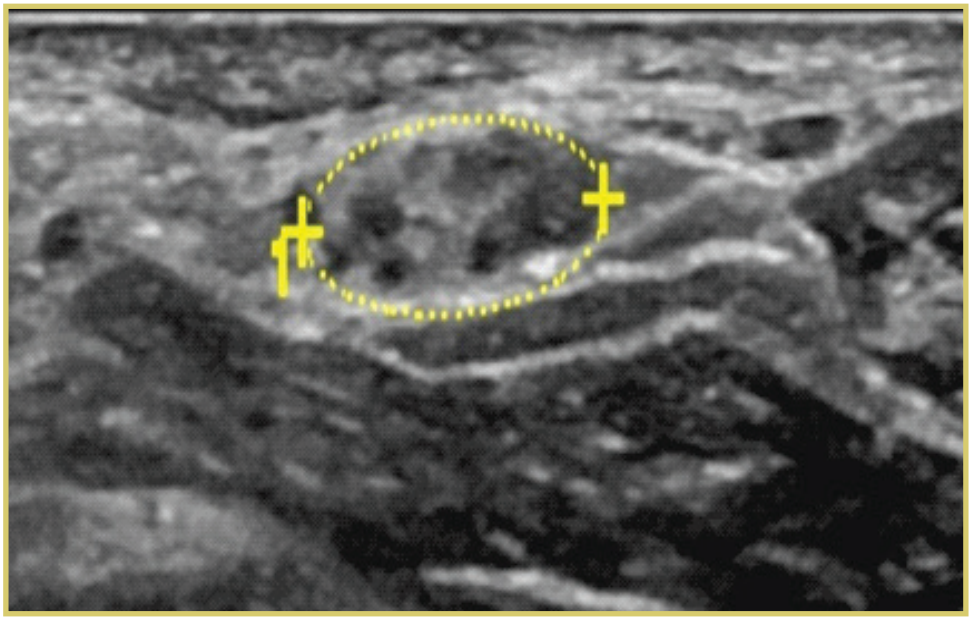

Figure 1 : Image showing the normal HRUS findings of an uninvolved nerve

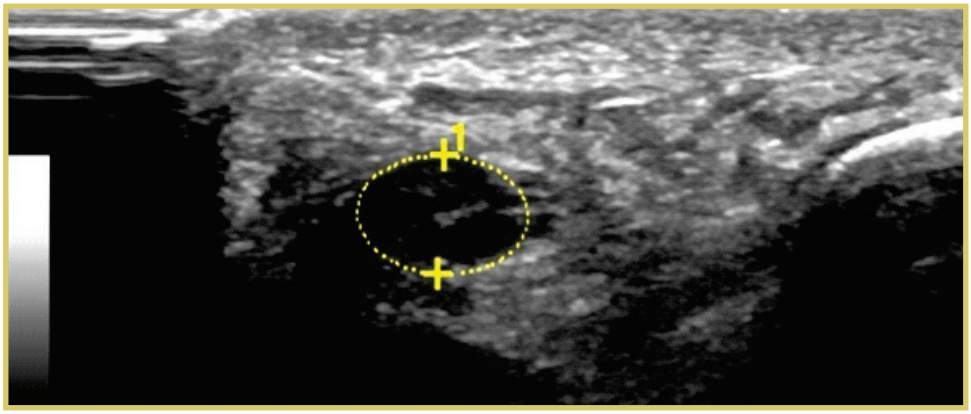

Figure 2 : 14-18 MHz Sonogram showing hypoechoic nerve with loss of fibrillary echotexture and distorted echogenic rim in transverse view.

Among the ulnar nerves, 17(53.1%) nerves were thickened, 9(28.1%) nerves were tender on right side, and 15(46.9%) nerves were thickened, 10(31.3%) nerves were tender on left side.

Table 2 shows HRUS findings of individual nerves.

Increased cross-sectional area (CSA) of the right ulnar nerve was found in 13(40.6%) cases. All these nerves have lost internal fibrillary echo-texture, but one nerve (3.1%) showed hyperechogenicity (Figure 3). Decreased

Table 2 : HRUS findings of individual nerves

Nerve	Side	Increased CSA	Loss of fibrillary echo-texture	Echogenicity		Echogenic Rim Lost	Blood flow in CD
				Hypo-echoic	Hyper-echoic		
Ulnar nerve	Right	13(40.6%)	14(43.8%)	12(37.5%)	2(6.3%)	14(43.8%)	7(21.9%)
	Left	13(40.6%)	10(31.3%)	10(31.3%)	0	10(31.3%)	9(28.1%)
Median nerve	Right	6(18.8%)	5(15.6%)	5(15.6%)	0	5(15.6%)	0
	Left	2(6.3%)	1(3.1%)	1(3.1%)	0	1(3.1%)	0
Common peroneal nerve	Right	8(25%)	6(18.8%)	7(21.9%)	0	7(21.9%)	2(6.3%)
	Left	9(28.1%)	5(15.6%)	6(18.8%)	0	4(12.5%)	2(6.3%)
Posterior tibial nerve	Right	0	0	0	0	0	0
	Left	1(3.1%)	2(6.3%)	2(6.3%)	0	2(6.3%)	1(3.1%)
Great auricular nerve	Right	4(12.5%)	4(12.5%)	4(12.5%)	0	4(12.5%)	4(12.5%)
	Left	2(6.3%)	2(6.3%)	0	2(6.3%)	2(6.3%)	0
Total		58 (18.12%)	49 (15.31%)	47 (14.7%)	4 (1.25%)	49 (15.31%)	25 (7.81%)

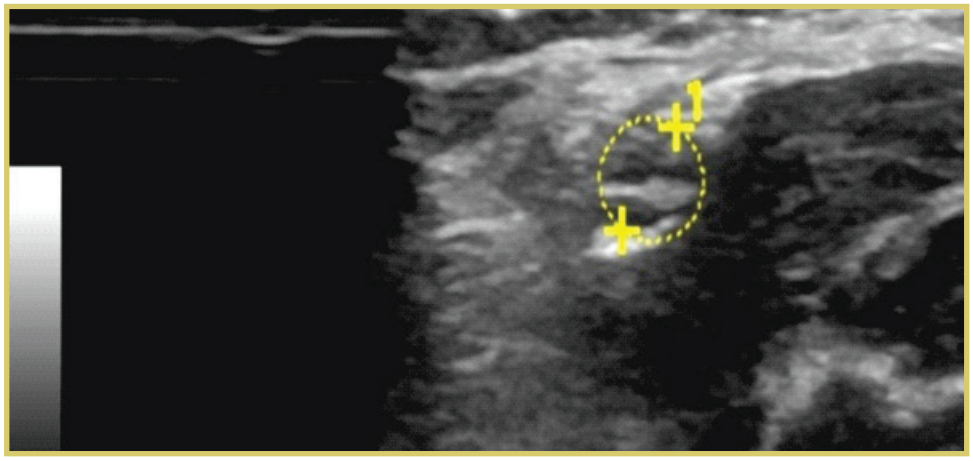

Figure 3 : 14-18 MHz Sonogram showing hyperechoic nerve with loss of fibrillary echotexture in transverse view

echogenicity, i.e. hypoechoic pattern, was observed in 12(37.5%) nerves and hyperechoic pattern in 2(6.3%) nerves. In all these nerves echogenic rim was distorted (Figure 2); however, blood flow in colour doppler was observed in only 7(21.9%) nerves. Increased left ulnar nerve CSA was seen in 13(40.6%) cases, but the loss of fibrillary pattern, decreased echogenicity,

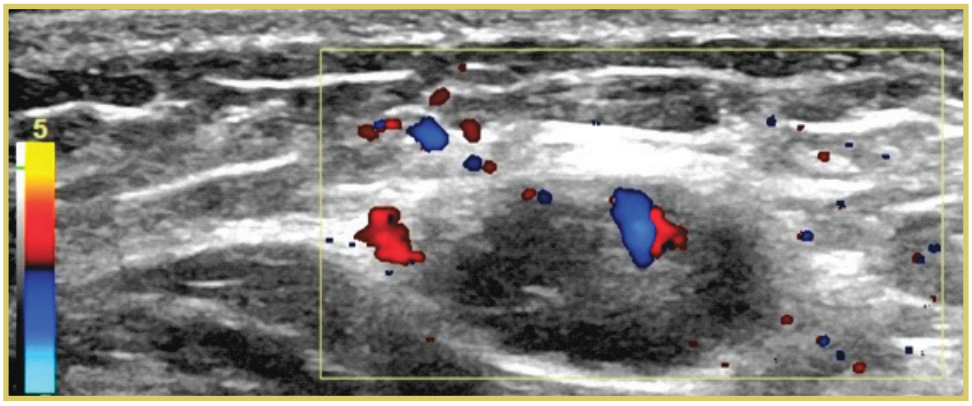

Figure 4 : The image shows perineural and intraneural blood flow in the colour doppler of Figure 2.

Table 3 : Clinical thickness and High-Resolution USG findings correlation

Nerve	Side	Increased CSA	Loss of fibrillary echo-texture	Echogenicity		Echogenic Rim Lost	Blood flow in CD
				Hypo-echoic	Hyper-echoic		
CSA	Thickened	48	65.8	10	4	144.57	<0.001
	WNL	25	34.2	237	96		
Fibrillary echotexture	Lost	43	58.9	6	2.4	138.58	<0.001
	maintained	30	41.1	241	97.6		
Echogenicity	Hyperechoic	3	4.1	1	0.4	147.90	<0.001
	Hypoechoic	42	57.5	5	2		
	WNL	28	38.4	241	97.6		
Echogenic rim	Lost/ distorted	43	58.9	6	2.4	138.58	<0.001
Maintained Blood flow in CD	Present	25	34.2	0	0	91.758	<0.001
	Absent	48	65.8	247	100		

and distorted echogenic rim was observed in only 10(31.3%) nerves. Blood flow in colour doppler was only seen in 9(28.1%) nerves. Increased right median nerve CSA was seen in 6(18.8%) cases and decreased fibrillary echotexture, hypoechogenicity, and distorted echogenic rim were observed in 5(16.6%) cases. However, none of the nerves showed blood flow in colour doppler. Increased CSA of the left median nerve was observed in 2(6.3%) cases. Decreased echogenicity, loss of internal fibrillary echotexture and distorted echogenic rim around

Table 4 : FNAC findings

Stain	Findings		No.	%age
H & E Stain	Cellularity	Necrotic Material	2	6.3
		Plenty	16	50
		Few	13	40.6
		Acellular	1	3.1
	Nerve Fragments	Present	18	56.3
	Lymphocytes	Present	29	90.6
	Polymorphs	Present	7	21.9
	Epithelioid Cells	Present	18	56.3
	Granuloma	Present	10	31.3
	Giant Cells	Present	4	12.5
Modified Z-N Stain	AFB-	Present	4	12.5
	Morphology	Single	3	75
		Few	1	25
	Number	Solid	2	50
		Fragmented	2	50

nerves were seen in 1(3.1%) case only. Blood flow in colour doppler was not detected in any nerves. Increased CSA of the right common peroneal nerve was observed in 8(25%) cases, and loss of fibrillary echotexture in 6(18.8%) cases, but the hypoechoic pattern and distorted echogenic rim was observed in 7(21.9%) cases. Blood flow in CD was observed in 2(6.3%) nerves only. CSA of the left common peroneal nerve was increased in 9(28.1%) cases. Fibrillary echotexture was found to be lost in 5(15.6%) cases, hypoechoic pattern in 6(18.8%) cases, distorted echogenic rim in 4(12.5%) cases and flow in Colour Doppler in 2(6.3%) cases were noted. None of the right-sided posterior tibial nerves was found to be involved in any patients. CSA was increased in 1(3.1%) case of the left posterior tibial nerve, but the distorted fibrillary pattern and hypoechogenicity were observed in 2(6.3%) cases. Blood flow in CD was found in 1(3.1%) case**.** A total of 25 nerves had increased blood flow in colour doppler (Figure 4).

Correlating observed thickness by clinical examination with the increased cross-sectional area (CSA) by HRUS examination, the correlation was found to be significant ($p<0.001$). The measure of agreement, i.e. kappa value between these correlations, is 0.113. The results are summarised in Table 3. 25 (34.2%) nerves out of 73 nerves which show normal CSA by HRUS were taken thickened clinically, whereas 10 (4%) nerves out of 58 nerves which lead increased CSA as measured in HRUS were taken to be within normal limits clinically. The correlation between clinically thickened nerves and loss of fibrillar echotexture was significant ($p<0.001$). Of 73 thickened nerves, 43(58.9%) nerves have lost fibrillar echotexture, and 30(41.1%) nerves have

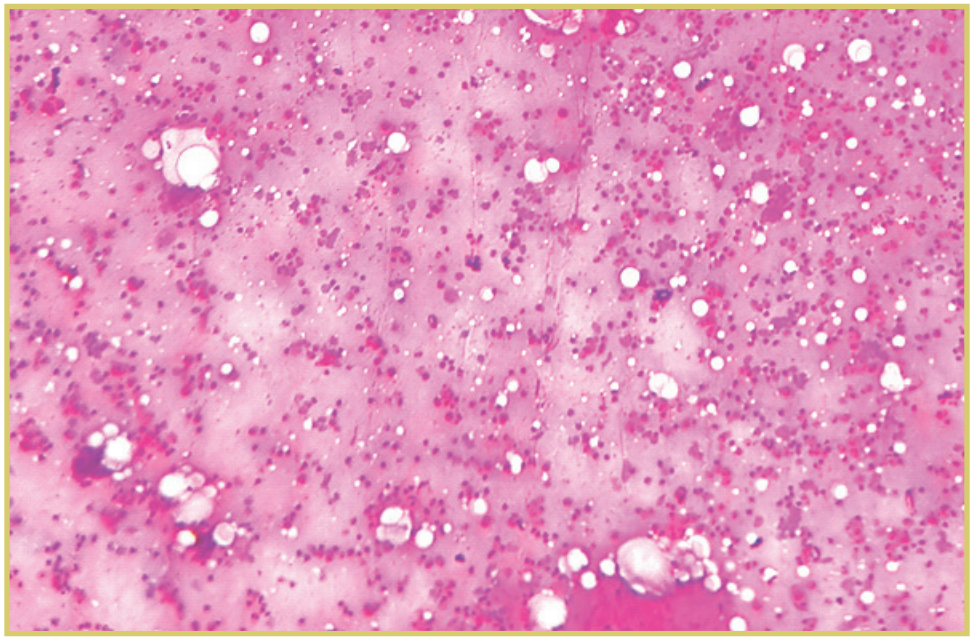

Figure 5 : Image showing high cellular aspirate, plenty of lymphocytes, and epithelioid cells in H&E stain (40x)

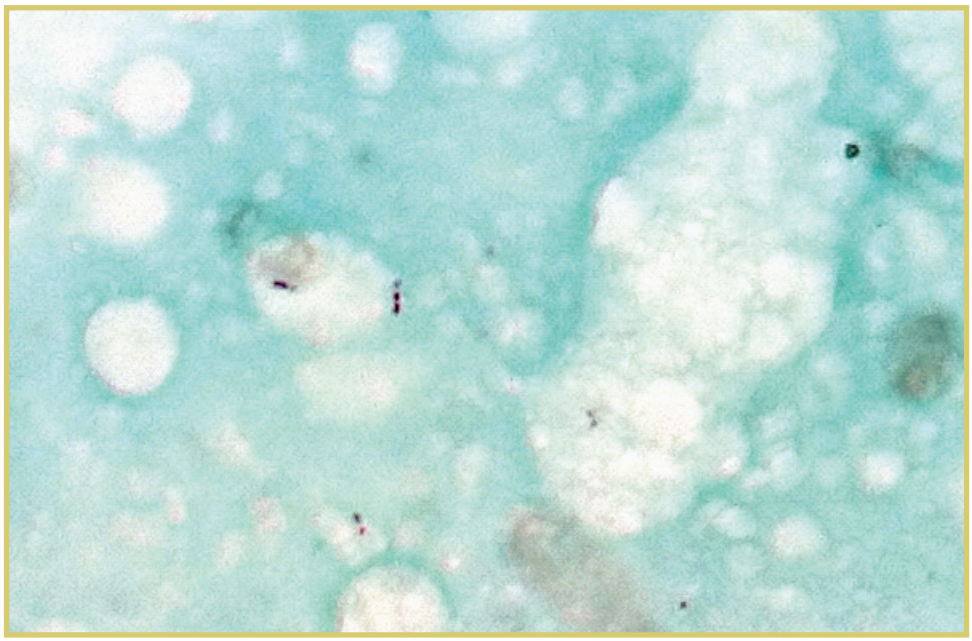

Figure 6 : Smear showing multiple fragmented acid-fast bacilli (AFB stain, oil immersion)

Table 5 : Clinical neuritis grade and FNAC findings correlation

Clinical neuritis	No. of cases	FNAC findings					
		H&E stain					AFB
		Cellularity		Epitheliod cells	Epithelioid granuloma	Macrophage granuloma	
		plenty	few				
Grade 0	8 (25%)	1 (12.5%)	4 (50%)	1 (12.5%)	1 (12.5%)	0	0
Grade I	19 (59.4%)	11 (57.8%)	8 (42.1%)	12 (63.1%)	6 (31.6%)	0	4 (21%)
Grade II	5 (15.6%)	4 (80%)	1 (20%)	5 (100%)	3 (60%)	0	0
GradeIII	0	0	0	0	0	0	0
Total	32	16 (50%)	13 (40.6%)	18 (56.2%)	10 (31.2%)	0	4 (12.5%)

Table 6 : High-Resolution USG Echogenicity and FNAC findings correlation

HRUS Echogenicity	No. of cases	FNAC findings					
		H&E stain					AFB
		Cellularity		Epitheliod cells	Epithelioid granuloma	Macrophage granuloma	
		plenty	few				
Hyperechoic	2 (6.3%)	0	2 (100%)	1 (50%)	1 (50%)	0	0
Hypoechoic	30 (93.7%)	16	11 (36.7%)	17 (56.7%)	9 (30%)	0	4 (13.3%)
Total	32	(53.3%)	13 (40.6%)	18 (56.2%)	10 (31.2%)	0	4 (12.5%)

normal echotexture. There were 6(2.4%) nerves which have lost fibrillar echotexture despite being clinically not thickened. Correlating thickness by clinical examination with echogenicity, the correlation was significant ($p<0.001$). While 42(57.5%) nerves were hypoechoic in the thickened category, 5(2%) were not thickened but still hypoechoic. 3(4.1%) nerves were hyperechoic and thickened, and 1 (0.4%) nerve was hyperechoic, but thickness was within normal limits. 28(38.4%) nerves were clinically thickened, but echogenicity was within normal limits. Correlating the clinical thickness with a distorted echogenic rim around nerves, the correlation was significant ($p<0.001$). There were 43(58.9%) nerves which were thickened and had distorted echogenic rims, 30(41.1%) nerves that were clinically thickened but still the echogenic

rim was preserved. 6(2.4%) nerves were not thickened, but the echogenic rim was distorted. Correlating thickness clinically to colour doppler (CD), the correlation was found to be significant ($p<0.001$). Blood flow was present in 25(34.2%) clinically thickened nerves and was absent in 48(65.8%) thickened nerves.

FNAC findings are tabulated in Table 04. Hematoxylin and Eosin stain of fine needle aspirate showed plenty of cellularity in 16(50%) cases, few cellularities in 13(40.6%) cases, and necrotic material in 2(6.3%) cases. The aspirate was acellular in 1(3.1%) case. Fragments of nerves were seen in 18(56.3%) cases and Schwann cells in 13(40.6%) patients. In the aspirate, lymphocytes were seen in 29(90.6%) and polymorphs in 7(21.9%) cases. Epithelioid cells in 18(56.2%), granuloma in 10(31.3%), and giant cells in 4(12.5%) patients were observed (Figure 5).

In modified Z-N staining of the aspirate, AFB was seen in only 4(12.5%) cases. Of those 4 cases, single bacilli were seen in 3(9.4%) cases and few bacilli in 1(3.1%) case. In 2(50%) cases, the morphology of bacilli was found to be solid, and in 2(50%), it was fragmented. The morphology of bacilli was solid in 2(50%) cases and fragmented in 2 cases (50%) (Figure 6).

The correlation between clinical neuritis grade and FNAC findings is tabulated in Table 5. Most of the patients n=19 (59.4%) had grade I neuritis in which plenty of cellularities was found in 11(57.8%) nerves, epithelioid cells in 12(63.1%) nerves, epithelioid granuloma in 6(31.6%) nerves in H&E stain. AFB was present in 4(21%) nerves in grade I neuritis.

HRUS echogenicity and FNAC findings correlation are noted in Table 6. Out of 32 patients, 30 patients showed hypoechoic texture with plenty of cellularity in 16 cases, epithelioid cells were found in 17(56.7%) cases, epithelioid granuloma in 9(30%) cases and AFB were found in 4 (13.3%) patients.

Discussion

In India, the incidence of PNL (Pure Neuritic Leprosy) has been reported to range from 5.5% to 18% of leprosy cases (Wilder-Smith 2002). Though this constitutes a considerable fraction of total leprosy patients, diagnosis of PNL has been purely subjective in the absence of simple and less invasive laboratory investigation. In our study, we tried to find out simple and effective diagnostic tool for PNL by combining High-Resolution Ultrasonography with ultrasound-guided FNAC. HRUS is a noninvasive modality to study structural changes, exact location, severity and extent of involvement of peripheral nerves that cannot be biopsied for histopathology (Gupta et al 2016). There is growing interest in ultrasonography as a diagnostic tool for diseases of the peripheral nervous system, including mononeuropathies and polyneuropathies (Beekman & Visser 2004, Beekman et al 2004). There is considerable inter-observer variability in assessing the presence of an enlarged nerve by palpation (Chen et al 2006). Furthermore, an inter-observer agreement between sonographic measurements is excellent (Beekman et al 2004).

In our study, out of 320 nerves, 73(22.5%) nerves were thickened clinically, and 58 (18.12%) nerves had increased CSA in HRUS. Venugopal et al (2021) also observed out of the 320 nerves, 71 (22.18%) were clinically involved, which is close to our study findings. Of the 320 peripheral nerves, 63 (19.7%) were sonologically abnormal. In a study by Ashwini et al (2018), out of the 210 nerves examined, 86 (41%) were clinically thickened, and 138 (65.7%) were sonologically thickened. Jain et al (2009) examined 152 nerves in leprosy patients; 50% of the nerves had normal

echotexture, and demonstrated a significant correlation between CSA and clinical thickness [P= 0.001]. They also showed 39 out of 152 nerves [26%] had endoneural or perineural blood flow demonstrating increased neural vascularity by colour doppler. 80% of the nerves had increased blood flow signals without being enlarged. In our study, we observed 25(7.81%) nerves had increased blood flow. Fibrillary echotexture and echogenic rim loss were observed in 49(15.31%) nerves. When compared with thickened nerves, the number was 43 out of 73 thickened nerves.

Lawande et al (2014) described a radiological picture of a normal nerve. The transverse section showed small hypoechoic areas separated by hyperechoic septae, giving a "honeycomb-like" appearance. The hypoechoic area represented nerve fascicles, whereas the echogenic septae represented interfascicular perineurium. The longitudinal sections also revealed the fascicular architecture, showing a "bundle of straws" appearance. The nerves show sliding movement over the muscles and tendons on dynamic examination. An altered movement or contour deformity during nerve movement gave a clue to diagnose the pathology.

Nerve biopsy is the gold standard for diagnosis of PNL, but complications and permanent nerve damage may occur. FNAC of the thickened nerve may be a lesser intrusive and generally more secure demonstrative methodology than nerve biopsy in cases of PNL.

Vijaikumar et al (2001) reported a 92% diagnostic yield from FNAC nerve in PNL cases; the reported yield by various authors ranges from 38.7% to 66.6% (De et al 2017, Reja et al 2003). Sandhu et al (2021) found FNAC of nerve in PNL to be diagnostic in 10 (37%) cases out of 27 cases. In the remaining 17 (63%) cases, the aspirates were paucicellular, and no conclusive diagnosis on cytology was possible. We found plenty of cellularity in 16 cases, epithelliod cells in 18 cases, epithelliod granuloma in 10 cases and AFB in 4 cases out of 32 cases.

The nerves being an immune-privileged site, *M leprae* in nerves may not be recognised immunologically, leading to a higher bacillary density inside nerve lesions than in skin histology (Ridley & Ridley 1984). This emphasises the significance of FNAC of nerve in the precise classification of the cases as a higher range was seen in nerve FNAC than the skin biopsy.

Conclusion

PNL is a diagnostic challenge. With increasing awareness, HRUS and FNAC become additional tools to improve the diagnosis. Our study has a significant correlation between clinical observation and HRUS findings. The presence of epitheloid cells and epitheloid cell granuloma on FNAC shows a good correlation with HRUS and clinical observations.

Moreover, the correlation of FNAC with the hypoechogenicity of nerve on HRUS increases the importance of USG-guided FNAC for getting a higher probability of cytological findings from hypoechoic focus minimising trauma. In the absence of skin lesions, HRUS and USG-guided FNAC are the best options for early diagnosis and timely management of new disability.

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