Egyptian Journal of Medical Microbiology Volume 30 / No.1 / January 2021 45-52 Online ISSN: 2537-0979

ORIGINAL ARTICLE

The value of Real time PCR and High Resolution Ultrasound in Diagnosis of Suspected Pure Neural Leprosy

[1]Mohammad A. Rashed, [1]Fatma Eldesoky, [1]Ahmed G. Selim, [2]Hamed A. Gobran, [3]Nissreen E. ELBadawy

[1]Departments of Dermatology and Venereology, Faculty of Medicine, Zagazig University
[2]Diagnostic Radiology, Faculty of Medicine, Zagazig University
[3]Medical Microbiology & Immunology, Faculty of Medicine, Zagazig University

Key words:
Pure neural leprosy, real time, PCR, High resolution ultrasound

Corresponding Author:
Nissreen Elbadawy
Assistant professor of medical microbiology & Immunology
Faculty of Medicine, Zagazig University
Tel.: 01010200956
nissreenbadawy77@gmail.com

ABSTRACT

Background: *Cases with pure neural leprosy (PNL) are difficult in their diagnosis and usually loss the early management opportunity.* ***Objective:*** *to evaluate Real time PCR and high resolution ultrasonography of peripheral nerves as diagnostic tools in PNL.* ***Methodology*** *The study included 25 suspected PNL and 25 healthy controls. All patients were subjected to ultrasonography cross-sectional area (CSA) measurement. Fine needle aspiration (FNA) was obtained, and followed by Ziehl-Neelsen (ZN) staining, and real time PCR.* ***Results:*** *ZN staining demonstrated M leprae bacilli in only 8 cases while their DNA was detected in 92 % cases (Sensitivity of both were 100%, and 44.4%, respectively). Only the left median and right and left posterior tibial nerves CSA measurements showed good performance in distinguishing patients of PNL. Median nerve cut off=15.5 mm^2, with a sensitivity of 72%), and right and left posterior tibial nerves CSA Cut Off were 12, 11.5 mm^2 with sensitivity of 76-84%.* ***Conclusions:*** *ultrasonography of suspected nerves and real time PCR of nerve aspirates are simple accurate tests for diagnosis of PNL.*

INTRODUCTION

Leprosy or Hansen's disease is a granulomatous disease caused by *Mycobacterium Leprae (M. leprae)*[1]. It is considered as an ancient disease affecting humanity since thousands of years and regardless of all the efforts, the disease remains a major healthcare distress in many underdeveloped and developing countries[2].

In the last twenty years, more than sixteen million people have been cured for leprosy globally[3]. Classification of leprosy depends upon the cell mediated and humoral immune responses of the host, from tuberculoid to lepromatous stage [4].

Leprosy is commonly associated with nerve inflammation. The nerves swelling as a result of inflammation may be the principal of neuropathy that causes neurological deficits. Late diagnosis of these cases may lead to loss of opportunity of management and resolving [5]. Moreover, PNL, defined as a peripheral neuropathy in which the patient has no skin lesions, is difficult to diagnose [6].

PCR could be used for diagnosis of PNL and depends on amplification of specific sequences of *M. leprae* genome, either by conventional PCR techniques [7] or other molecular methods as nested-PCR, total genomic amplification, and real-time PCR. Real-time PCR allows achieving a better result as a rapid, sensitive, specific and quantification technique [8].

The study of infection by *M. leprae* using PCR has been conducted in multiple sources of samples such as slit skin smear, skin biopsy fragments, nasal swab, oral mucosa, urine, nerve; blood, lymph node, and hair [9]. There are specific *M. leprae* DNA targets that has been identified by amplification techniques including Proline-rich antigen (pra-36 KDa), 18-kDa antigen, Ag 85B, 65-kDa antigen, 16S rDNA and complex 85 [10].

High-resolution ultrasonography (HRUS) in cases with PNL is safe, less risky technique but less accurate than histological investigation and also requires a matching information from the clinical characterization[11]. It was proposed that HRUS can describe the involved nerve and determine the neurological manifestations [12].

The current research aimed to assess the role of Real time PCR and high resolution ultrasonography of peripheral nerves as a new additional trustable diagnostic tool in detection of cases with PNL.

METHODOLOGY

Study design:

The present work is a case control study and performed at Medical Microbiology and Immunology laboratory, Zagazig University Hospitals and Mansoura Leprosy Specialized Hospital from October, 2015 to October, 2019. Informed consent was obtained from all

DOI : https://doi.org/10.51429/EJMM30106

patients and the study protocol was approved by the local ethical committee IRB #2100/10-5-2015.

Study groups:

The study involved 25 subjects with suspected PNL and 25 healthy controls. Inclusion criteria of suspected PNL include cases with sensory only or both affection of ≥ one of these nerves ulnar, median, posterior tibial and/or great auricular nerves without neurological confirmed diagnosis and negative investigations of leprosy.

Exclusion criteria were Patients with systemic or neurological diseases affecting nerve morphology or physiology. Exclusion criteria of controls were free on examination and not contacts to leprosy patients.

All patients were subjected to 1) sonography (NCS), 2) fine needle aspiration (FNA) from the peripheral nerves followed by Ziehl-Neelsen (ZN) staining of the aspirates, and 3) extraction of DNA from the FNA samples, followed by real time polymerase chain reaction.

***Clinical characterization*:**

All participants were subjected to careful history taking. The ulnar, median, posterior tibial and great auricular nerves were examined for neurological functions in case and control groups. Each subject was screened for the current symptoms of specific nerve lesion, i.e., numbness, hypothesia, anesthesia, weakness and paralysis. For sensory testing we used Semmes-Weinstein monofilaments which is a clinical test that measures touch sensation in response to monofilaments ranged from 0.86 gm to 448gm. The test done by holding monofilaments to skin surface in perpendicular manner with smooth, steady motion for 1 to 2 seconds. Sensory loss was determined by the patient inability to perceive 2 grams of target force on the finger [13]. Grading of disability was done according to the world health organization (WHO) classification as grade 0: no impairment – grade1: sensation loss and absence of any visible impairment in the extremities, grade2: visible impairment in the hands and/ or feet [14].

Nerve Ultrasonography CSA measurement (HRUS):

The ulnar, median, posterior tibial, great auricular nerves were imaged by HRUS blinded to the clinical findings of the cases in all groups. The nerves transverse sections were measured at the point of maximum thickness.

Fine needle aspiration biopsy and ZN staining:

HRUS guided fine needle aspiration (FNA) using sterilized 5ml syringes was obtained from suspected involved nerves of cases with suspected neural leprosy. Biopsies were collected in sterilized Eppendorf tubes containing buffer media and examined with ZN staining[15]. The aspirate was immersed in carbol fuchsin solution and heated for 30 minutes at 63^{o} C in the oven. Then, decolorizing with 0.3% acid alcohol, and counterstaining with methylene blue by 5-6 dipping, the acid-fast bacilli stain red to bright red [15].

Each of the specimen was read by two independent evaluators under oil immersion microscopy. The sample is considered positive when ≥ one acid-fast bacteria is even detected in a single part of the tissue sample [15].

***Genomic DNA Extraction and Real time PCR*:**

Genomic DNA was extracted from the FNA sample aspirates, using i-genomic BYF DNA Extraction Mini Kit (Korea iNtRON Biotechnology, Korea) according to manufacture instructions. Quantification of DNA was determined by optical density at 260 nm (A260) in a spectrophotometer. An absorbance of 1 unit corresponds to 50 µg genomic DNA per ml. Purity of DNA was checked by calculation of the ratio of the readings at 260 nm and 280 nm (A260/ A280). Pure DNA has an A260/ A280 ratio of 1.7–1.9.

For PCR examination, a specific primer (RNA polymerase beta subunit (rpo B)) and probe mix was used (primerdesign™ genesig® Kit for Leprosy, UK). Each time the kit was used, a positive control reaction must be incorporated in the run. Positive control was DNA template of *M. lepra*e provided by the kit and also generated the standard curve of Leprosy copy number / CT value. Negative control was included by addition of RNAse/DNAse free water instead of template [16]. The PCR mixture was run on the Stratagene Mx3000P system (Agilent Tech., Washington, USA).

The test sample result was calculated using the delta CT method by subtracting the target CT value from the negative control CT value (NC CT value – sample CT value). The sample was considered positive when it exhibited Ct ≤ 38.5 in first fluorescent signal detection Cycle Threshold (C_T)[16]

Statistical Analysis

Data derived from the current study are expressed mean ± standard deviation (SD) or number and percent. Comparison between numerical variables was obtained using one-way ANOVA while categorical data were differentiated using chi-square test. Receiver operator characteristic (ROC) analysis was applied to characterize the diagnostic value of HRUS and real time PCR in detection of neural leprosy.

RESULTS

The demographic characteristics of the studied groups are shown in table 1. No differences of statistically significance were observed between the studied groups regarding age and sex distribution. Clinically, in patients with PNL, positive reaction was reported in 72.0% of patients. disability type was sensory in sixty-four percent of the cases, motor in only four percent and mixed in thirty-two percent of cases. The disability grade was grade I in 64% of these patients and grade II in 9 patients (36.0 %) (tables 1, 2, figure 1).

Table 1: Demographic characteristics in the studied patients

	PNL n=25	**Controls n=25**	**P value**
Age (years) mean ± SD	44.0 ± 12.4	37.8 ± 12.9	0.22
Male	16 (64.0)	14 (56.0)	0.57
Female	9 (36.0)	11 (44.0)	

PNL: pure neural leprosy

Table 2: Leprosy reaction and disability type and grade in patients with pure neural leprosy (n=25)

Clinical reaction	**n (%)**
Leprosy reaction n (%)	
+ve	**18 (72.0)**
-ve	**7 (28.0)**
Disability type n (%)	
Sensory	**16 (64.0)**
Motor	**1 (4.0)**
Mixed	**8 (32.0)**
Disability grade n (%)	
I	**16 (64.0)**
II	**9 (36.0)**

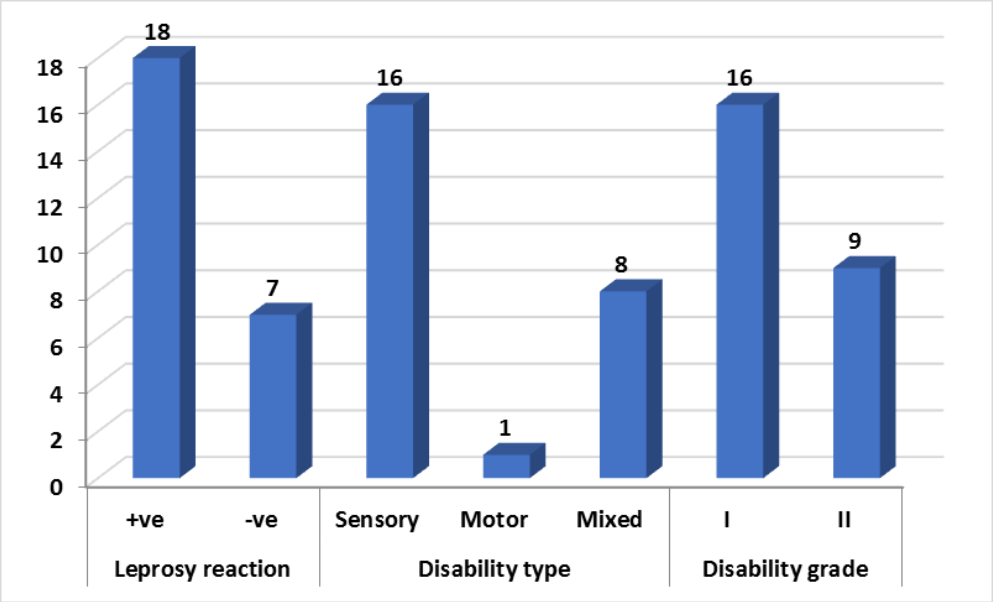

Fig. 1: Leprosy reaction and disability type and grade in patients with pure neural leprosy: disability type was sensory in 64.0 % of cases, motor in 4.0 % and mixed in 32.0 % of cases. The disability grade was grade I in 16 patients (64.0 %) and grade II in 9 patients (36.0 %).

Comparison between HRUS CSA measurements in the studied groups shows significantly higher CSA measurements of all nerves in suspected PNL group. PNL patients had significantly higher CSA of median nerve, ulnar nerve CSA and posterior tibial nerve than the measurements of controls (P<0.005) (table 3). In addition, great auricular nerve and ulnar nerve CSA measurements was shown to have low sensitivity (56%) in distinguishing PNL from healthy controls followed by median nerve HRUS CSA measurements (table 4, figures 2,3).

Table 3: Comparison between high-resolution ultrasound CSA measurements in the studied groups:

Nerve Mean ± SD (mm^2)		PNL n=25	Controls n=25	P value
Great auricular	**RT**	13.7 ± 7.1	5.0 ± 1.0	0.0001
	LT	12.4 ± 6.0	5.2 ± 1.4	0.0001
Median	**RT**	22.6 ± 10.1	9.4 ± 1.6	0.0001
	LT	22.2 ± 10.6	9.7 ± 2.2	0.0001
Ulnar	**RT**	24.0 ± 12.9	4.4 ± 1.9	0.0001
	LT	24.0 ± 11.2	9.0 ± 1.5	0.0001
Posterior tibial	**RT**	19.9 ± 10.9	6.2 ± 1.7	0.0001
	LT	18.8 ± 10.2	6.3 ± 1.8	0.0001

Table 4: Value of HRUS CSA measurements in diagnosis of suspected pure neural leprosy

Statistic	Great auricular nerve		Median nerve		Ulnar nerve		Posterior tibial nerve	
	Right	Left	Right	Left	Right	Left	Right	Left
Cut-off (mm^2)	10.5	10.5	15.5	15.5	18.5	20.5	12.0	11.5
AUC	0.63	0.63	0.63	0.7	0.59	0.61	0.7	0.71
P	0.08	0.08	0.12	0.015	0.26	0.19	0.015	0.011
Sensitivity	68.0 %	56.0 %	68.0 %	72.0 %	56.0 %	56.0 %	76 %	84.0 %
Specificity	68.0 %	68.0 %	64.0 %	72.0 %	64.0 %	68.0 %	64 %	64 %

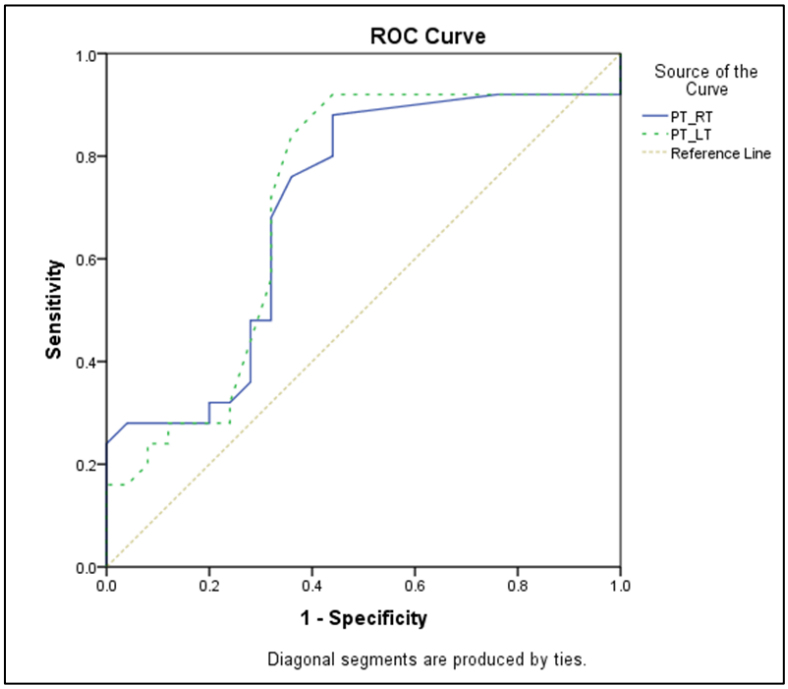

Fig. 2: ROC curve of high resolution ultrasonography for right and left posterior tibial nerves: Receiver Operating Characteristic (ROC) curve presented with plotting the true positive rate of median nerve thickness (Sensitivity) by HRUS versus the false positive rate (100-Specificity) for different cut-off points. The cut off for the right and left nerves with best sensitivity and specificity =12, 11.5 mm^2, respectively

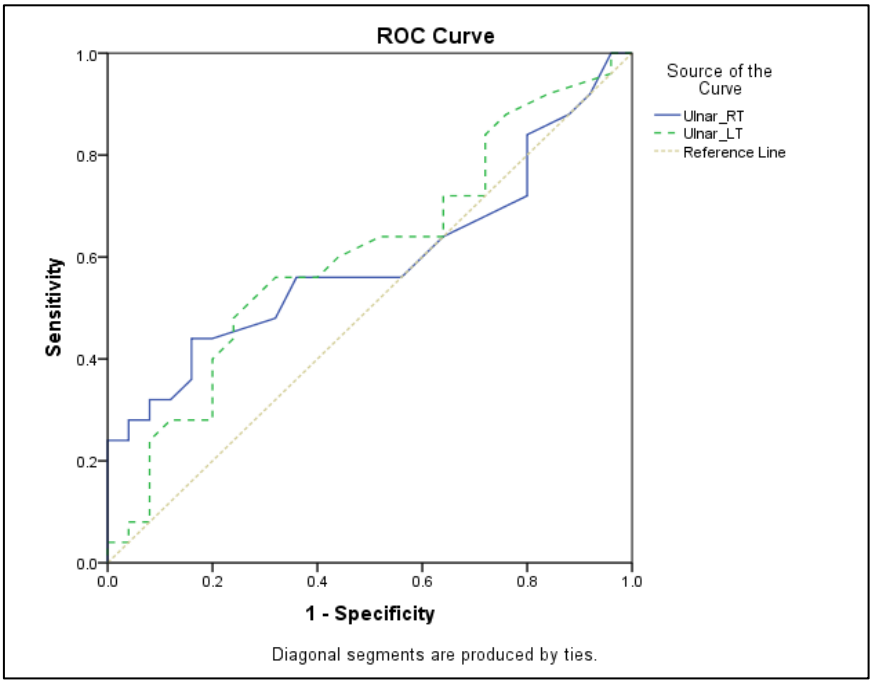

Fig. 3: ROC curve of high resolution ultrasonography for right and left ulnar nerves: Receiver Operating Characteristic (ROC) curve presented with plotting the true positive rate of ulnar nerve thickness (Sensitivity) by HRUS versus the false positive rate (100-Specificity) for different cut-off points. The cut off with best sensitivity and specificity =18.5 mm^2 for right ulnar, and =20.5 for the left one

Zeil Nelssen staining of different nerves aspirates from PNL patients revealed positivity of 8 suspected PNL by a percentage of (32%). Real time PCR of nerve aspirates in patients with PNL revealed the presence of *M. leprae* DNA were in 23 patients out of 25 of the suspected individuals (92%) (figure 4). Real time PCR CT values and their association with clinical criteria of PNL patients revealed high association between the bacterial DNA copies as indicated by CT and disabilities grading of different types (OR=7.2, 95% CI=0.54 –16.64, P=0.018). In addition, ZN staining morphological grading revealed significant association with neurological disabilities grading (table 5).

Table 5: Association between ZN and real time PCR with clinical disabilities grading

	Clinical diagnosis						
	-ve		+ve		OR 95% CI	Chi square test	
	n	%	n	%		χ^2	p
ZN Stain							
-ve	7	28.0	10	40.0			
+ve	0	0.0	8	32.0	5.6 [0.57 – 15.43]	4.575	0.032
PCR							
-ve	2	8.0	0	0.0			
+ve	5	20.0	18	72.0	7.2 [0.54 – 16.64]	5.590	0.018

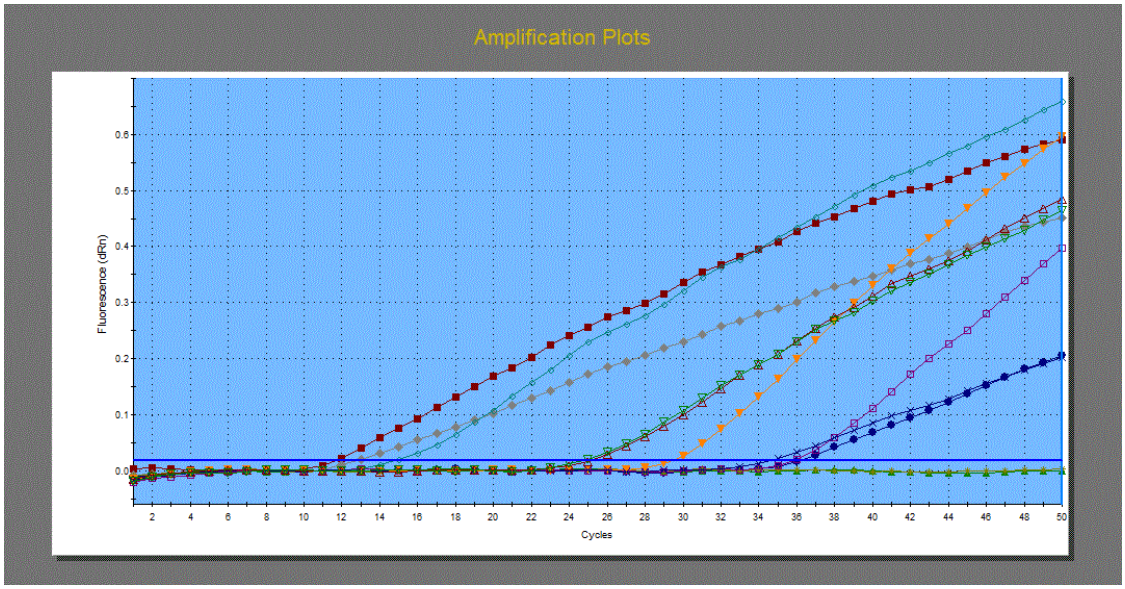

Fig. 4: Amplification plot of eight *M leprae* DNA templates from suspected pure neural leprosy patients: A graph of ΔRn versus cycle number (CT). ΔRn ; Fluorescence intensity of the reporter dye that was normalized by CT of actin gene. CT; the cycle at which each sample exceeds the baseline fluorescence background to a detectable level of fluorescence.

It was demonstrated that Real time PCR in diagnosis of PNL had the highest sensitivity (100%) with 95% CI =81.47% to 100.00%. However, ZN staining revealed a poor sensitivity (44.4%) and 95% *CI*=21.53% to 69.24% (table 6).

Table 6: Diagnostic value of Real time PCR in detection of clinically suspected pure neural leprosy

Statistic	Value	95% CI
Sensitivity	100.00%	81.47% to 100.00%
Specificity	58.57%	3.67% to 70.96%
Positive Likelihood Ratio	1.40	0.88 to 2.24
Negative Likelihood Ratio	0.00	0.00
Positive Predictive Value	78.26%	69.26% to 85.19%
Negative Predictive Value	100.00%	100%
Accuracy	80.00%	59.30% to 93.17%

DISCUSSION

Regarding HRUS CSA measurement of the involoved nerves as a diagnostic tool for PNL, our findings are supported by the previous studies which demonstrated evaluated HRUS CSAs of peripheral nerves, as ulnar CSAs, and median nerves. The study found that the CSA measurements in healthy controls were lower than those in PNL cases ($p < 0.0001$). The optimum CSA sensitivities that were analyzed statistically by ROC curve were ranged from 68 to 85% for the median nerve and from 81 to 72% for the common fibular nerves [17, 18].

Likewise, the research of Afsal et al.[12] included a heterogeneous groups of patients with different nerve injuries. Sonography showed significant thickening of both median nerve and ulnar nerve in leprosy patients. The current study and in agreement with the study of Bathala et al.[19], analyze the association between nerve thickness as determined by CSA and clinical characteristics in cases with ulnar neuropathy ($P < 0.0001$).

More recently, the study of Chen et al.[20] revealed that CSAs of the upper limbs were significantly increased in the patients than the controls. In contrast, the current study demonstrated that the posterior tibial nerve of the lower limb was of the highest sensitivity of 76-84% (cut off = 12.5 mm^2). All the previous finding demonstrated that HRUS offered a better diagnosis and monitoring of leprosy reactions and associated neuritis.

In agreement with an earlier study [21], Zeil Nelssen staining of different nerves aspirates by FNA in suspected patients of PNL revealed the presence of *M leprae* bacilli in 8 suspected PNL by a percentage of (32%). However, ZN staining in our study revealed a poor sensitivity (44.4%) and 95% *CI*=21.53% to 69.24%. In contrast, the previous observations of Reja et al[22] obtained higher sensitivity of 60%. These the

advantages of nerve FNAC over nerve biopsy for PNL diagnosis: less risky, and very little expert personnel to be obtained[23]. In addition to previous advantages, another study by fine needle aspiration cytology proved the presence of acid-fast bacilli from involved nerves in more cases (18 of the 27) suspected to have leprosy with pure neuritis [24].

Polymerase chain reaction in a previous research confirmed the detection of DNA of *M. leprae* bacilli in the nerve aspirate [25]. In the current study, Real time PCR of nerve aspirates in patients with PNL detected DNA in 92% of the patients with a sensitivity of 100%. PCR in another research diagnosed 75% of PNL cases[26]. However, another published study presented a systematic review analysis concluded that, PCR is a good diagnostic test with the highest sensitivity using multiplex procedure (82%) followed by RT-PCR (78%) and traditional PCR (63%) [9].

Association of the Real time PCR CT values with clinical criteria of PNL patients of the present research was detected and revealed significant relation between bacterial DNA copies as indicated by CT and disabilities grading of different type (OR=7.2, 95% CI=0.54 – 16.64, P=0.018). Also, another study [27] noted a moderate positive correlation (p = 0.047) between the values of Ct (DNA levels) and infection.

PCR amplification of the 16S ribosomal RNA in a previous research detected 50% of the cases with a specificity of 100 percent[28]. In a study performed in Eastern India, PCR confirmed *M. leprae* in 84% of these aspirates [22]. All the previous finding proved that PCR is a valuable tool for confirmation of leprosy specially in difficult circumstances such as PNL, paucibacillary leprosy and patients with uncharacteristic clinical features [29].

However, lack of the tests that could be a gold standard for leprosy diagnosis and failure to discriminate the cases with PNL, limits the diagnosis to the clinical features [30]. In general, fine needle aspiration followed by staining and PCR, is a simple, and less invasive technique that can be tried and suitable when PNL is suspected [31].

Zeil Nelseen staining failed to attain high sensitivity in PNL confirmation. So, PCR was considered as the diagnostic gold standard in diagnosis of PNL, helped to reduce the diagnostic problem of PNL by decreasing bias of the diagnosis[21]. Finally, real time PCR allows the identification of asymptomatic cases and serve as a better diagnostic tool for early case detection and treatment to achieve faster control of leprosy[28].

CONCLUSION

In conclusion, HRUS CSA can help to diagnose nerve affection in suspected PNL. FNAC technique followed by PCR could substitute the invasive hazardous nerve biopsy as they are relatively less invasive and simpler method with high sensitivity for detection of cases with PNL.

- The authors declare that they have no financial or non financial conflicts of interest related to the work done in the manuscript.
- Each author listed in the manuscript had seen and approved the submission of this version of the manuscript and takes full responsibility for it.
- This article had not been published anywhere and is not currently under consideration by another journal or a publisher.

REFERENCES

1. Alemu W, Naafs B. Position statement: LEPROSY: Diagnosis, treatment and follow-up. J Eur Acad Dermatol Venereol. 2019 Jul;33(7):1205-1213.
2. Sarode G, Sarode S, Anand R, Patil S, Jafer M, Baeshen H, et al. Epidemiological aspects of leprosy. Dis Mon. 2019 Dec 2:100899.
3. Reinar LM, Forsetlund L, Lehman LF, Brurberg KG. Interventions for ulceration and other skin changes caused by nerve damage in leprosy. Cochrane Database Syst Rev. 2019 Jul 31;7:CD012235.
4. Dwivedi VP, Banerjee A, Das I, Saha A, Dutta M, Bhardwaj B, et al. Diet and nutrition: An important risk factor in leprosy. Microb Pathog. 2019 Dec;137:103714.
5. Balestrino A, Fiaschi P, Riccardi N, Camera M, Anania P, Martinoli C, et al. Neurosurgical treatment of leprosy neuropathy in a low-incidence, European country. Neurol Sci. 2019 Jul;40(7):1371-1375.
6. Serrano-Coll H, Mieles O, Escorcia C, Díaz A, Beltrán C, Cardona-Castro N. A case series of pure neural leprosy in patients diagnosed in a specialized center for the control of Hansen's disease in Colombia]. Biomedica. 2018 Jun 15;38(2):153-161.
7. Beissner, M., Woestemeier, A., Saar, M., Badziklou, K., Maman, I., Amedifou, C., et al.. Development of a combined RLEP/16S rRNA (RT) qPCR assay for the detection of viable M. leprae from nasal swab samples. BMC infectious diseases, 2019;19(1):753.
8. Acosta L, Torres P. Leprosy Diagnosis: An Update on the Use of Molecular Tools. Acosta and Torres, Mol Biol 2015, 4:4
9. Tatipally S, Srikantam A, Kasetty S. Polymerase Chain Reaction (PCR) as a Potential Point of Care Laboratory Test for Leprosy Diagnosis-A Systematic Review. Trop Med Infect Dis. 2018 Oct 1;3(4):107.
10. Machado A, Lyon S, Rocha-Silva F, Assunc C, Hernandez M, Jorge D, et al. Novel PCR primers

for improved detection of M. lepraeand diagnosis of leprosy. Journal of Applied Microbiology 128, 1814--1819

11. Machet L, Ossant F, Bleuzen A, Grégoire JM, Machet MC, Vaillant L. High-resolution ultrasonography: utility in diagnosis, treatment, and monitoring dermatologic diseases]. J Radiol. 2006 Dec;87(12 Pt 2):1946-61.
12. Afsal M, Chowdhury V, Prakash A, Singh S, Chowdhury N. Evaluation of peripheral nerve lesions with high-resolution ultrasonography and color Doppler. Neurol India. 2016 Sep-Oct;64(5):1002-9.
13. Brakel WH, Nicholls PG, Wilder-Smith EP, Das L, Barkataki P, Lockwood DN; INFIR Study Group. Early diagnosis of neuropathy in leprosy--comparing diagnostic tests in a large prospective study (the INFIR cohort study). PLoS Negl Trop Dis. 2008 Apr 2;2(4): e212. doi: 10.1371
14. Rathod SP, Jagati A, Chowdhary P. Disabilities in leprosy: an open, retrospective analyses of institutional records. An Bras Dermatol. 2020 Jan-Feb;95(1):52-56.
15. Cabic E, Cabic AG, Esposo SM, Dizon F, Quinones GJ, Guia A. Histopathological Detection of Mycobacterium Tuberculosis and M. lepraeusing a Modified Acid-Fast Technique. Philippine Journal of Pathology, 2018; 3(1), 29
16. Turankar RP, Pandey S, Lavania M, Singh I, Nigam A, Darlong J, ET AL.. Comparative evaluation of PCR amplification of RLEP, 16S rRNA, rpoT and Sod A gene targets for detection of M. leprae DNA from clinical and environmental samples. Int J Mycobacteriol. 2015 Mar;4(1):54-9.
17. [17] Frade MA, Nogueira-Barbosa MH, Lugão HB, Furini RB, Marques Júnior W, Foss NT. New sonographic measures of peripheral nerves: a tool for the diagnosis of peripheral nerve involvement in leprosy. Mem Inst Oswaldo Cruz. 2013 May;108(3).
18. Chaduvula MV, Visser LH, Suneetha S, Suneetha L, Devaraju B, Ellanti R, et al. High-Resolution Sonography as an Additional Diagnostic and Prognostic Tool to Monitor Disease Activity in Leprosy: A Two-Year Prospective Study. Ultraschall Med. 2018 Feb;39(1):80-89.
19. Bathala L, Kumar K, Pathapati R, Jain S, Visser LH. Ulnar neuropathy in Hansen disease: clinical, high-resolution ultrasound and electrophysiologic correlations. J Clin Neurophysiol. 2012 Apr;29(2):190-3.
20. Chen X, Zhang L, Huang M, Zhai X, Wen Y, Pan C. Coexistence of Nerve Enlargement and Neuratrophy Detected by Ultrasonography in Leprosy Patients. Sci Rep. 2018 May 17;8(1):7812.
21. De A, Hasanoor Reja AH, Aggarwal I, Sen S, Sil A, Bhattacharya B, Sharma N, et al. Use of Fine Needle Aspirate from Peripheral Nerves of Pure-neural Leprosy for Cytology and Polymerase Chain Reaction to Confirm the Diagnosis: A Follow-up Study of 4 Years. Indian J Dermatol. 2017 Nov-Dec;62(6):635-643.
22. Reja AH, De A, Biswas S, Chattopadhyay A, Chatterjee G, Bhattacharya B, et al. Use of fine needle aspirate from peripheral nerves of pure-neural leprosy for cytology and PCR to confirm the diagnosis: a pilot study.. Indian J Dermatol Venereol Leprol. 2013 Nov-Dec; 79(6):789-794
23. Siddaraju N, Sistla SC, Singh N, Muniraj F, Chahwala Q, Basu D, et al. Pure neritic leprosy with nerve abscess presenting as a cystic, soft tissue mass: Report of a case diagnosed by fine needle aspiration cytology. Diagn Cytopathol. 2009; 37:355-8.
24. Rao P N, Suneetha S. Pure neuritic leprosy: Current status and relevance. Indian J Dermatol Venereol Leprol [serial online] 2016 [cited 2020 Aug 27]; 82: 252-61.
25. Kumar B, Pradhan A. Fine needle aspiration cytology in diagnosis of pure neuritic leprosy. Patholog Res Int 2011; 11: 158712.
26. Kamal NM, Hashem O, Mohamed H, Abd Alsamee H. Polymerase Chain Reaction versus Slit Skin Smear in Diagnosis of Leprosy; A Cross Sectional Study. International Journal of Medical Arts. 2020; 2 (3): 650-654
27. Gama RS, Gomides TAR, Gama CFM, Moreira SJM, de Neves Manta FS, et al. High frequency of M. leprae DNA detection in asymptomatic household contacts. BMC Infect Dis. 2018 Apr 2;18(1):153.
28. Bang PD, Suzuki K, Phuong le T, Chu TM, Ishii N, Khang TH. Evaluation of polymerase chain reaction-based detection of M. lepraefor the diagnosis of leprosy. J Dermatol. 2009; 36:269-76.
29. Martinez AN, Talhari C, Moraes MO, Talhari S. PCR-based techniques for leprosy diagnosis: From the laboratory to the clinic. PLoS Negl Trop Dis 2014; 8: e2655.
30. Tiwari V, Malhotra K, Khan K, Maurya P, Singh A, Thacker A, et al. Evaluation of polymerase chain reaction in nerve biopsy specimens of patients with Hansen's disease. J Neurol Sci. 2017 Sep 15; 380:187-190.
31. Vijaikumar M, D'Souza M, Kumar S, Badhe B. Fine needle aspiration cytology (FNAC) of nerves in leprosy. Lepr Rev 2001; 72:171-8