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Original Article

Microbiology Section

Comparative Evaluation of RLEP-PCR and Conventional Laboratory Methods for Confirmation of Clinically Diagnosed Early-stage Leprosy in Eastern India

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ABSTRACT

Introduction: Leprosy is an infectious disease caused by *Mycobacterium leprae* (*M. leprae*). Diagnosis of leprosy is confirmed based on cardinal signs and symptoms along with supportive laboratory investigations. Early diagnosis and treatment of new cases are essential to prevent disease burden and spread in community.

Aim: To evaluate the efficacy of currently available laboratory tests like *M.leprae*-specific repetitive element Polymerase Chain Reaction (RLEP-PCR) and conventional methods like, Slit Skin Smears (SSS), Histopathology (HP) and Fite-Faraco (FF) staining for detection of *M. leprae* in clinically diagnosed cases of early-stage leprosy.

Materials and Methods: A cross-sectional study of 82 newly diagnosed cases of leprosy was carried out in S.C.B Medical College, a tertiary care hospital of Odisha, India from September 2018 to August 2020. For every case, skin tissue was punched out to get biopsy for HP and FF staining and incised for SSS. At least two SSS slides were prepared per case. One SSS slide was stained by modified Ziehl-Neelsen (ZN) staining to demonstrate Acid Fast Bacilli (AFB). The other SSS slide was air dried and the dried material was scraped off to perform PCR. Data were analysed by Statistics software IBM Statistical Package for the Social Sciences (SPSS), version 24.0. Association between categorical variables was studied by using Chi-square test. Comparison of mean±SD and median (IQR) were done by using independent sample't' test. Agreement on different types of laboratory methods was done by using Kappa test.

Results: Among 82 cases, 68.3% were RLEP-PCR positive, 56% had relevant chronic granulomatous features histopathologically, 39% were FF stain positive, and only 29% were ZN stain positive. Improvement in case detection of 39%, 29.3%, and 12.2% by PCR over SSS, FF Stain and HP respectively proved superiority of PCR over other procedures.

Conclusion: PCR was most sensitive to detect leprosy both in pauci and multibacillary groups, thus considered as investigation of choice for diagnosis of early-stage leprosy.

Keywords: Fite faracco staining, Histopathology, *Leprae*-specific repetitive element-polymerase chain reaction, Slit skin smear

INTRODUCTION

Leprosy is an age old chronic infectious disease that affects skin, mucosa and peripheral nerves. It is caused by *Mycobacterium leprae*, an obligate intracellular AFB, transmitted via droplets of mouth and nasopharyngeal secretions of untreated cases to their close contacts [1]. Worldwide leprosy prevalence is 0.24/10000 population whereas new case detection rate is 2.74/100000 population [2]. Although the trend of new case detection in most countries is declining from 2009 to 2018, India documented an increment. India has maximum number of new cases in world accounting for 57.7% of the global case load [3]. Number of new cases indicates continued transmission of infection. National leprosy prevalence of India has been maintained <1 per 10,000 population but few states/Union territories (Odisha, Chandigarh, Delhi, and Lakshadweep), have shown a prevalence of >1 per 10,000 population, which is a matter of concern [4]. Clinical manifestations of leprosy are varied and diverse. It ranges from discolored skin patches to profound deformity or disability and often mimic with other skin diseases that challenge accurate diagnosis. Ridley D and Jopling W categorised leprosy clinicopathologically into a spectrum ranging from tuberculoid (TT) to lepromatous (LL) while World Health Organisation (WHO) categorised leprosy into multibacillary (MB) and paucibacillary (PB) based on number of skin lesions, involvement of peripheral nerves and microscopic demonstration of Lepra bacilli [5,6].

The laboratory test in common practice to support clinical diagnosis is SSS in most of healthcare settings. Despite of low sensitivity and risk of subjective errors in microscopy SSS is the most preferred test for diagnosis because of highest specificity and convenience to perform but, many cases of early leprosy are missed in SSS and treatment is delayed which is a threat to community spread [7]. Other tests like HP and FF staining of skin biopsy or IgM Anti-Phenolic Glycoloipid-1 (PGL1) ELISA have also low sensitivity in TT and PB cases compared to LL and MB cases of leprosy [8]. Thus, a diagnostic tool of high sensitivity is desperately needed in high endemic country like India. PCR has revolutionised laboratory diagnosis of leprosy in past few years by detecting bacilli in clinical samples like lymph, blood, nasal swab, tissue and urine [9]. Since decades SSS was being considered as gold standard as it was highly specific for diagnosing leprosy although it has low sensitivity. Recently PCR is claimed to have higher sensitivity in early stage leprosy. Therefore, in our regional context there was a need to evaluate efficacy of PCR over conventional tests for early-stage leprosy. Keeping in view of the above facts, the current study was designed to compare interprocedural sensitivity and early detection ability among tests like SSS, FF staining, HP study and PCR in clinically diagnosed cases of leprosy.

MATERIALS AND METHODS

The current cross-sectional study was undertaken in S.C.B Medical College and Hospital, a tertiary care Institute of Odisha, a high endemic state for leprosy in Eastern India from September 2018 to August 2020. Before specimen collection approval from Institutional Ethical Committee (IEC) was obtained (IEC/IRB no.981/14.10.2019). Written informed consent was also obtained from participants in case of adults and guardians of participants in case of children.

Inclusion criteria: All new cases of leprosy above 5-years of age diagnosed in Dermatology department during given time period were included in the study.

Exclusion criteria: Patients having past history of receiving antileprosy treatment, lepra reaction, tuberculosis, haematological malignancies, immunodeficiencies and those who were unwilling to give consent are excluded from study.

While enrolling a patient in study, the case definition criteria was strictly adhered which included well demarcated hypopigmented macular skin patches having partial or complete loss of sensation and peripheral nerve involvement with or without thickening and sensory loss [10].

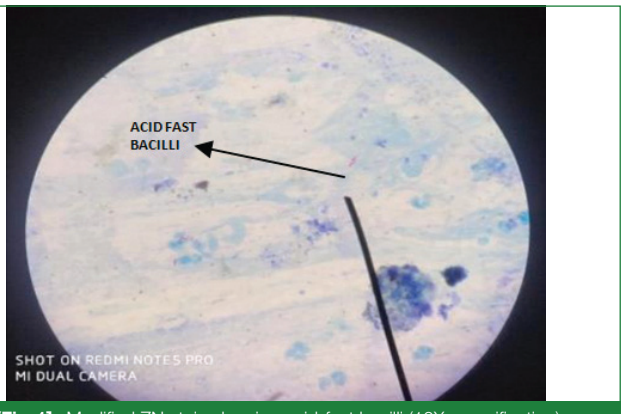
Specimen collection: A total of 82 cases of clinically diagnosed leprosy were included in the study. Relevant history, clinical features and demographic data of each individual were documented.

Procedure

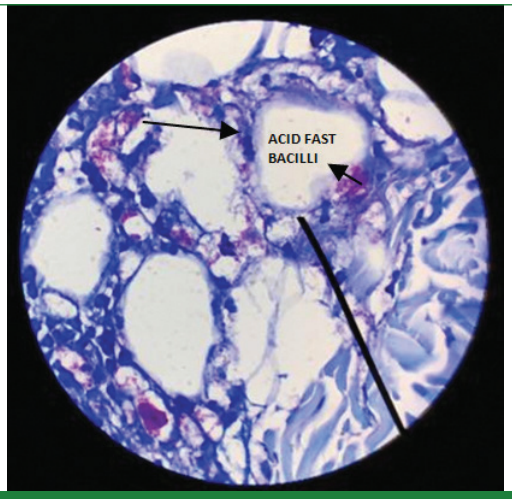
Laboratory confirmation of cases by microscopy was carried out in bacteriology and histopathology units. Molecular characterisation was conducted in a collaborated molecular lab of Heredity Biosciences Ltd., Bhubaneswar, India.

Specimens like SSS and skin biopsies were collected from each case. By giving an incision of 2 mm deep and 5 mm long in a scalpel (No 15), SSS were collected at least from three sites including both ear lobes and edge of active lesions as per standard protocol [10]. From each patient two smears were collected. One of the smears was preserved for molecular characterisation and the other one was stained by modified ZN stain for detection of AFB. Skin biopsies were taken by 6 mm punch from active lesions or from anaesthetic areas. While collecting biopsies, plaques and nodules were preferred over patches where all were present concomitantly. Biopsy samples were fixed in 10% formalin for 8-10 hours followed by routine processing. Paraffin embedded tissue sections of 4-5 µm thickness were stained with Haematoxylene & Eosin (H&E) stain for HP and modified FF stain for detection of AFB as per standard procedure [10].

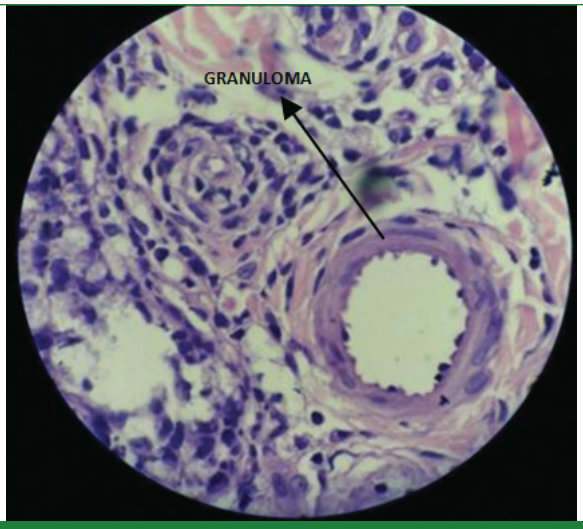
Microscopy: Bacteriological status of SSS stained by ZN and biopsies stained by FF staining were noted as positive by demonstrating AFB [Table/Fig-1,2]. SSS were graded (1+ to 6+) based on number of bacilli per oil immersion fields to obtain bacteriological index [11]. Histopathological status of tissue sections were noted for features of chronic inflammation to confirm leprosy [Table/Fig-3]. After clinicopathological association patients were categorised as Tuberculoid (TT), Border line tuberculoid (BT), mid Border line (BB), Border line Lepromatous (BL) and Lepromatous (LL) according to Ridley-Jopling classification and also categorised as PB and MB as per WHO classification. Ridley-Jopling classification was done based on clinicohistological association and WHO classification was done based on number of skin lesions, involvement of peripheral nerves and microscopic demonstration of Lepra bacilli ,as mentioned earlier [5,6].



[Table/Fig-1]: Modified ZN stain showing acid-fast bacilli (10X magnification).



[Table/Fig-2]: Fite Faracco stain showing acid fast bacilli (40X magnification).



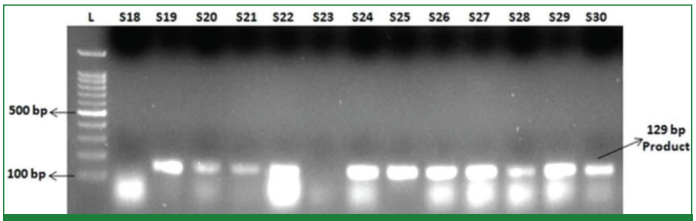
[Table/Fig-3]: Histopathology (HE Stain) of skin tissue showing granuloma (40X magnification).

DNA extraction: The unstained SSS was scrapped of the slides gently by bevelled edge of an 18-gauge needle into a 1.5 mL Microcentrifuge tube containing 700 µL of 70% ethanol and stored at -20°C until DNA extraction [12].

M. leprae DNA was extracted using commercial DNeasy Blood & Tissue mini spin column kit (Qiagen, Germany) as per the manufacture's instruction and DNA was quantified on Nano Drop™ -2000 spectrophotometer (Thermo Fisher, U.S.A). The ratio of absorbance at 260 nm and 280 nm was used to access the purity of DNA. A ratio of ~1.8 to 2 was accepted as pure DNA. The extracted DNA was stored at -20°C until used for PCR amplification.

Amplification of DNA by PCR (RLEP-PCR): RLEP was targeted in sample DNA by conventional PCR assay. Before conducting PCR, gene specific forward primer of sequence 5'TGCATGTCATGGCCTTGAGG3'and reverse primer of sequence 5'CACCGATACCAGCGGCAGAA3' were procured commercially (IDT, USA) to amplify 129 bp fragment in test samples [13]. PCR was conducted in a 25 µL volume of reaction mixture consisting of 10 ng of sample DNA, 1×Taq DNA polymerase buffer (10 mM Tris-HCl, 50 mM KCl, and 1.5 mM MgCl2 pH 8.3) (MP Biomedicals, UK), 200 mM each of dNTPs (MP Biomedicals, UK), 5U of Taq DNA

polymerase (MP Biomedicals, UK) and 10 pM of each primer. PCR was carried out by using a master cycler gradient thermal cycler (Bio Rad, USA). The cycling conditions were as follows: i) One cycle of pre denaturation done at 95°C for 5 minutes followed by 36 cycles of amplification, ii) Each cycle consisted of denaturation at 95°C for 45 seconds, annealing at 55°C for 45 seconds and extension at 72°C for 1 minute, iii) A final step of extension at 72°C for 10 minutes. The amplified products were resolved in 2% Agarose gel in TAE buffer, stained with ethidium bromide (0.5 µg/mL) and photographed under a gel documentation system (gel doc XR+, Biorad, USA). A 100 bp ladder was included on every gel to determine the RLEP-PCR product size of 129 bp. While conducting PCR, positive and negative controls were included in each run. A SSS positive sample of high-grade bacteriological index and DNA free milli-Q water were considered as positive and negative control respectively. The assay was considered positive when the 129 bp amplicon was detected and negative when it was not detected [Table/Fig-4]. Assay results were compared with microscopic findings of SSS, F-F staining and HP.



[Table/Fig-4]: Gel electrophoresis following PCR amplification of 129 bp fragment of RLEP of *M. leprae* DNA.

STATISTICAL ANALYSIS

Data collected under the study were scrutinised, codified and entered into the IBM SPSS Statistics, 24.0 software(www.spss.co.in) for analysis. Categorical variables were classified following frequency procedure and their association was studied by using Chi-square test of independence. Comparison of mean ± SD and median (IQR) were done by using independent sample 't' test. Agreement on different types of method was done by using Kappa test. Considering PCR as most sensitive, agreement analysis of other tests with PCR was done and degree of agreement (Kappa value) was found out using Landis & Koch scale [14]. The scores were divided as, no agreement (<0); slight (0.0-0.20); fair (0.21-0.40); moderate (0.41-0.60); substantial (0.61-0.80); almost perfect (0.81-0.99) and perfect. The p-value <0.05 was considered to indicate cut-off for statistical significance.

RESULTS

Out of 82 cases, males [60 (73.17%)] outnumbered females, with male to female ratio of 2.7:1. Majority of the cases were in 30-49 years (56%). The mean age ± SD was 41.0±14.2 years and median (IQR) was 42 (30.8-48.3). In male, older age group proportions were significantly higher than the females with p<0.05. Males have significantly higher mean±SD and median (IQR) value as compared to females (p=0.002) [Table/Fig-5]. According to R-J clinical types of leprosy classification, 2 cases (2.4%) were TT, 40 (48.8%) were BT, 2 (2.4%) were BB, 20 (24.4%) were BL and 18 (22.0%) were LL. In the less than 30 years age group, majority proportion were BL (44.4%) followed by BT (33.3%). In the 30-49 years and ≥ 50 years age group the proportion of BT was maximum. According to WHO clinical typing majority of leprosy cases were PB [50 (61%)] and few were MB [32(39%)]. Among younger (<30 years) age group the proportion of MB was higher (55.6%) and among elder age group (≥ 50 years), proportion PB was higher (88.9%) [Table/Fig-6]. In SSS, no case of BT, all 2 cases of BB, 30% cases of BL and 88.9% cases of LL were +ve for AFB. Similarly in HP, 35% cases of BT, 60% of BL and all cases of BB and LL were +ve. In F-F staining also all 2 cases of BB, 5% of BT, 60% of BL and 88.9% of LL cases were +ve. Lastly in PCR, 50% cases of BT, 80% cases BL and all cases of BB and LL were tested + ve. The differences in positivity through SSS, HP, F-F and PCR method among R-J clinical types were found significant (p-value <0.05) [Table/Fig-7]. Similarly, among WHO clinical types, in SSS: 75% cases of MB but no case of PB, in HP: 93.8% cases of MB and 32% of PB, in FF staining: 87.5% cases of MB and 8% of PB and in PCR: all the cases of MB and 48% of PB were tested +ve. The differences in positivity through the tests among MB and PB were significant (p-value<0.05). Results obtained from classical tests were compared with results obtained from PCR and percentage of agreements were calculated [Table/Fig-8]. Categorising SSS positive cases by grades of Bacteriological Index (BI), 8/24 were 2+, 6/24 were 3+, 7/24 were 4+, 1/24 was 5+ and 2/24 were 6+ [Table/Fig-9].

Age group (years)	Gender				Total		p' value
	Male		Female				
	No.	%	No.	%	No.	%	
<30	6	10	12	54.5	18	22	<0.001
30-49	38	63.3	8	36.4	46	56	
≥50	16	26.7	2	9.1	18	22	
Total	60	100	22	100	82	100	
Mean±SD	43.8±12.2		33.4±16.5		41.0±14.2		0.002#
Median (IQR)	43.5 (34-52)		29 (22-44)		42 (30.8-48.3)		

[Table/Fig-5]: Age and gender distribution of leprosy cases.
*Chi-square test 'p' value; #Independent sample 't' test; A p-value <0.05 is considered to be statistically significant

Age group (years)	R-J Clinical types of Leprosy					WHO Clinical types of Leprosy	
	TT	BT	BB	BL	LL	MB	PB
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
<30 (N=18)	2 (11.1)	6 (33.3)	0	8 (44.4)	2 (11.1)	10 (55.6)	8 (44.4)
30-49 (N=46)	0	20 (43.5)	2 (4.3)	10 (21.7)	14 (30.4)	20 (43.5)	26 (56.5)
≥50 (N=18)	0	14 (77.8)	0	2 (11.1)	2 (11.1)	2 (11.1)	16 (88.9)
Chi-square test 'p' value	0.008					0.015	
Gender							
Male (N=60)	2 (3.3)	28 (46.7)	2 (3.3)	16 (26.7)	12 (20)	22 (36.7)	38 (63.3)
Female (N=22)	0	12 (54.5)	0	4 (18.2)	6 (27.3)	10 (45.5)	12 (54.5)
Chi-Square test p-value	0.638					0.470	
Total (N=82)	2 (2.4)	40 (48.8)	2 (2.4)	20 (24.4)	18 (22.0)	32 (39)	50 (61)

[Table/Fig-6]: Distribution of age and gender with R-J and WHO clinical type of leprosy. A p-value <0.05 is considered to be statistically significant; TT: Tuberculoid; BT: Border line tuberculoid; BB: Mid border line; BL: Border line lepromatous; LL: Lepromatous; PB: Paucibacillary; MB: Multibacillary

DISCUSSION

Though considered to be eliminated from most parts of world, leprosy is still prevalent in India where early diagnosis and treatment is the key component to limit disease morbidity and community spread. Several diagnostic modalities like SSS microscopy, tissue biopsy for HP or FF staining, PGL-1 ELISA, and PCR are in practice to diagnose leprosy. In most of the community healthcare settings leprosy is confirmed by SSS as it has high specificity and easy to perform. But SSS has poor sensitivity as AFB staining requires presence of at least 10^4 bacilli per gram of tissue for microscopic detection. Therefore, SSS is unsuitable for early diagnosis as majority of paucibacillary leprosy are missed as they have low bacilli burden [15]. Tissue biopsy for HP study and FF staining has high specificity but has moderate sensitivity, more turnaround time and difficult to accomplish in most of the health care settings. But, compared to classical methods PCR

	Different Methods							
	SSS		HP		FF		PCR	
	Negative	Positive	Negative	Positive	Negative	Positive	Negative	Positive
R-J Clinical types of leprosy	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
TT (N=2)	2 (100)	0	2 (100)	0	2 (100)	0	2 (100)	0
BT (N=40)	40 (100)	0	26 (65)	14 (35)	38 (95)	2 (5)	20 (50)	20 (50)
BB (N=2)	0	2 (100)	0	2 (100)	0	2 (100)	0	2 (100)
BL (N=20)	14 (70)	6 (30)	8 (40)	12 (60)	8 (40)	12 (60)	4 (20)	16 (80)
LL (N=18)	2 (11.1)	16 (88.9)	0	18 (100)	2 (11.1)	16 (88.9)	0	18 (100)
Chi-square test 'p' value	<0.001		<0.001		<0.001		<0.001	
WHO Clinical types of leprosy								
MB (N=32)	8 (25)	24 (75)	2 (6.3)	30 (93.8)	4 (12.5)	28 (87.5)	0	32 (100)
PB (N=50)	50 (100)	0	34 (68)	16 (32)	46 (92)	4 (8)	26 (52)	24 (48)
Chi-square test 'p' value	<0.001		<0.001		<0.001		<0.001	
Total	58 (70.7)	24 (29.3)	36 (43.9)	46 (56.1)	50 (61)	32 (39)	26 (31.7)	56 (68.3)

[Table/Fig-7]: Association of R-J and WHO clinical type of leprosy with different methods.
SSS: Slit skin smear; HP: Histopathology; FF: Fite-faracco; PCR: Polymerase chain reaction

	PCR				Total			
	Negative		Positive				Agreement	Kappa test
SSS	No.	%	No.	%	No.	%		
Negative	26	44.8	32	55.2	58	100	61.00%	k=0.322 <0.001
Positive	0	0	24	100	24	100		
HP								
Negative	24	66.7	12	33.3	36	100	82.9.7%	k=0.643 <0.001
Positive	2	4.3	44	95.7	46	100		
FF								
Negative	22	44	28	56	50	100	60.10%	k=0.278 p=0.003
Positive	4	12.5	28	87.5	32	100		
Total	26	31.7	56	68.3	82	100		

[Table/Fig-8]: Comparison of results obtained by classical methods versus PCR.

		SSS: Z-N +VE: Bacteriological Index (RIDLEY SCALE)					
Types of leprosy	SSS: Z-N-ve	1+	2+	3+	4+	5+	6+
TT (02)	02	0	0	0	0	0	0
BT (40)	40	0	0	0	0	0	0
BB (02)	0	0	2	0	0	0	0
BL (20)	14	0	6	0	0	0	0
LL (18)	2	0	0	6	7	1	2
Total (82)	58	24					

[Table/Fig-9]: Bacteriological profile in SSS.

assay is useful for early diagnosis of leprosy with high sensitivity of detecting even 10-30 fg of *M. leprae* component which is equivalent to 2.8-8.3 bacilli [16]. In past two decades several sequences such as 16S rRNA, sodA, pra gene, RLEP, genes encoding antigen 85B, ML1545 etc. has been used as targets for PCR [17]. But among them RLEP PCR act as a better target because it is a region of specific repetitive sequence having 28-32 repeats per genome dispersed in *M. leprae* chromosome [18].

In the present study, all 82 cases were subjected for SSS, FF staining, HP study and PCR assay. Sensitivity of SSS, HP and F-F stain were compared with PCR. Male preponderance [60 (73.17%)] with male to female ratio of 2.7:1 was observed. Similar gender ratio with male preponderance was also observed by Khatoon S et al., (71%male vs 29% female with male to female ratio of 2.4:1) [19]. In our study, BT leprosy was maximum (48.8%) followed by BL, LL, BB and TT. Similar to present finding, another study from eastern India conducted by Banerjee S et al., also documented highest (51.8%) number of BT cases of leprosy among study groups [20]. Present study observed significant difference in different age groups of R-J clinical type leprosy (p=0.008) and WHO clinical type of leprosy (p=0.015) [Table/Fig-6]. However, this may be validated with larger sample size. The association of R-J clinical type with gender was not significant (p=0.638). That implied the distribution of leprosy types among males and females did not differ significantly [Table/Fig-6]. Comparing diagnostic ability of tests among cases in terms of positivity, 68.3% of RLEP-PCR, 56.1% of HP, 39% of Fite-Faraco stain, and only 29.3% of SSS were found positive in present study [Table/Fig-7]. The low sensitivity of SSS microscopy can be explained by lower bacilli load in smears and individual observer variation. Present study finding of 68.3% PCR positivity was close to findings of Kamal R et al., with 72% PCR positivity on SSS [21]. But present study for PCR result was much lower as compared to the studies conducted by Wichitwechkarn J et al., where 87% PCR positivity was reported in the skin biopsy [22]. This significant difference can be explained by the presence of low level of DNA in SSS of present study due to less amount of tissue as compared to skin biopsy. In present study FF stain detected AFB in more number of cases compared to SSS (39% vs 29%) as more amount of tissue is collected in biopsy than slit skin smear. However, the earlier mentioned Indian study by Patil AB et al., [10] reported high AFB positivity in SSS than F-F stain (21.5% vs11.3%). This significant difference can be explained by subjective variation in sampling procedure, staining technology and microscopic observation. In comparison to other tests, present study found PCR to be most sensitive to detect leprosy not only in different spectra i.e., BT (50%), BB (100%), BL (80%) and LL (100%) but in clinical sub groups of MB (100%) and PB (48%) also. Improvement in case detection of 39%, 29.3%, and 12.2% by PCR over SSS, FF Stain and HP respectively proved superiority of PCR over other procedures. Compared to present study results Goulart IM et al., using 130bp primer found better PCR positivity of 40% in TT, 55% in BT, 100% in BB, BL and LL [23]. In the previously mentioned study, Banerjee S et al., [20] using 372 bp primer performed PCR of skin biopsy and found positivity of 100% in LL,90.9% in BL followed by 82.3% in BT and TT which is better than present study results. This may be attributed to use of skin biopsy instead of slit skin for PCR and their methods of PCR assay. When results of SSS microscopy and PCR were observed, present study found PCR to be positive in all (100%) AFB positive cases and 55.2% (32/58) of AFB negative cases, which implies no false negative PCR among AFB positive cases. This was almost similar to finding of Siwakoti S et al., where PCR positivity was 100% in AFB positive and 65% in AFB negative cases [24]. When overall agreement of results of different methods with PCR were analysed, substantial agreement between HP and PCR (Kappa=0.643 with p<0.05) and moderate agreement between SSS /F-F staining and PCR was observed [Table/Fig-8]. Silva AR et al., in Brazil also

reported moderate agreement between SSS and PCR (k=0.57) [25]. However, in the current study disparity in two of HP study and four of FF staining positive cases which were PCR negative was observed. Possible explanation to negative PCR in these cases could be that the bacilli in tissue samples may have become non viable or degraded due to improved host immunity, but yet not cleared off completely, thereby there was persistence of granuloma in HP and appearance of bacilli fragments in FF stain. Another possibility to negative PCR may be inadequate collection of SSS sample as they were also AFB negative in SSS microscopy. Lastly for reasons unclear to us presence of PCR inhibitors or the method of PCR adopted in present study could have hindered detection of *M. leprae* DNA. There were also 2 MB cases in present study, which were PCR positive along with AFB positive in SSS and FF Staining, but did not demonstrate significant chronic granulomatous features in HP. This disparity may be explained by possibility of poor cell mediated immunity in them which did not demonstrate significant tissue rection in HP.

Limitation(s)

Real time PCR or nested PCR was not done which would have achieved higher sensitivity than classical PCR. PCR of skin biopsy was not done which would have shown higher positivity than PCR of SSS. Nerve biopsy could not be performed in cases having no skin lesion which may have confirmed pure neuritic varieties of leprosy that are missed in the study. Indeterminate and histoid variants of leprosy were also not enrolled in the study.

CONCLUSION(S)

Present study infers that PCR is more sensitive than microscopy in diagnosing leprosy. The study also revealed the fact that PCR can detect a greater number of early-stage leprosy like PB cases or BT/TT pole of leprosy which are usually missed by SSS and other conventional lab procedures. In endemic areas like India this PCR characteristic is need of the hour to diagnose and treat leprosy at the earliest to limit disease morbidity and community spread. PCR being expensive and technically demanding is not feasible to implement in all suspected cases of leprosy in resource constraint health centers. However, in the post COVID-19 pandemic era most of the tertiary care hospitals are now well equipped with PCR equipment and trained man power. Therefore, conventional microscopy of bacteriological indexing and histopathology should be used as first choice to support clinical diagnosis but early and clinically indeterminate cases must be referred to tertiary care centres for PCR to increase rate of case detection, which will help the clinicians for better case management and decrease disease burden in the society.

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