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EVALUATION OF THE DIAGNOSTIC ROLE OF RLEP PCR AND PGL-1 IN PAEDIATRIC LEPROSY PATIENTS

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ABSTRACT **Introduction-** Leprosy an ancient stigma still present as a major social health question even after its near elimination, so its advance detection and efficient treatment is salient predominantly in children because they stand as a symbol of unbroken transmission of the disease. **Aims and Objetives-** To study and compare diagnostic efficacy of RLEP PCR (MYCOBACTERIUM LEPRAE SPECIFIC REPETITIVE ELEMENT), PGL -1(PHENOLIC GLYCOLIPID-1). **Methods-** We included 31 untreated early leprosy cases equal to or less than 16 years during the period of year 2017 to 2019. After examining clinically their skin smear, skin scraping for RLEP PCR and serum for Anti PGL -1 antibodies were studied. **Results-** All results were compared between cases and controls. The outcome was significant (P value <0.005) for RLEP PCR. Sensitivity and specificity of RLEP PCR was 72.10% and 96.50, that for anti PGL-1 was 41.00% and 93.37% respectively. Only 25.79% cases were slit skin smear positive. **Conclusion-** RLEP PCR is highly sensitive and useful tool for advance detection of leprosy.

KEYWORDS : Skin smear, RLEP PCR, Anti PGL-1 Antibody.

INTRODUCTION

Being a chronic disease, which especially involve the skin and nerves. As the way of spread is contact, many other organs may also be involved.

Although Leprosy has a worldwide distribution, but as WHO stated India contributes about 60% of newly detected leprosy cases in the world. Out of 135485 new cases detected in India, 11,792 were children (8.7%)[1].

Hypopigmented patches with or without loss of sensation and/or thickened cutaneous nerves are the major clinical presentation. The major obstacle in early detection is that while acid fast bacilli in the skin smears is verifying, but in early stages it usually comes negative. Amplification by polymerase chain reaction (PCR) has shown very promising results in detecting M. leprae in past years. These molecular methods are more specific and sensitive for recognizing mycobacterium DNA. During the last three decades, molecular methods have been used widely to amplify different gene targets of M. leprae.[(2)]

PCR technique has been applied on several specimens inclusive of skin. PCR was used to test different sequences of mycobacterium genome.

After substantial studies on these molecular tests in has shown that DNA-based PCR assays are highly specific, while the sensitivity range can differ from 34 to 80% in PB forms to greater than 90% in MB forms of the disease.[(3)]

RLEP region of M. leprae has shown highest PCR Positivity among all kind of samples.
RLEP sequence is repeated 28 times in the M leprae genome therefore highly sensitive and specific which can detects 10 fg of purified M. leprae DNA.[(2)]

Antibodies to phenolic glycolipid-1 (PGL-1) in leprosy[(4)] has been extensively evaluated in community, diagnosis and monitoring therapy[(4,5,6,7,8,9)].Its synthetic analogs containing antigenic carbohydrates[(10)] has been used in ELISA (Enzyme linked immunosorbent assay) to identify antibodies particularly of IgM class [(11,,12)] These T-cell independent antibody production can be both IgM isotype and IgG isotype[(8)]. Previous PGL-1 studies have shown that the technique is sensitive for multibacillary leprosy but detection in paucibacillary cases ranges from 30% to 60% [(7)].

This study is aimed for evaluating the diagnostic efficacy of RLEP PCR and PGL-1 in early paediatric leprosy patients.

MATERIAL AND METHODS

Recruitment was done from outpatient department of National JALMA Institute for Leprosy and Other Mycobacterial Diseases, Agra after consent taken from their parents.

Total 31 children were recruited from the OPD and 31 age matched controls were taken from S.N medical college Agra.

Inclusion Criteria

Only untreated having Age less or equal to 16 years with characteristic skin lesions and or nerve involvement were included in this study.

Exclusion Criteria

Age more than 16 years
Suffering from other diseases.

Recording Of Clinical Data- After taking history regarding family and duration of symptoms complete examination was done focusing on site, details of skin lesions and degree of nerve involvement.

Slit skin smear was done for acid M. leprae by staining with Zeihl - Neelsen's method.

RLEP PCR

Scraping was taken from three sites -
From the lesion
From both ear lobes.

DNA extraction from samples was done using the method described by van Embden et al (1993).

Sterile tubes and plugged tips were used to avoid cross contamination.

Reactions were executed in 25µl reaction mixture having-
1. DNA template-5 µl
2. Deoxynucleoside triphosphate- 0.2 m mol
3. RLEP gene primer- 0.5 mol
4. Taq polymerase- 1U

The 129 bp fragment amplification was done by using F-'TGCATGTCATGGCCTTGAGG3' as forward primer and 5'CACCGATACCAGCGGCAGAA3' as reverse primer respectively. Initial de-naturation was done at 95°C for 2 minutes then for 45 cycles at 94°C for 30 seconds; 58°C for 2 minutes and 72°C for 2 minutes, final extension was done at 72°C for 8 minutes and kept at 4°C.

A band of 129 bp on 2% Agarose gel electrophoresis was considered as

a positive result (Figure-1).

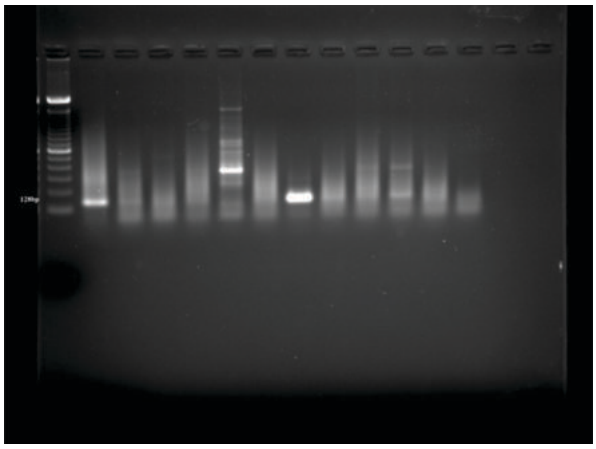

Fig.-1: Showing The Appearance Of RLEP PCR 129 bp Amplicon Of M Leprae On 2% Agaraose Gel Electrophoresis

ELISA FOR ANTI PGL-1 ANTIBODY- PGL-1 antigen and Anti Human IgM antibody were used and the absorbance was measured at 450 nm using a Spectramax-M2 Reader. The mean± 2 S.D. of the Optical density values of the controls were taken as the cut-off values.

Statistical Analysis

Statistical analysis was done with the help of SPSS version 26. Chi square test was done for comparison.

RESULTS

Out of thirty one cases 15 cases (48.38%) were of BT (Borderline Tuberculoid), 8 of TT (Tuberculoid Leprosy) and 1 of BL (Borderline Leprosy), LL (Lepromatous Leprosy) and I (Indeterminate Leprosy) each, the rest 5 cases were of BB (Borderline Borderline leprosy). The clinical profile of cases have been described in Table 1.

Table-1: Clinical Profile Of Cases

Clinical type	No. of cases (%) (Total= 31 cases)
TT	8 (25.80)
BT	15 (48.38)
BB	5 (16.12)
BL	1 (3.22)
LL	1 (3.22)
I	1(3.22)

Further it was observed that male cases (58.06%) were more as compared to female (41.94%) cases, and M:F (ratio) was 1.39:1.The age and sex profile of cases have been described in Table 2.

Table-2: Age And Sex Profile Of Cases

Age	
Preschool and school age (<10 years)	11 (35.48)
Adolescents (11-16 years)	20 (64.52)
Sex	
Boys	18 (58.06)
Girls	13 (41.94)

We also found that 28 cases (92.33 %) had both skin lesions and nerve trunk thickening and only 3 cases (9.67 %) had skin lesions without nerve trunk thickening. There were no case having only nerve involvement in this study.

Out of 15 BT cases, 2 cases were smear positive for AFB. 6 out of 8 cases with BB/BL/LL/I type of leprosy were skin smear positive for AFB. Rest of the cases were smear negative.

Cut off Optical density for Anti PGL-1 Antibody was taken as 0.0446. Value above the cut off were taken as positive and that below it was considered negative.

In this study 2 and 14 cases were such that who had negative skin smears for AFB but were positive for Anti PGL-1 based ELISA and RLEP PCR respectively.

There was one case which is smear positive but Anti PGL-1 negative. There were no cases where skin smear for AFB was positive but RLEP PCR was negative.

In this study 4/24 PB cases were found seropositive for PGL-1 while (5/7) MB cases showed seropositivity. RLEP PCR was found in 15/24 PB cases and all MB cases (7/7).

In this study 22/31 (71.00%) cases were positive for RLEP PCR whereas 9/31(29.03%) cases were positive by PGL-1 based ELISA. Only 8/31 (30.00%) cases showed positive skin smears (Table-3).

Table-3: Correlation Between Skin Smear, RLEP PCR and ANTI PGL-1 Antibody

Clinical Types	Number Of Cases	History Of Contacts	Smear Positive	Positive For Rlep Pcr	Positive For Anti Pgl -1 Antibody
TT	8	3	NIL	5	2
BT	15	5	2	10	2
BB	5	2	4	5	3
BL	1	0	1	1	1
LL	1	0	1	1	1
I	1	0	NIL	0	0
TOTAL	31	10	8	22	9
%	100	32.24	25.79	71	29.03

When results of cases were compared with age matched controls, out of 31 controls none was positive for RLEP PCR and 1 was positive for anti PGL-1. The result for RLEP PCR was significant (P value <0.05).

When results of both tests (RLEP PCR and Anti PGL-1) were combined together we found that they could detect 22 out of 31 cases and 1 out of 31 controls. Senstivity and specificity of for RLEP PCR were 72.10% and 96.50 % respectively while for Anti PGL-1 were 41.00 % and 93.37 % respectively. It clearly shows that sensitivity of RLEP PCR is higher than PGL-1.

DISCUSSION

India even after having leprosy elimination has several leprosy prevalent pockets. It denotes ongoing spread and existence of a high morbidity causing disease. Still new cases are identified as same rate. Its alarming us that more efforts are required for early diagnosis. The past of this disease has caused so much disability and deformity, so for a future free from leprosy early detection and stopping of ongoing transmission is mandatory.

WHO global leprosy strategy 2016-2020 aimed for zero disability among new paediatric leprosy patients for which early case identification is the major weapon. Hence this study is focussed on finding rapid diagnostic test of early detection of in paediatric leprosy.

Ganapati et al[13] who reported leprosy is more common in school age group as compared to preschool children. In our study (Table -2) 20 cases (64.52%) were between 11 -16 years of age and 11 cases (35.48 %) in ≤ 10 years of age group. Most of the cases are in adolescent age group. Our observation is similar to their study.

Further it was observed (Table -1) that male cases (58.06%) were more as compared to female (41.94%) cases, and M:F (ratio) was 1.39:1. This observation was comparable to the results of the study done by Dayal et al, Nigam et al and Dave et al who reported male preponderance in their studies[14,15,16]

We also noted that overall 32.24 % of cases had leprosy in family. This observation is similar to study done by Dayal et al 2007 who reported 31.8 % cases having family history (more in mutibacillary type)[14,17] .

We observed in our study RLEP PCR was positive in 22/31(71%) cases (TT 5/8 BT 15/10, BB 5/5,BL 1/1,LL 1/1) these results of were similar to those of Kamal et al[18] (2016) who observed 76.71% positivity. Kang et al (2003), Donoghue et al[19] (2001) and Martinez et al (2011) observed positivity of 73%, 87% and 100% respectively.

In comparison to AFB which is positive in 25.79% cases clearly demonstrates advantage of RLEP PCR over AFB for the diagnosis of leprosy, similar results has been demonstrated by previous studies (Cox et al 1991,Phetsukisiri et al 1994,Jamil et al 1994,Kang et al 2003,Wichitwechkarn et al 2004,Martinez et al 2006,Bannerjee et al 2010,Yan et al 2014,Turankar et al 2015).

9/31 cases (29.03%) [2/15 cases of BT type, 3/ 5 cases of BB type, 2/8 cases of TT, 1/1 case of BL and 1/1 cases of LL type of leprosy] was found serorpositive for anti PGL antibody. Study conducted by Buhrer-Sekula S et al[20] , Cellona RV et al[21],Cho SN et al[22]for MB and PB patients where average seropositivity among was 78% and 23%, respectively, varying between 51.2% and 97.4% in the MB group and from 6.9% to 57.3% in PB. Further low rate of detection was seen in BT type of leprosy (Table –3).

CONCLUSION

We found that RLEP PCR in leprosy is more sensitive than skin smear and PGL-1. It emerges as a minimally invasive and brilliant advance detecting tool for paediatric leprosy. Molecular methods are the vision for breaking active circulation and leprosy free future of india however we require further more studies to achieve this target.

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