Original Research Article

Evaluation of diagnostic role of IP-10 (Interferon Gamma Inducible Protein) and anti PGL-1 antibody in pediatric leprosy

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Abstract

Background: Leprosy is among the world's oldest and most dreaded diseases and it has been synonymous with stigma and discrimination due to the hideous deformities it produced, mystery around its aetiology and transmission and lack of any effective remedy till recently. Leprosy is characterized by a long and variable incubation period and a chronic clinical course. Diagnosis of leprosy is essentially based on clinical features. Although the majority of cases can be diagnosed clinically yet alternative methods for diagnosis are required especially for early cases. The present study is aimed to assess the diagnostic value of anti PGL-1 antibody and Interferon Gamma Inducible Protein (IP-10) and to compare these techniques with skin smear. **Materials and Methods:** A prospective study was done on 30 patients below 16 years of age between March 2014 to March 2015. **Results:** In this study 13 / 30 (43.33%) cases were positive by Anti PGL-1 antibody based ELISA and 12/30 (40%) by IP-10 based ELISA against 9/ 30 (30.00%) cases by skin smears for AFB. Hence Anti PGL-1 and IP-10 based ELISA has more diagnostic value than slit skin smear for AFB in confirmation of leprosy cases. **Conclusion:** This study supports that Anti PGL-1 antibody and Interferon Gamma induced protein (IP-10) enhance the diagnostic yield of leprosy when compared to routine skin smears stained by Z.N staining. They are important diagnostic tools for definitive diagnosis in early cases of leprosy.

Keywords: Anti PGL-1 antibody, Interferon Gamma Inducible Protein (IP-10), Leprosy, Skin smear

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Introduction

Leprosy is one of the world's oldest and most dreaded diseases that have tormented humans throughout history, leaving lasting impressions on religion, literature and art. Leprosy was earliest described in Asia (India and China) around 6th century B.C. and has been believed to have spread from India to Europe in 4th century B.C. In older days it was described as "KUSTHA ROGA" (which in Sanskrit means eating away), in "*Sushruta Samhita*" which dates back 600 B.C.[1.2.3] and was believed as wrath of God on those who had done some evil deeds (paap) in their present and past life, as a result they were abandoned by the family and society[4]. Dr. Gerald Henrik Armaeur

Hansen of Norway in 1873 discovered *M. leprae* as a causative agent of leprosy, therefore leprosy is also known as Hansen's disease [5,6].

Leprosy has a worldwide distribution. According to WHO weekly epidemiological report published on September 4, 2015 [7] number of new cases among children was 18,861. In india a total of 1,25,785 new cases were detected during the year 2014-15, which gives Annual New Case Detection Rate (ANCDR) of 9.73 per 100,000 population. 88,833 cases were on record as on 1st April 2015, giving a Prevalence Rate (PR) of 0.69 per 10,000 population.

A total of 11,365 child cases were recorded during the year 2014-15, indicating the Child Case rate of

0.88/100,000 population. Among the pediatric cases 3737 (2.97%) were Multibacillary Leprosy and 7628 (6.06%) were Paucibacillary Leprosy.

Materials and Methods

Patients were recruited from out patient department of National JALMA Institute of Leprosy and Other Mycobacterial Diseases, Agra after formal written consent from their parents. The ethical approval for this study was taken from the ethics committee of National JALMA Institute of Leprosy and Other Mycobacterial Diseases and also from ethics committee of S.N. Medical College, Agra.

Inclusion Criteria-

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

- Thirty age matched controls were taken from inpatients admitted in Department of Pediatrics, S. N. Medical College, Agra.

Exclusion Criteria- All patients more than 16 year of age and other diseases were excluded from this study.

Sample size (n): Total of 30 children was recruited from OPD of National JALMA Institute of Leprosy and 30 age matched controls were taken from Department of Pediatrics, S.N. Medical College, Agra.

Recording of clinical data: A detailed clinical history of the patient was taken.

- Patient's age / sex.
- History of contact with Leprosy patient.
- Duration and site of lesion.
- Charting and description of skin lesion and nerve involvement

Results

Out of thirty cases included in the present study (Table 1) 12 cases (40.00%) were of Borderline Tuberculoid (BT) type and 10 of Tuberculoid TT and 1 of Borderline Leprometous (BL) and Leprometous (LL) each, the rest 6 cases were of Borderline Borderline (BB) type of leprosy. In this study we have recruited more cases of the early forms of TT, BT, BB of leprosy.

The clinical and demographic profile of cases is summarized in tables 1 and 2 . Out of the total cases, 40% patients were of BT type and rest 60.00% of cases were of BB /BL/LL/TT type.13 out of 30 (43.33%) cases had positive family history of leprosy.

In this study 16.67% cases had only skin lesions, while 83.33% had both skin lesions and nerve involvement. Out of 30 cases, 2 cases with BT type of leprosy were smear positive for AFB and 7 out of 8 cases with BB/BL/LL type of leprosy were skin smear positive for AFB. Rest of the cases were smear negative.

In this study 4 and 3 cases were such that who had negative skin smears for AFB but were positive by Anti PGL-1 based ELISA and IP-10 respectively.

There were no cases where skin smear for AFB was positive but Anti PGL-1 and IP-10 based ELISA was negative. There were 9 cases who were positive by both slit skin smear for AFB and Anti-PGL-1 Antibody and IP-10 based ELISA.

In this study 5 /22(22.7%) PB cases were found seropositive for PGL-1 while all cases(8/8) MB cases showed seropositivity. IP-10 seropositivity was found in 4/22(18.2%) PB cases and all MB cases (8/8).

In this study 13 / 30 (43.33%) cases were positive by Anti PGL-1 antibody based ELISA and 12/30 (40%) by IP-10 based ELISA against 9/30 (30.00%) cases by skin smears for AFB.

Hence Anti PGL-1 and IP-10 based ELISA has more diagnostic value than slit skin smear for AFB in confirmation of leprosy cases.

Cut off point for reactivity was calculated by Mean + 2SD Method. Cut off Optical density for PGL- 1 antibody was taken as 0.0424 and that for IP-10 was taken as 169.24 pg/ml. Value above the cut off was taken as positive and that below it was considered negative.

Table-1: Clinical and demographic profile of cases.

Clinical Type	No. of Cases (%)
TT	10 (33.3)
BT	12 (40.00)
BB	6 (20.00)
BL	1 (3.33)
LL	1 (3.33)
Age	
Preschool & School age (≤ 10 yrs)	9 (30)
Adolescent (11-16 yrs)	21 (70)
Sex	
Boys	19(63.33)
Girls	11(36.67)
No of Skin Lesions	
Single lesion	7 (23.33)
2-4 lesions	15 (50.00)
>5	8 (26.67)
Type of nerve involvement	
Ulnar nerve only	13 (52)
Lat. popliteal nerve only	1 (4)
Radial nerve	1 (4)
Tibial nerve	2 (8)
Both ulnar & lateral Popliteal nerve	8 (32)

Table-2: Correlation between skin smear for AFB and ELISA based detection of antibody against anti PGL-1 antibody and IP-10 of cases in this study.

Clinical Type	No. of cases	History of contacts	Smear positive	ELISA based AntiPGL-1 Antibody	Human Interferon Inducible Protein
TT	10	4	NIL	3	0
BT	12	5	2	2	4
BB	6	3	5	6	6
BL	1	1	1	1	1
LL	1	0	1	1	1
Total	30	13	9	13	12
%	100	43.33	30	43.33	40.00

Discussion

In this study (Table-1) 21 cases (70.00%) were between 11 -16 years of age and 9 cases (30.00 %) in ≤ 10 years of age group. Most of the cases were in adolescent age group, and this may be explained by the fact that leprosy has long incubation period and needs prolonged exposure. Preschool children were less as compared to school going age group and this observation is comparable to study done by Ganapati et al[8] who reported leprosy is more common in school age group as compared to preschool children. Further it was observed (Table -1) that male cases (63.33%) were more as compared to female (36.67%) cases, and M:F (ratio) was 1.72:1. This observation was comparable to the results of the study done by Dayal et al[9], Nigam et al[10] and Dave et al[11] who reported male preponderance in their studies.

This is a small study sample and therefore further comments cannot be made on sex ratio of patients included in this study. We also noted that overall 43.33% of cases had leprosy in family of which 5/1 3 cases were of BT type & 8/13 cases were BT/BL/LL type of leprosy (Table-2). This observation is similar to study done by Dayal et al 2007 who reported 31.8% cases having family history (more in multibacillary type).

In this study there were 7 cases having single lesion. Maximum number of cases had 2-4 skin lesions [15 cases (50.00%)] and 8 cases had >5 skin lesions (26.67%) which was shown in Table -1.

We also found that 25 cases (83.33%) had both skin lesions and nerve trunk thickening and only 5 cases (16.67%) had skin lesions without nerve trunk thickening (Table- 1).

Further we noted that, 25 cases had nerve involvement & 13/25 (52%) cases had only ulnar nerve thickening & 8/25 (32%) cases had both ulnar & lateral popliteal nerve thickening. There was no child with pure neuritic leprosy (Table-1). The results are comparable to the finding of Dayal et al [9].

All the cases of TT of leprosy were smear negative for AFB. Skin smear were positive for AFB in 9/30 cases (30%) of BB & BL type of leprosy. 5 out of 6 (16.67%) of BB type of leprosy cases were positive for AFB , whereas for all BL and LL cases were slit smear positive as shown in Table-1.

ELISA based antibody against Anti PGL-1 antibody positivity was observed in 13/30 cases (43.33%) of leprosy [2/12 cases of BT type, 6/ 6 cases of BB type, 3/10 cases of TT, 1/1 case of BL and 1/1 cases of LL type of leprosy]. Among age matched control 1 was positive for anti PGL-1 antibody. There were no cases which were positive for skin smear for AFB, and ELISA was negative. In this study low rate of detection was seen in BT type of leprosy as compared to other types (Table - 2).

These finding correlate well with the studies conducted by Buhrer-Sekula et al [12], Cellona RV et al[13] , Cho SN et al [14] for MB and PB patients where average sero positivity 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. Analysis of the results showed that the use of serology as a tool for patient classification would lead to a reduction in the number of patients treated as MB. This is because the counting of skin lesions is a functional operational tool, but has not been well-received by health professionals. When laboratory tests like bacilloscopy and histopathology are not available, there is a strong tendency to classify patients as MB, as seen in the Nigerian study, where a large proportion of patients received the MB treatment regimen unnecessarily as shown by Buhrer-Sekula et al [12].

Barreto JG [15] investigated the prevalence of antibodies against PGL-I in people affected by leprosy (PAL) who were diagnosed and treated between 2004 and 2010, their household contacts (HC) and school children (SC) from a hyperendemic municipality in the Brazilian Amazon, and determined the prevalence of previously undiagnosed leprosy (PPUL) among both the HC and SC. The level of anti-PGL-I antibodies was significantly higher in multibacillary (MB) than in paucibacillary (PB) cases (P < 0.05). Thirty-nine percent of HC were positive for anti-PGL-I, and eight (2.6%) new cases were detected among these individuals. One hundred and twenty-five SC (66.5%) were seropositive, and we nine (4.8%) new cases of leprosy (eight PB and one MB) were detected in this group. In the homes of SC affected by leprosy, 31 contacts were clinically examined, and three (10%) new cases were detected (one PB and two MB). The mean age of students with leprosy was 14.1 years (SD = 2.5; min = 10, max = 18).

Douglas JT [16] monitored contacts over a period of 6 years and showed that there is a 7.2- fold greater risk of developing leprosy (MB or PB) in seropositive contacts with antibodies to PGL-I when compared to seronegative contacts, increasing to 24-fold greater risk of developing MB leprosy. Cellona RV found that percentage of contacts that progress to disease among seropositive contacts suggests that serology with anti-PGL-I could be useful as a prognostic test [13].

ELISA based antibody against human interferon inducible protein (IP-10) positivity was observed in 12/30 cases (40.00%) of leprosy [4/12 cases of BT type, 6/ 6 cases of BB type, 0/10 cases of TT, 1/1 case of BL and 1/1 cases of LL type of leprosy]. Among age matched control 3 were positive for anti PGL-1 antibody. There were no cases which were positive for skin smear for AFB, and ELISA was negative. In this study low rate of detection was seen in BT type of leprosy as compared to other types(Table -2). IP-10 is a potential biomarker of TB exposure in children; however, like IFN-γ, IP-10 cannot be used to discriminate between active and latent

TB in this population as stated by Whittaker E et al. [17] As with TB, IP-10 levels are elevated within tissues and sera of leprosy patients as studied by Schuller I et al [18] and Scollard DM et al [19] . Schuller I et al also stated that Type 1 reactions, a systemic inflammatory syndrome of borderline leprosy patients, are associated with a significant increase in serum IP-10 but not IFN-γ. In study of Geluk A et al [20] to develop diagnostic approaches for subclinical/early-stage leprosy, IP-10 was shown to augment the diagnostic potential of IFN-γ/M. leprae peptide-based tests .

Overall ELISA based antibody detection has good sensitivity and specificity in diagnosing leprosy cases. Earlier antibody based ELISA was used for diagnosis of pediatric TB cases and it showed good diagnostic potential. Our results are similar to those findings. In this study when we compared the results of slit skin smear for AFB and Anti PGL-1antibody based ELISA (Table 2), it was found that Anti PGL-1 antibody based ELISA was positive in 13/30 (43.33%) cases and slit skin smear for AFB was positive in 9/30 cases (30.00%). Similarly IP-10 based was positive in 12/30 (40%) cases. This shows that Anti PGL-1 antibody and IP-10 based ELISA can significantly enhance diagnosis as compare to slit skin smear.

Conclusion

The observations in this study shows that Anti PGL-1 antibody and human interferon inducible protein based ELISA on serum can be useful as research tool in confirming early leprosy cases where slit skin smears for AFB are negative and skin biopsy is not feasible. However since the number of cases in our study was small, there is a need for further studies.

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