

Diagnostic Role of Anti PGL-1 Antibody, Antigen 85-C and IP-10 (Interferon Gamma Inducible Protein) in Pediatric Leprosy

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ABSTRACT

Introduction: Early cases of pediatric leprosy are difficult to diagnose when characteristic skin lesions are not present and where Acid Fast Bacilli are difficult to detect. It is important to detect these early cases of leprosy in children to reduce the rate of transmission and prevent disability as emphasized by WHO Global Leprosy Strategy 2016-2020. Our study analyzed the detection of Anti- 85C antibody, Anti PGL-1 antibody and Human interferon inducible protein (IP-10) using ELISA (Enzyme Linked Immunosorbent Assay) in early pediatric leprosy cases.

Material and Methods. A prospective study was conducted in 30 untreated early paediatric leprosy cases equal to or less than 16 years. Their clinical profile, slit skin smear examination and serological test results using ELISA were studied.

Result: Serological testing results were compared between cases and controls. The outcome was significant (P value <0.005) for all the three serological markers. Sensitivity and specificity of anti-85C was 76.67% and 90%, that for anti PGL-1 was 46.67% and 90% while that for IP-10 was 43.33% and 93.33% respectively. Sensitivity of Ag85C was higher than PGL-1 and IP-10. Only 30% cases were slit skin smear positive.

Conclusion: Anti PGL-1 antibody, anti-85C antibody and IP-10 based ELISA on serum can be useful research tool in confirming early diagnosis of leprosy in cases where slit skin smears for AFB is negative and skin biopsy is not feasible.

Keywords: Anti PGL-1 antibody, Antigen 85C, Interferon Gamma Inducible Protein (IP-10), Leprosy, Skin smear

INTRODUCTION

Leprosy is a chronic granulomatous disease which usually presents with characteristic skin lesions, thickening of peripheral nerves with variable sensory and or motor loss.

Leprosy has a worldwide distribution. According to the WHO India contributes about 60% of newly detected leprosy cases in the world. Out of 127326 new cases detected in India, 11,389 were children (8.94%).¹

According to Global Leprosy Strategy 2016-2020 the focus is on early case detection before visible disabilities occur. A special focus will be on children as a way to reduce disabilities and reduce transmission. The target is zero disabilities among new pediatric leprosy patients by 2020.

Clinically leprosy is diagnosed by the presence of hypopigmented patches showing partial loss of sensation in that affected area and/or presence of thickened cutaneous nerves. Presence of acid fast bacilli in the skin smears and/or biopsy specimens is helpful in confirming the diagnosis. The finding of characteristic skin lesions and detection of

acid fast bacilli is easily evident in established forms of leprosy, but in early cases, these diagnostic findings may not be present which creates a hurdle in early case detection.

To solve this problem, the role of immunological markers has been studied in recent past and attempts are being made to make serodiagnosis an easy and useful test.

Antigen 85 complex, which is a complex of 3 proteins namely 85A (32kDa), 85B (30kDa) and 85C (32.5kDa). The complex is encoded by 3 genes located at different sites in the mycobacterial genome.^{2,3} The Antigen 85 complex molecules are major stimulants of the humoral and cellular immune responses of tuberculosis and leprosy patients.⁴ Kumar et al found Anti-85C to be a useful marker for tuberculosis patients but its role in leprosy needs further studies.⁵

The detection of the species-specific antibody to Phenolic glycolipid-I (PGL-1) in leprosy and its synthetic analogs containing antigenic carbohydrates have been used in ELISA to detect antibodies, mainly of the IgM class.^{6,7} Cho et al demonstrated that the technique is 96% sensitive for multibacillary leprosy but detects 62% of paucibacillary cases.⁷

The role of Interferon Gamma Inducible protein (IP-10) in leprosy has been examined as a part of cross sectional study of reactions in leprosy. As with tuberculosis IP-10 levels are elevated within tissues and sera of leprosy patients.^{8,9} IFN-g production induced by *M. leprae*-unique proteins can identify individuals highly exposed to *M. leprae* and therefore more

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at risk for developing disease and/ or transmitting the bacterium.¹⁰

Our study analyzed the detection of Anti- 85C antibody, Anti PGL-1 antibody and Human interferon inducible protein (IP-10) using ELISA (Enzyme Linked Immunosorbent Assay) in early pediatric leprosy cases.

MATERIAL AND METHODS

Patients were recruited from out patient department of S.N. Mediacal College and National JALMA Institute of Leprosy and Other Mycobacterial Diseases, Agra after formal written consent from their parents.

Inclusion Criteria

- Age less or equal to 16 years with characteristic skin lesions 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 years of age and those suffering from tuberculosis or other diseases were excluded from this

Clinical type	No. of cases (%) (Total= 30 cases)
TT	8 (26.66)
BT	13 (43.33)
BB	7 (23.33)
BL	1 (3.33)
LL	1 (3.33)
Age	
Preschool and school age(≤10 years)	10 (33.33)
Adolescents(11-16 years)	20 (66.66)
Sex	
Boys	18 (60)
Girls	12 (40)
Number of skin lesions	
Single	7 (23.33)
2-4	14 (46.66)
≥5	9 (30.00)
Type of nerve involvement	
Ulnar nerve	12 (50)
Lateral popliteal nerve	2 (8.33)
Radial nerve	1 (4.16)
Tibial nerve	2 (8.33)
Both ulnar and lateral popliteal nerve	7 (29.16)

Table-1: Clinical and demographic profile of cases.

Clinical Type	No. of cases	History o contacts	Smear positive	Ag85C	PGL-1	IP-10
TT	8	2	NIL	2	3	0
BT	13	6	2	12	3	5
BB	7	3	5	7	6	6
BL	1	1	1	1	1	1
LL	1	0	1	1	1	1
Total	30	12	9	23	14	13
%	100	40	30	76.66	46.66	43.33

Table-2: Correlation between skin smear for AFB and ELISA based detection of antibody against Ag85C, ANTI PGL-1 Antibody and IP-10 of cases in this study

study.

Total of 30 children were recruited from the OPD and 30 age matched controls were taken.

Recording of clinical data: A detailed clinical history was taken and thorough examination done. Duration, site and description of skin lesions and nerve involvement was recorded.

Slit skin smear was done for acid *M. leprae* by staining with Zeihl -Neelsen's method. The study was approved by Institutional human Ethics Committee.

ELISA for antibody detection against antigen 85c was done using recombinant Antigen 85C (derived from Ag85 complex of *M. tuberculosis* strain) and Antihuman IgG peroxidase-conjugated antibody and the absorbance was measured at 492 nm using a Spectramax-M2 Reader (Molecular devices, Sunnyvale, CA, USA). The mean \pm 2 S.D. of the OD values of the age matched controls were taken as the cut-off values.⁵

ELISA For Detection of Anti Pgl-1 Antibody using PGL-1 antigen (derived from *M. leprae* strain) and Anti Human IgM antibody and the absorbance was measured at 450 nm using a Spectramax-M2 Reader (Molecular devices, Sunnyvale, CA, USA). The mean \pm 2 S.D. of the OD values of the age matched controls were taken as the cut-off values.⁷

ELISA to detect Interferon Gamma Inducible Protein (IP-10): IP-10 was estimated using sandwich ELISA method through commercially available kit from R&D system, USA and absorbance was measured at 450 nm using a Spectramax-M2 Reader (molecular devices, Sunnyvale, CA, USA). The mean \pm 2 S.D. of the OD values of the age matched controls were taken as the cut-off values. Ag85c and PGL-1 were procured from BEI Resources, 10801, University Blvd, Manassas, VA20110, USA. All the above experiments were carried out two times.

STATISTICAL ANALYSIS

Statistical analysis was done with the help of SPSS version 21. Chi square test was done for comparison.

RESULT

Out of thirty cases included in the present study, 13 cases (43.33%) were of BT (Bordeline Tuberculoid) type and 8 of TT (Tuberculoid leprosy) and 1 of BL (Bordeline Lepromatous) and LL (Lepromatous Leprosy) each, the rest 7 cases were of BB (Borderline Borderline) type of leprosy.

In this study we found more cases of early forms (TT, BT, BB) of leprosy. 12 out of 30 (40.00%) cases had positive family history of leprosy. The clinical and demographic profile of cases have been described in Table 1.

In this study 20.00% cases of leprosy had only skin lesions, while 80.00% had both skin lesions and nerve involvement. Out of 13 BT cases, 2 cases were smear positive for AFB. 7 out of 9 cases with BB/BL/LL type of leprosy were skin smear positive for AFB. Rest of the cases were smear negative.

Cut off point for reactivity was calculated by Mean \pm 2SD Deviation Method where mean was calculated from O.D of controls. Cut off Optical density for Ag85C Antibody was taken as 0.4103, cut off for PGL- 1 antibody was taken as 0.0511 and that for IP-10 was taken as 172.34 pg/ml. Value above the cut off were taken as positive and that below it was considered negative.

As shown in Table-2, 14, 5 and 4 cases were such that who had negative skin smears for AFB but were positive by Ag85C, PGL-1 and IP-10 based ELISA respectively.

There were no cases where skin smear for AFB was positive but Ag85C, PGL-1 and IP-10 based ELISA were negative. There were 9 cases who were positive by both slit skin smear for AFB and Ag85C, PGL-1 and IP-10 based ELISA.

In this study, seropositivity for Anti-85C was seen in 14/21 PB (Paucibacillary includes TT and BT) cases and all MB (Multibacillary includes BB, BL and LL) cases (9/9) were positive. 6 /21 PB cases and 8/9 MB cases were found seropositive for PGL-1. IP-10 seropositivity was found in 5/21 PB cases and 8/9 MB cases.

In this study 23/30 (76.66%) cases were positive for Ag85C based ELISA whereas 14 / 30 (46.66%) cases were positive by PGL-1 based ELISA and 13/30 (43.33%) by IP-10 based ELISA. Only 9/30 (30.00%) cases showed positive skin smears. When results of cases were compared with age matched controls, 3 out of 30 controls were positive for anti-85C and anti PGL-1 antibody while 2 out of 30 were seropositive for IP-10. The results for all the three serological markers were significant (P value <0.05).

When results of all the three tests (anti 85C, anti PGL-1 and IP-10) were combined together we found that they could detect 24 out of 30 cases and 6 out of 30 controls. Sensitivity and specificity of anti-85C was 76.67% and 90%, that for anti PGL-1 were 46.67% and 90% respectively while that for IP-10 were 43.33% and 93.33% respectively. It shows that sensitivity of ag85C is higher than PGL-1 and IP-10.

DISCUSSION

Leprosy is the oldest scourge of mankind and still present in many parts of the world as public health problem including India. Although several attempts have been taken to control the disease but still it continues to remain an enigma.

To achieve the target of zero disability among new paediatric leprosy patients (in compliance with WHO global leprosy strategy 2016-2020), early case detection is the key to reach the target. Hence our study is focussed on finding an easy and rapid diagnostic method of early detection of leprosy in

paediatric cases.

In this study 20 cases (66.66%) were between 11 -16 years of age and 10 cases (33.33%) in \leq 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 children as also observed by Ganapati et al.¹¹

Further it was observed that male cases (60.00%) were more as compared to female (40.00%) cases, and M:F (ratio) was 1.5:1. Similar results have been reported by Dayal et al¹², Nigam et al¹³ and Dave et al.¹⁴

Positivity for anti PGL-1 antibody was observed in 14/30 cases (46.66%) of leprosy. Among age matched controls 3 controls were positive for anti PGL-1 antibody. There were no cases who were positive for skin smear and negative for antibody. When the result was compared between MB and PB cases, MB cases showed higher rate of detection of antibody (P value <0.05).

These finding correlate well with the studies conducted by Buhner-Sekula et al¹⁵, Cellona RV et al¹⁶, Cho SN et al¹⁷ for MB and PB patients where average sero positivity was 78% and 23%, respectively. 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 Buhner-Sekula et al.¹⁵

Barreto JG¹⁸ observed that 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 JT¹⁹ 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.¹⁶ Raj et al found 43.33% sensitivity for PGL-1 in pediatric leprosy.²⁰ Hardly any studies are available in the world literature on the role of Antigen 85C in diagnosis of leprosy cases. Antibody positivity against Ag85C was observed in 23/30 cases (76.66%). 3 out of 30 age matched controls were positive. There were no cases who were positive for skin smear but negative for antibody. Overall antibody detection has good sensitivity and specificity in diagnosing leprosy cases and

observations are statistically highly significant ($p < 0.05$). In the study conducted by Kumar et al, antigen 85C showed highest sensitivity of 89.77% and specificity of 92% among different secretory antigens of mycobacterium tuberculosis (CFP10, Ag85A,B,C).⁵ Positivity with antigen was 95% in smear and culture negative patients. Antibody reactivity was noted in 92.62% of patients who were positive for IS6110 by PCR.⁵ Kumar et al similarly found Ag 85C a good marker in paediatric tuberculosis With sensitivity of 83.65%.²¹ Further studies are required in leprosy cases for evaluating its role. Positivity for IP-10 was observed in 13/30 cases (43.33%) while among age matched controls, only 2 showed IP-10 positivity (P value < 0.05). MB cases showed higher rate of detection than PB cases (P value < 0.05). The role of Interferon Gamma Inducible protein (IP-10) in leprosy has been examined as a part of cross sectional study of reactions in leprosy. 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.²² As with TB, IP-10 levels are elevated within tissues and sera of leprosy patients as studied by Schuller I et al²³ and Scollard DM et al.⁸ 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 al²⁴ 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. Raj et al found 40.00% sensitivity for IP-10 in pediatric leprosy.²⁰ Further studies are needed in leprosy cases for evaluating its role.

CONCLUSION

The observations in this study shows that Anti PGL-1 antibody, Anti-85C antibody and human interferon inducible protein (IP-10) based ELISA on serum can be useful as a research tool for early diagnosis leprosy cases with paucity of clinical signs where slit skin smears for AFB are negative and skin biopsy is not feasible. Further studies are needed to evaluate our findings.

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