Original article

Clinico-histopathological correlation and detection of anti PGL-1 antibodies in leprosy patients

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Abstract:

Background: Leprosy (Hansen's disease) is one of the major health problems of the world especially in developing countries. In India, it was first described in "Sushruta Samhita" & treated by Chaulmoogra oil and caused by Mycobacterium Leprae. Early diagnosis of leprosy, an absolute necessity for control as well as effective therapy. For this, clinical diagnosis, skin smear examination is adequate coupled with histo-pathological examination of skin and nerve lesions with modified Fite Faraco stain for demonstration of acid fast bacilli. Moreover, bacillary index is required for adequate combined chemotherapy regimen. Detection of anti PGL-1 antibodies in serum gives an added advantage for detection and monitoring treatment.

***Materials & Methods* :** A total of 85 cases of leprosy who attended outpatient department of Skin & VD, Shri Sayaji General Hospital Baroda chosen for study during '07-08' period with 75 cases from leprosy hospital, Baroda which included 50 detected patients and 25 child contacts with 25 healthy voluntary blood donors from blood bank, SSGH selected.Clinical, past and family history taken with slit skin smears stained with Z-N stain,graded and histopathological evaluation done. Serological study done from serumof leprosy patients and healthy blood donors; tested by serodia kitsand interpretation made.

Results : Most cases were in 2nd to 4th decade and males dominated. Clinically most cases were of indeterminate and tuberculoid type and histologically indeterminate and borderline tuberculoid. Clinico-histopathological correlation was found most in indeterminate followed by histoid type. Voluntary blood donors were seronegative. 21 out of 48 multibacillary cases and 6 out of 28 paucibacillary showed seropositivity for anti PGL-1 antibodies ($p<0.001$).

Conclusion : . All suspected leprosy cases clinically should be subjected to slit skin smear examination with histopathological evaluation; bacillary study which helps in diagnosis and adequate treatment of patients. Detection of antibodies to PGL-1 in patients indicate pres-ence of leprosy bacilli and useful in preclinical diagnosis and determining progress of therapy.

Key words: Fite Faraco; anti PGL-1 antibody; gelatin agglutination; MLPA

DOI: http://dx.doi.org/10.3329/bjms.v13i3.9153
Bangladesh Journal of Medical Science Vol. 13 No. 03 July '14. Page: 316-322

Introduction

Leprosy is one of the major health problems of the world, especially in developing countries[1]. Early diagnosis of leprosy is an absolute necessity for control as well as effective therapy[1]. Most of the time, clinical diagnosis and skin smear examination are adequate but difficulty is encountered in early, indeterminate and borderline cases[1,2]. Leprosy is a disease of skin and nerves. So, histopathological examination of skin and nerve lesion is essential for confirmation of diagnosis and classification .Modified fite-faraco's stain is useful for demonstration of lepra bacilli in paucibacillary case (Indeterminate & Borderline) & bacillary content on the basis of which treatment is given[3]. Serological test for Phenolic glycolipid antibodies (Anti PGL-I) are

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helpful for determining prognosis of leprosy[4].

Thus, clinico-pathological correlation along with evaluation of anti PGL-I antibodies in sera can give us a complete package of leprosy disease confirmation with treatment[1-4].

Objective:-

1. Clinico-histopathological correlation in leprosy cases.
2. Bacillary study by modified Fite Faraco method .
3. Comparison of data with those available in India.
4. Detection of Anti PGL-I antibodies by MLPA (gelatine particle agglutination assay).

Materials and methods :-

The cases chosen for study included 85 cases of leprosy who attended the Outpatient Skin –V.D. Shree Sayaji General Hospital Vadodara with 75 Leprosy patients(50 detected cases and 25 children contacts) taken from leprosy hospital, Vadodara,Gujrat. Cases selected for study included new leprosy casesdetected during the period of study and none showed sign & symptoms of reactions. The study was approved by Shree Sayaji General Hospital Vadodara

Detailed clinical history (skin lesions and sensory disturbances) with past and family history was asked for ; general and systemic examination were carried out. The cases were diagnosed clinically utilizing the criteria of Indian Consensus classification(Indeterminate, Tuberculoid, Borderline Lepromatous, Lepromatous and Pure neuritic forms).The diagnosis of leprosy was based on clinical examination, slit skin smear /bacteriological examination, histology of skin and nerve tissues[1,2,5,6].

The cardinal signs of leprosy are anesthesia [which may be of individual skin lesion,may be in distribution of large peripheral nerve (happens in tuberculoid leprosy),may be over areas of fine nerve involvement (happens in lepromatous leprosy)]; thickened nerve; skin lesions and acid fast bacilli in slit skin smears[1,5,6].

Clinically, lepromatous leprosy patients present with nasal stuffiness, pedal edema , multiple, symmetrical cutaneous lesion, lost eyebrows, nasal septal collapse, hoarse voice, glove and stocking sensation and nerve thickening with associated motor and sensory dysfunction[1,4].

Tuberculoid leprosy patients show loss of feeling, tingling, muscle weakness/paralysis, erythematous lesion with dry surface, deficient hair growth over lesion and pain, touch & temperature sensation impaired with presence of thickened nerve [1,2].

Borderline leprosy patients usually present with asymmetrically damaged nerves.Borderline tuberculoid lesions are less numerous with anesthesia and impaired hair growth more marked but never as complete as in Tuberculoid leprosy cases[1,4].

Indeterminate cases have small, hypopigmented or erythematous asymmetrical lesions , no anesthesia , late cases present with palpable thickened nerve which heal spontaneously or shift to borderline or lepromatous types[1,2].

In addition to routine laboratory investigation, skin smears for acid fast bacilli (AFB) were taken from skin lesions, eye brows or ear lobes by slit and scrape method and stained by Ziehl-Neelsen method and graded according to Dharmendra's scale[3,6].

Slit skin smears made from suspect lesions as well as from sites commonly affected in lepromatous leprosy, usually ear lobes and forehead. Blade - Bard Parker number 15 used for tissue scraping and smear fixed by flaming the slide and stained by Ziehl Neelsen's method[2,3].

The method comprise of adding 1% aqueous solution of carbol fuchsin over heat fixed slit skin smears,differentiating with acid alcohol mixture and counterstaining with 1% methylene blue[3]. Slides examined under oil immersion lens, show bacilli as red rods against blue background that diagnose leprosy[1,6].

Smears are read following different indices used namely, bacteriological index (BI); Morphological Index (MI); Solid fragmented granular percentage (SFG).Bacteriological Index (BI) indicates density of leprosy bacilli in smears known for measuring bacteriological index termed as Ridley's logarithmic scale and Dharmendra's scale.Here, Dharmendra's scale is used.Dharmendra's scale includes slight[(1+) present in anoccasional field]; Moderate[(2+) present in every field]; Heavy [(3+) numerous bacilli and globi in every field]; Massive[(4+) innumerable bacilli and many globi in every field] [1,3,6].

Bacteriological index is obtained by adding degrees of positivity of all smears and dividing total by the numbers of smear examined.Morphological index is percentage of presumably living bacilli in relation to total number of bacilli in smears and 200 pink stained bacilli calculated. The percentages added up

and divided by number of smears[3,6].

Histological features of leprosy : Punch biopsy of skin lesion taken from active part of lesion ; biopsy material fixed in FMA fixative (Zenker's fluid) or 10% formol saline and transported to pathology department of SSG Hospital, Vadodara.Fixed tissue specimens processed as per standard procedure for paraffin section in histokinette and sections stained with hematoxylin and eosin for histology study and modified Fite Faraco's stain used in doubtful cases[3,7-9].

Indeterminate (IL) leprosy cases show atrophy of epidermis with loss of rete pegs (Hasselman et al) and lymphohistiocytic infiltrate around capillaries, sweat glands, pilosebaceous structures. Involvement of nerves by chronic inflammatory cells constitutes strong evidence in histological diagnosis of IL[9].

Tuberculoid leprosy (TT) casesshow atrophied epidermis with no free sub-epidermal zone; dermis contain well defined granulomas containing epithelioid cells surrounded by small lymphocytes with diffuse infiltrate obliterating cutaneous nerve twigs[9].

Borderline leprosy cases are divided into borderline tuberculoid (BT); pure borderline (BB) and borderline lepromatous (BL). BT cases show more diffuse epithelioid granulomas than in TT. Here ,we get free but a narrow papillary zone. Giant cells are of foreign body type with dermal nerves affected by cellular infiltrate,bacilli usually absent in dermis.In BB cases; diffuse epithelioid cell granuloma with scanty lymphocytes found with absence of giant cells; clear papillary zone; slight swelling of dermal nerves with moderate number of bacilli. BL cases show macrophage granuloma with foamy change,and presence of lymphocytes with onion skin perineurium; clear papillary zone (Grenz zone)visualised,plenty of lepra bacilli[9]

Lepromatous leprosy (LL) cases show perineurium infiltration with histiocytes and plasma cells, swollen macrophages packed with bacilli which are known as lepra cells(globi).Epidermis thinned with flattened rete ridges; macrophage granuloma with foamy changes found with absent or scanty lymphocytes. Dermal appendages intact with clear sub-epidermal zone. Histoid leprosy (HL) cases present high bacillary index (usually 6) ; show spindled cells with arrangment in storiform pattern, similar to fibro-histiocytoma. Epidermis is stretched over such dermal expansion nodules[9,10].·

Sections for histopathological study stained by hematoxylene and eosin stain and modified Fite Faraco stain for detection of bacilli. In Hematoxylene and eosin stain method; stain with hematoxylene for 5-7 min;blueing with 1% acid alcohol and counterstain with eosin done[1,6].

In ambiguous cases; sections stained with modified Fite Faraco's staining method to demonstrate bacilli. Sections deparaffinised with xylene and groundnut oil (in a ratio of 2 parts of xylene and 1 part groundnut oil) with 2 changes of 12 mins. each.Staining in Ziehl Neelsen carbol fuchsin ; decolourizing in 5% sulphuric acid and 25%ethyl alcohol and counterstain with 0.1 % methylene blue done and looked for presence of bacilli if any[3,7,8].

Serodiagnosis of leprosy carried out by qualitative test for detection of antibodies to M. Leprae antigen (anti PGL-1 antibodies by serodia leprae method) which is helpful for diagnosis and evaluating progress of the disease.The priniciple of the test is gelatine particle agglutination test.Controls tested were 25 healthy voluntary donors without known contacts with leprosy patients from Blood Bank, SSGH. Study group included 75 leprosy patients from leprosy hospital, Vadodara(50 detected cases and 25 child contacts).Patients included 15 detected and 13 child contacts of paucibacillary group ; 35 detected and 12 child contacts of multibacillary group which are further classified according to Ridley Jopling scale as 47 multibacillary subjects(BL21, LL26) and 28 paucibacillary subjects (BT 16,TT 12) From each group, 5cc blood samples aseptically collected , serum separated and preserved at- 20?C and testing carried in batches.Samples tested by "SERODIA KITS" in which gelatine particles were sensitized with " NT-BSA " antigen.Negative control was with unsensitized gelatine particles at 1/16 final dilution of each serum and test sera screened with sensitized particles at 1/32 final dilution..[11,12]

Wells taken were three ;serum diluents of 75 microl and 25 microl dropped in first and second wells leaving the third vacant. Test serum (25 microl) then poured in the first well only. Diluted serum (25 microl) removed from first well and poured in the second well followed by removal of 25 microl of diluted serum from second well which was further poured in the third well and finally discarded.Serum

dilution was in a ratio of 1:4; 1: 8; 1:16 respectively in the three wells.Now, the gelatine particles were poured in . Unsensitized particles poured in second well and sensitized particles poured in third well both in a volume of 25 microl leaving the first well.Final dilution thus becomes 1:16 in the second well and 1: 32 in the third well.Wells with their respective mixtures in them mixed thoroughly , covered with cover plate ; incubated for two hours and interpretation made.If the well showed no agglutination or inconclusive the result given was negative and positive result was given in cases of complete agglutination[11,12].

Observation**:**

We analysed our data of 85 clinically diagnosed leprosy cases. Age, sex and family history noted. Condition was to correlate clinical types with histopathological types. Clinically, maximum cases (23 cases-27.05%) presented in indeterminate spectrum followed by TT, BT (both 22 cases-25.88%), Histoid leprosy (10 cases-11.76%), lepromatous leprosy (5 cases-5.88 %), BB (3 cases – 3.52 %).

Maximum number of leprosy cases found in 2nd to 4th decade of life followed by 5th -6th decade.55 cases (64.69 %) were in 2nd to 4th decade and 30 cases (35.29%) were in 5th to 6th decade.Incidence in males (51 cases – 60%) was greater by 1.5 times than that in females (34 cases-40%).Out of 85 cases of leprosy only 7 cases (8.23%) gave positive family history of contact with infectious .cases of leprosy.

Out of 85 skin biopsy cases, 22 were diagnosed clinically as TT among which 16 were diagnosed histopathologically as TT while 05 grouped under borderline tuberculoid and 01 under indeterminate group.Out of 22 clinically diagnosed BT, 16 (72.72%) were also histologically BT, 01 case showed features consistent with TT and 5 cases had features of indeterminate leprosy histologically confirmed by demonstration of bacilli.Out of 3 clinically diagnosed BB cases, only 01 case(33.33%) were also histologically BB; 1 case showed features consistent with BT and 1 case with BL.No case was clinically diagnosed as BL. Out of 5 clinically diagnosed LL cases, 02 (40%) cases were also histologically LL; 2 cases grouped under BL; 1 case showed features consistent with BT and no bacilli was found by special staining method. Out of 10 clinically diagnosed HL cases; 09 (90%) were histologically confirmed as HL . 1 case showed features consistent with LL; 23 cases were clinically diagnosed as IL; all(100 %) of which turned out to be IL histologically too which was confirmed by bacteriological examination by special staining techniques.22 cases were found to be bacteriologically positive..Here, we can see maximum disparity (66.67%) found in borderline cases.

There is 100% clinico-histopathological correlation in Indeterminate Leprosy cases followed by 90% in histoid leprosy cases.

Results of serological aspect of leprosy cases

Among 25 voluntary blood donors taken from blood bank SSGH, Vadodara none showed positive results by serodia kits. Among 25 children contacts 5 showed serodia positivity (20%). Among the 50 leprosy patients, 22 showed serodia positivity (44%).Thus, sera collected from voluntary blood donors showed negative results in all tested samples.Out of 47 patients(12 contacts and 35 multibacillary leprosy group), only 21 (17 leprosy patients and 04 contacts) showed seropositive results.However, in paucibacillary subjects out of 28 subjects(15 leprosy patients and 13 contacts);6 cases(5 leprosy patients and 01 contact)showed seropositive result which is considered statistically significant [P<0.001] in multibacillary than paucibacillary patients. Specificity of gelatine particle agglutination test (MLPA) was 91% in multibacillary patients and 21 % in paucibacillary patients.

Table : 1: **Correlation between Clinical & Histopathological classification in present study** .

Clinical type	No of cases	Histological type							% of agreement	% of disagreement
		TT	BT	BB	BL	LL	HL	IL		
1.TT	22	16	05	-	-	-	-	01	72.72	27.28
2.BT	22	01	16	-	-	-	-	05	72.72	27.28
3.BB	03	-	01	01	01	-	-	-	33.33	66.67
4.BL	-	-	-	-	-	-	-	-	-	-
5.LL	05	-	01	-	02	02	-	-	40	60
6.HL	10	-	-	-	-	01	09	-	90	10
7.IL	23	-	-	-	-	-	-	23	100	0
TOTAL	85	17	23	01	03	03	09	29	68.12	31.88

TT-tuberculoid leprosy;BT-Borderline tuberculoid; BB-Borderline ; BL-Borderline lepromatous; LL-Lepromatous leprosy; HL- Histoid leprosy; IL-Indeterminate leprosy.

Among 5 contact cases and 7 diagnosed leprosy cases in tuberculoid group (TT)only 2 leprosy cases showed MLPA reactivity (2 out of 12 – 16.67%). 3 leprosy cases and 1 contact cases out of 8 contact cases and 8 diagnosed case of borderline tuberculoid(BT) patients showed MLPA reactivity (4 out of 16 – 25%). In borderline cases (BB) 2 leprosy cases out of 10 diagnosed cases and no contacts found showed MLPA reactivity (2out of 10 -20%).In borderline lepromatous cases (BL) ; 2 out of 4 contact cases and 3 out of 7 test cases showed MLPA reactivity (5 out of 11 – 45.55%). Finally, in Lepromatous leprosy (LL) group ; 2 out of 8 contact cases and 12 out of 18 diagnosed test cases showed MLPA reactivity(14 out of 26 – 53.8%). Thus, in total , 27 cases including both diagnosed cases and contact cases out of total 75 cases showed seropositivity (36.0%)

Table 2a Observed frequency("o" table)

R &j group	Contact case	Leprosy total	Total	mlpa reactive		
				Contact Case	Leprosy Case	Total
Tt	5	7	12	-	2	2
Bt	8	8	16	1	3	4
Bb	-	10	10	-	2	2
Bl	4	7	11	2	3	5
Ll	8	18	26	2	12	14
Total	25	50	75	5	22	27

Table :2 :Contingency tables

(Tests of significance using chi-square test)

Table 2b -Expected frequency("e"table)

R &j group	Contact Case	Leprosy Case	Mlpa contact case	Reactive leprosy cases
TT	4	8	-	1.6296
BT	5.3333	10.6667	0.7407	3.2593
BB	-	6.6667	-	1.6296
BL	3.6667	7.3333	0.9359	4.0791
LL	8.6667	17.3333	2.5926	11.4074

Table – 2c.O2 /E table for derivation of Chi-Square (x^2 test)

R & j group	Contact cases	Leprosy Cases	Mlpa contact cases	Reactive Leprosy Cases
TT	6.25	6.125	-	2.4536
BT	12	5.9999	1.3501	2.7613
BB	-	14.9999	-	2.4546
BL	4.3636	6.6818	4.3201	2.2064
LL	7.3846	18.6923	1.5428	12.6234

	TOTAL CASE	MLPA REACTIVE	
O^2/E	82.4971	29.7133	
$\mathcal{O}^2/E = X^2_o$	7.4971	1.2032	
	5% level	5% level	$X^2_{0.05,4}$ is 9.49.

$X^2_o < X^2_{0.05}$ => H_0 = **accepted at 5% level of significance**.

Table 3: showing comparative study of clinico-histopathological correlation in different types of leprosy with present study (showing present study at par with that of Kar, Arora):

Clinical types	Varma 1981 (%)	Dubey 1981(%)	Singh 1983(%)	Shenoy Sidappa 1988(%)	Kar Arora 1994(%)	Nadkarni Rege 1999(%)	Present study2007-2008. (%)
1 TT	100	76.9	100	77.8	87.5	97.2	72.72
2 BT	40	100	-	62	60.9	95	72.72
3 BB	100	85	16.7	20.0	54.5	89	33.33
4 BL	40	100	-	14.4	53.8	87	-
5 LL	83.3	93.5	66.7	100	714	98.2	40
6 HL	-	-	-	-	-	-	90
7 IL	-	-	100	85.7	81.2	19	100
Total	60.5%	76%	47.23%	57.1%	68.2%	80.9%	68.12%

[TT-Tuberculoid leprosy; BT-Borderline tuberculoid leprosy; BB-Borderline leprosy; BL-Borderline leprosy; LL-Lepromatous leprosy; HL- Histoid leprosy ; IL- Indeterminate leprosy.]

Discussion:-

Leprosy still remains one of the most serious cause of morbidity and mortality in developing countries of the world despite lots of advancement in the field of diagnosis and management of leprosy[14,15]. Comparison of studies are carried out by different authors in the past and still continuing till date.

In the present study, out of 85 cases ; 52 were between 2nd – 4th decade of life followed by 25 in 5th to 6th decade of life.In study carried out by Kar and Arora ('94) out of 120 cases, 91 cases were between 2nd – 4th decade of life[2,15].

In the present study, 51 were males and 34 were females.In the study carried by Kar and Arora , 108 (90%) were males and 12(10%) were females. Our study is at par with study carried out by Kar and Arora[2,15].

In the present study , out of 85 cases, only 7(8,23%) gave positive family history of contact.In study carried out by Kar and Arora, out of 120 cases ,only 2 cases (1.6%) gave positive family history[2,15]

Clinical diagnosis of early leprosy lesions offers difficulties ; thus biopsies are done in doubtful cases in a hope of a definite diagnosis by histopathological examination coupled with bacillary study by special stain method.Many studies were carried out in the past trying to correlate clinical features of early lesions with histopathological findings.The histopathological findings coincide more often with clinical diagnosis than clinical diagnosis with histopathological diagnosis, in the whole leprosy spectrum, except in indeterminate group where it was the other way round[2,5,6].

In our present study of 22 cases diagnosed clinically as TT,16 cases (72.72%) were histologically diagnosed as TT while 05 cases (22.72%) were diagnosed BT & one (4.54%) diagnosed as IL.In study carried out by Kar & Arora(CS1), parity was found in 87.5% in study of 120 cases while study carried out by Nadkarni and Rege(CS2), parity was found in 97.2% in 264 cases[2,13,15].

Out of 22 cases diagnosed clinically as BT, 16cases(72.72%) were histologically diagnosed as BT while 5 cases(22.72%) were diagnosed as IL and one case(4.54%) diagnosed as TT.In CS1 clinicopathological agreement in BT was in 60.9% and in CS2 it was in 95.0%.Out of 3 borderline cases , one case (33.3%)was diagnosed histologically as BB; one case(33.3%) as BT; one case(33.3%) as BL.Parity in BB cases in CS1 was 54.5% and CS2 was 89.0%. No case was diagnosed clinically as BL, however parity was found in BL cases in CS1 to be 53.8% & in CS2 as 87%.

Out of 5 clinically diagnosed LL,2 cases(40%) were consistent histologically with LL while 2 cases(40%) were consistent with BL and rest single case (20%)found to be consistent with BT which was smear negative for leprosy bacilli. In CS1,LL had 71.4% parity and CS2 LL had 98.2% parity.Out of 23 clinically diagnosed IL cases, all were histologically consistent with addition of 6 cases histologically diagnosed as IL which were clinically diagnosed different[2,13,15].

In this study of 29 cases diagnosed histologically , we have observed epidermal atrophy in 41.37% of cases; periappendageal infiltration in 100% of cases; 41.37% cases showed perineural infiltration and Schwann cell proliferation seen in 31.03% cases. Study of 27 histologically confirmed cases were carried out by Nadir Paksoy, observed epidermal atrophy in 40% of cases; periappendageal infiltration in 100% of cases, 51% cases showed perineural infiltration and Schwann cell infiltration seen in 11% of cases. So, our study correlates well with this study[14].

Among 29 cases,22 were bacteriologically positive in skin smears.Remaining 7 cases were bacteriologically negative.IL parity was 81.2% in CS1 and 19,0% in CS2 respectively.In our present study,acid fast bacilli was found in skin biopsies in 22(75.86%)cases while Nadir Paksay (study at Karagiri, South India) observed bacilli positivity in 63.0% cases.Remaining 7 cases in present study which were bacteriologically negative were diagnosed as IL on basis of histopathological and clinical supporting features.In his comparative study , Nadir Paksay had seen 10 cases bacteriologically negative out of 27 cases, which were diagnosed as IL on basis of clinical and histological features.Maximum parity was found in indeterminate and histoid leprosy cases in our study.In spite of greater disparity in borderline cases, patients treated on basis of paucibacillary BT or multibacillary BL on clinical grounds.Clinically BT cases which turned out to be BB on histopathol-

ogy and showed bacteriological positivity were treated as multibacillary cases, on basis of histopathology report[14].

Overall parity between clinical and histopathological types of leprosy in the present study is 68.12 %.The histopathological features in leprosy indicate the accurate response of tissues while clinical features indicate gross morphology of lesions caused by underlying pathological change.Since tissue response varies in disease spectrum, because of variability of CMI, which is maximum in borderline leprosy, it is logical to expect some disparity between clinical and histological features (Ridley & Jopling 1966) .

ELISA method considered to be the most sensitive and specific techniques for detection of anti PGL1 antibodies. Gelatin particle agglutination test (MLPA) is less specific than ELISA but equally sensitive for multibacillary cases than paucibacillary cases. Paucibacillary cases generally display a very low level of anti PGL1 antibodies that cannot be detected by MLPA. Usefulness of detection of anti PGL1 antibodies has been extensively evaluated for serodiagnosis of clinical or subclinical infection, identification of individual with high risk, early detection of disease among contacts and patients with suspected clinical symptomatology , prognosis of disease in patients under observation and evaluation of effects of treatment.It is an additional tool for diagnosis of multibacillary patients and also as an alternate tool to bacillary index determination for surveillance of multibacillary patients after treatment[11,12,16].

Conclusion : Leprosy though reported to be eliminated still continue to be one of the common inflammatory disease in Gujrat and necessitate revival of knowledge regarding disease. Skin biopsy , to be taken from proper representative area of lesion is tool to confirm clinical diagnoses with serological detection of anti PGL-1 antibodies helpful for classification, therapeutic efficacy,risk of relapse, selection of contacts for high risk of contracting disease. 17,18.

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