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Diagnostic significance of MLSE Elisa in indeterminate leprosy

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Abstract
Introduction: Early detection and treatment can be beneficial for the check and progress of the disease in the community so we planned to tackle the disease in very early stage. Indeterminate stage is the earliest stage so it is very easier to treat this disease at this stage.
Materials and Methods: The study was carried out at the skin outpatients department (OPD) and immunology unit of Department of Pathology, K.G.'s Medical College, Lucknow from October 1998 to Dec. 2001.
Result: In our study we observed MLSE ELISA had a higher sensitivity in cases of indeterminate (87%) while specificity was 100% ELISA for detection of IgM antibodies to PGL-1 antigen had a lower sensitivity of 62.6% and specificity was 100%. Detection of IgM antibody against MLSE antigen of total case serum of spectral group had higher sensitivity 89% than sensitivity of PGL-1 antigen 87% specificity of both antigen were same.
Conclusion: Detection of antibody levels appeared to be useful in the diagnosis and to confirm the indeterminate leprosy from other groups of spectrum.

Keywords: indeterminate leprosy, MLSE, ELISA, PGL-1

1. Introduction

Leprosy is one of the oldest human bacterial disease recognized by a Norwegian scientist Armauer Hansen working in Bergen in 1873. Leprosy is still one of the infectious diseases and major health problem of developing countries.

Leprosy is caused by Mycobacterium leprae. M. leprae is pleomorphic, straight or slightly curved, rod shaped gram positive bacteria. It is strong acid fast 0bacilli and occurs in the human host intracellularly. They have an affinity for Schwann cells and reticuloendothelial cells. In more than one third of untreated or advanced cases, leprosy results in disabilities which increase with time and become permanent. The disabilities occur mainly due to infection of the peripheral nerves viz. ulnar, peroneal, greater auricular and dermal nerves. It also causes infection in the skin, testes and internal organs, resulting in serious impairment of working capacity which disrupts the social life of the patient, who becomes an outcast in the society.

Leprosy is not a single entity but rather a spectral disease with varied clinico-pathological presentations. It is generally agreed that multibacillary cases (lepromatous and borderline lepromatous cases) are the most infectious for the community. Lepromatous cases harbor millions of M. leprae in their nasal secretions. It is also transmitted from person to person by close contact between an infectious patient and a healthy but a susceptible person. Diagnosis and management of early leprosy posses a great challenge.

In India, leprosy is known since ancient times as kustha roga and attributed to punishment or curse from God. There are many species of Mycobacterium which affect directly and indirectly to man and animals.

Mycobacteria which are pathogenic to man are Mycobacterium leprae and Mycobacterium tuberculae. M. leprae caused leprosy but M. tuberculae caused tuberculosis. Mycobacterium lepraemurium causes leprosy in rats. Mycobacterium lepraemurium was first described by Stefansky in 1901 at Odessa.

Early detection and treatment can be beneficial for the check and progress of the disease in the community so we planned to tackle the disease in very early stage. Indeterminate stage is the earliest stage so it is very easier to treat this disease at this stage.

During recent years several tests have been developed and used with some success. These tests comprise of cross-immunoelectrophoresis (Rojas Epinosa *et al*., 1976), fluorescent antibody test (Abe *et al*., 1980), radioimmunoassay (Harboe *et al*., 1978). ELISA for antibodies against phenolic glycolipid derived from cell walls of M. leprae have been developed to detect anti M. leprae antibodies by Brett *et al*. in 1983. Further competitive radioimmunoassay and competitive Elisa using monoclonal antibody against M. leprae have been developed by Sinha *et al*. (1983, 1989) for the diagnosis. These tests have provided certain very useful information and may be used in detecting cases suffering from leprosy.

The present study has been undertaken to determine the diagnostic significance of MLSE Elisa over PGL-1 Elisa in early detection of indeterminate leprosy.

2. Materials and Methods

The study was carried out at the skin outpatients department (OPD) and immunology unit of Department of Pathology, K.G.'s Medical College, Lucknow from October 1998 to Dec. 2001. The present study has been carried out on suspected cases of leprosy with doubtful patches. The cases presented at the outpatient department of skin (OPD), Department of Medicine, K.G.'s Medical College, Lucknow, INDIA. A written informed consent was taken from every patient who was enrolled in this study.

A) Diagnosis criteria

Indeterminate cases will be selected according to the following criteria

i) Clinical Study: Early single or two-three hypopigmented or erythematous asymptomatic macular patch on skin with vague margins and sensory impairment.
ii) Histopathological study: Epidermis normal with well preserved retepegs and dermis infiltrated with scattered areas of mononuclear cell collections around skin appendages and nerve endings. The lesions are bacteriologically negative.

B) Control study

i) Disease controls were selected from multi-bacillary type, borderline, borderline lepromatous & lepromatous leprosy and paucibacillary type tuberculoid leprosy & borderline tuberculoid of leprosy subjects.
ii) Normal healthy controls were selected from the subjects those are not having contact history with leprosy patients.

Part I: Clinical assessment

The age and sex of the cases and disease controls has been recorded.

Part II: Enzyme linked immunosorbent assay

This technique was done as per the method of Ralhan *et al*. (1985) with some modification.

Experimental techniques

IgM antibody detection by indirect enzyme linked immunosorbent assay was performed for indeterminate leprosy cases, disease controls and normal healthy controls.

Standardization of the optimal dilution of antigen, serum and conjugate

For the standardization of the optimal dilution of antigen, serum and conjugate, positive reference pooled LL sera and negative reference pooled normal healthy sera were used.

Standardisation of optimal dilution of antigen (PGL-1 & MLSE) and serum

The determination of optimal dilution of antigen and serum was done by using Checker Board Titration.

For PGL-1 Antigen

Polystyrene micro-titration plates (Tarson) with flat bottom were coupled with 50 µl of PGL-1 antigen in carbonate bicarbonate buffer pH 9.6 at serially dilution – 5 µg/ml (1:200), 10 g/ml (1:100), 20 µg/ml (1:50), 25µg/ml (1:40). Plates were left overnight at 37^0C in a moist chamber and washed 10 times with phosphate buffered saline (pH 7.4) containing 0.1% Tween 20. Plates were blocked with wash buffer (PBS, pH = 7.4) containing 5% bovine serum albumin to avoid non-specific binding and incubated for 2 hour at 37^0C. After washing with wash buffer for three times 50 µl of serum dilution in wash buffer at a dilution of 1:100, 1:200, 1:300, 1: 400 were added in each well for a duration of 3 hours at 37^0C. Blank, negative control (normal healthy) and positive (LL) controls were also included simultaneously in each batch. The plates were then again washed 5 times with PBS containing 0.1% tween-20 followed by addition of 50 µl of rabbit antihuman IgM tagged with horse radish peroxidase conjugate at a dilution of 1:1000, which was added in each well and incubated for 90 minutes at 37^0C. After washing 5 times with wash buffer, it was followed by addition of 50 µl of tetramethylbenzidine (TMB) diluted to 1:20 in distilled water was added to each well and colour developed. The plate was incubated at 37^0C for 30 minutes. Finally the reaction was stopped by addition of 50 µl of stop solution 5N H_2SO_4. Later, optical density (OD) were read at 450 nm in an ELISA reader. Optical densities for cases were best at an antigen dilution of 1:100 and serum dilution of 1:500. ELISA was performed blindly with all test samples, serum including all cases and controls, using positive and negative control sera with each batch for standardization of ODs by the method described above.

For MLSE antigen

Polystyrene micro-titration plates (Tarson) with flat bottom were coupled with 50µl of MLSE antigen in PBS (pH 7.4) at serially dilution 5 µg/ml (1:200), 10 µg/ml (1:100), 20 g/ml (1:50), 25µg/ml (1:40). Plates were left 20 hours at 4^0C. After washing 3 times with phosphate buffered saline containing 0.1% Tween 20. Plates were blocked with wash buffer (PBS, pH = 7.4) containing 3% bovine serum albumin to avoid non-specific binding and incubated for one hours at 20^0C. After washing with wash buffer for three times 50µl of serum dilution in wash buffer at a dilution of 1:100, 1:200, 1:300, 1: 400 were added in each well for a duration of 3 hours at 37^0C. Blank, negative control (normal healthy) and positive (LL) controls were also included simultaneously in each batch. The plates were then again washed 3 times with PBST, followed by addition of 50µl of rabbit antihuman IgM tagged with horse radish peroxidase conjugate at a dilution of 1:1000, which has added in each well and incubated for 90 minutes at 37^0C. After washing 3 times with wash buffer, it was followed by addition of 50 µl tetra methyl benzidine (TMB) diluted to 1:20 in distilled water was added to each well and colour developed. The plate was incubated at 20^0C for 30 minutes. Finally the reaction was stopped by addition of 50µl of 5 NH_2SO_4 in each well. Later, optical density (OD) were read at 450 nm in an ELISA reader. Optical densities for cases were best at an antigen dilution of 1:100 and serum dilution of 1:100. ELISA was performed blindly with all test samples, serum including all cases and controls, using positive and negative control sera with each batch for standardization of OD's by the method described above.

Interpretation of Result

Cut off ODs (optical densities) were calculated as two times standard deviation above the mean of normal healthy control ODs. Values above cut off values were considered as positive and below were considered as negative for experimental ELISA.

3. Results

The patients under investigation, reported at the outpatient and inpatient skin & V.D. Department of Medicine at K.G.'s Medical College, Lucknow. Immunological study was done in all cases and controls to detect the IgM antibodies in serum by using the technique ELISA (as mentioned in materials and methods).

Table 1: Distribution of cases (Idt) and controls (disease controls and normal healthy controls)

A.	Cases (Indeterminate leprosy)	75
B.	Controls B.1 Disease Control	100
B.1.i	Tuberculoid leprosy	20
B.1.ii	Borderlind tuberculoid	20
B.1.iii	Borderline borderline	20
B.1.iv	Borderline lepromatous	20
B.1.v	Lepromatous leprosy	20
B.2	Normal healthy controls	75

Detection of IgM antibodies in serum (using PGL-1 antigen)

Sera of all cases (Idt), disease controls (TT, BT, BB, BL, LL) and controls (NHC) were evaluated for the presence of IgM antibodies against phenolic glycolipid antigen-1 (PGL-1) by using ELISA.

The cut off optical density (OD) was calculated mean of healthy control ±2SD (standard deviation) and found to be 0.228. The mean ±2 S.D. depicted observable limit as nil in normal healthy controls. Fig 1 depicts optical density of cases (Idt) and normal healthy controls. Fig 2 depicts optical density of disease controls and normal healthy controls. Bold ODs showing positive OD of cases.

Out of seventy five cases, 47 tested positive while 28 cases were negative. None of the subjects of normal healthy control group were positive for indeterminate leprosy (Table 2).

Sensitivity

True positive/Total diseased case × 100; 47/75 × 100 = 62.6%

Specificity

True negative/Total negative × 100; 75/75 × 100 = 100%

Positive predictive value (PPV)

True positive/Total test positive × 100; 47/47 × 100 = 100%

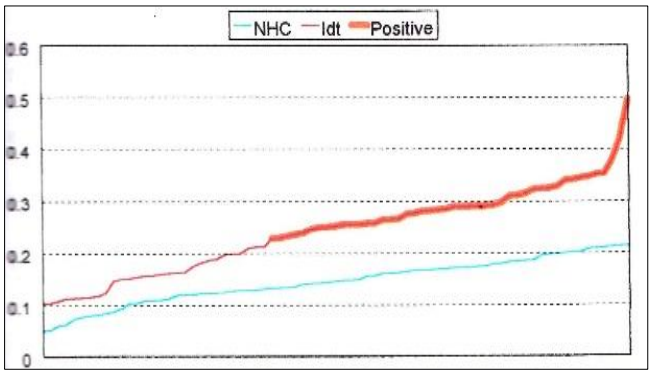
Negative predictive value (NPV)

True negative/Total test negative × 100; 75/103 × 100 = 72.8%

Table 2: Experimental IgM ELISA (using PGL-1 Ag) in indeterminate cases and normal healthy controls.

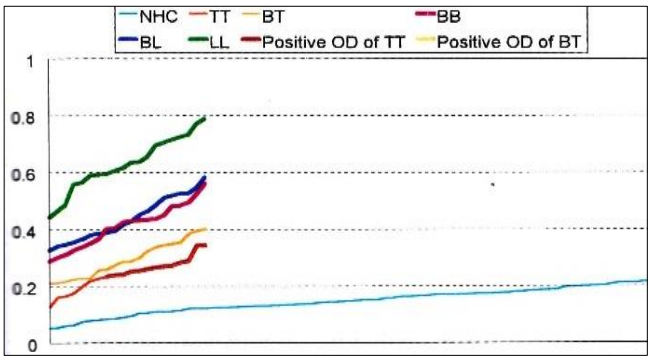
Test	Cases (Idt)	Controls (NHC)	Total Test
Positive	47	0	47
Negative	28	75	103
Total	75	75	150

Cut off O.D.: 0.228



Cut od = 0.228; positive value> cut off O.D. of normal healthy controls

Fig 1: depicts optical desity of indeterminate leprosy cases and normal healthy controls using PGL-1 antigen



Cut off OD=0.228 positive value > cut off O.D of normal healthy controls

Fig 2: Depict optical density of disease controls, and normal healthy control using PGL-1 antigen

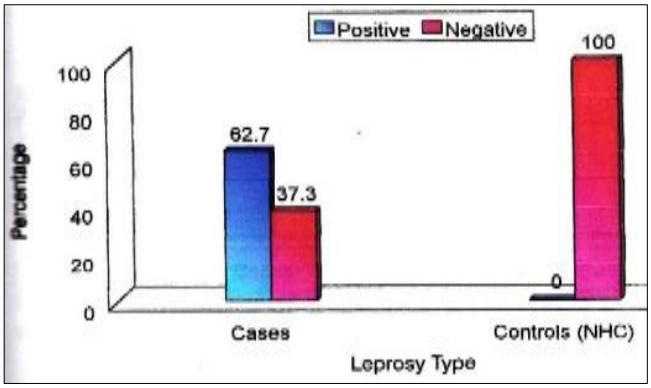


Fig 3: experimental igM ELISA (using PGL-1Ag) in cases of Idt and NHC

Out of 100 disease controls (TT-LL), 87 were positive while 13 cases were negative (Table 3).'

Table 3: Experimental Igm ELISA for disease control and normal healthy controls using PGL-1 antigen

Test	Disease controls	Healthy Controls	Total Test
Positive	87	0	87
Negative	13	75	88
Total	100	75	175

Cut off O.D: 0.228

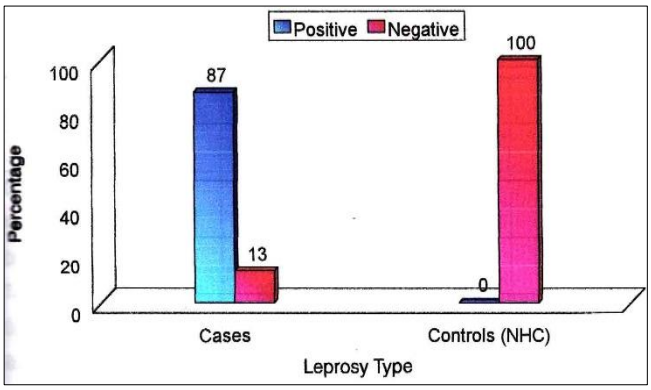


Fig 4: Experimental IgM ELISA (using PGL-1Ag) in disease controls and NHC

Sensitivity

True positive/Total diseased case × 100; 87/100 × 100 = 87%

Specificity

True negative/Total negative × 100; 75/75 × 100 = 100%

Positive predictive value (PPV)
True positive/Total test positive × 100; 87/87 × 100 = 100%

Negative predictive value (NPV)
True negative/Total test negative × 100; 75/88 × 100 = 85.2%

Table 4: Combined results of IgM ELISA in cases & controls using PGL-1 antigen against IgM antibody.

Study group	Number	Positive Number		Negative Number		Mean OD ± S.D.
		No.	%	No.	%	
Cases						
Indeterminate(Idt)	75	47	62.6	28	37.3	0.240±0.082
Disease Controls						
Tuberculoid leprosy (TL)	20	13	65	7	35	0.238±0.056
Borderline tuberculoid (BT)	20	14	70	6	30	0.291±0.063
Borderline borderline (BB)	20	20	100	-	-	0.409±0.076
Borderline lepromatous (BL)	20	20	100	-	-	0.429±0.078
Lepromatous leprosy (LL)	20	20	100	-	-	0.624±0.098
Normal Healthy Controls (NHC)	75	-	-	75	100	0.144±0.042

Cut off OD: 0.228

Reproducibility
The tests were repeated twice on two different occasions. Reproducibility was found to be 100%.

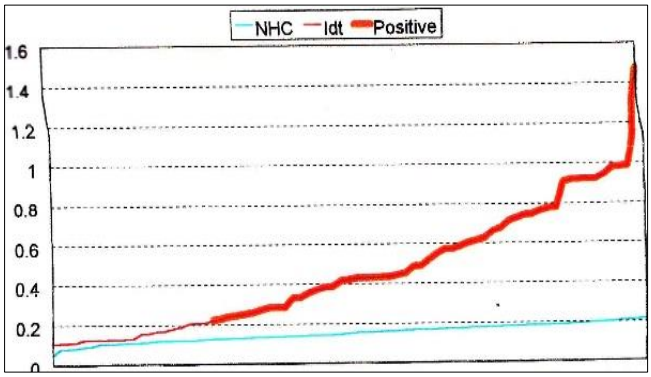
Detection of IgM antibodies in serum (using MSLE antigen)
Sera of all Idt cases (n=75), disease controls (n=100) and normal healthy controls (n=75) were evaluated for the presence of IgM antibodies using MLSE antigen by ELISA.
The cut off optical density (OD) was calculated as mean of healthy control ± 2SD, and was found to be 0.221. The mean ± 2SD depicts observable limit as nil in normal healthy controls. fig 3 depicts optical density of interminate leprosy and normal healthy controls. fig 4 depicts optical density of disease andcontrols and normal healthy controls. Bold ODs showing positive OD of cases.
Fifty five cases of the seventy five were tested positive for the disease indeterminate leprosy and twenty out of seventy five cases showed negativity (Table 5).

Sensitivity
True positive/Total diseased case × 100; 55/75 × 100 = 73.3%

Specificity
True negative/Total negative × 100; 75/75 × 100 = 100%

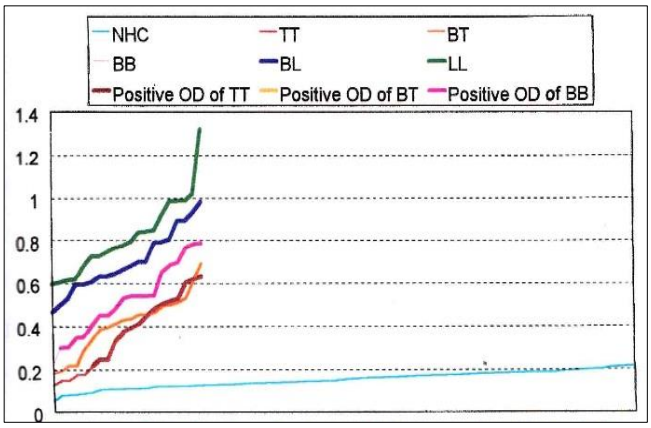
Positive predictive value (PPV)
True positive/Total test positive × 100; 55/55 × 100 = 100%

Negative predictive value (NPV)
True negative/Total test negative × 100; 75/95 × 100 = 78.9%



Cut off OD=0.221 positive value > cut off O.D of normal healthy controls

Fig 5: Depict optical density of disease controls, and normal healthy control using MLSN-1 antigen



Cut off OD=0.221 positive value > cut off O.D of normal healthy controls

Fig 6: Depict optical density of disease controls, and normal healthy control using MLSN-1 antigen

Table 5: Experimental IgM ELISA in indeterminate leprosy cases and normal healthy controls (NHC) (using MLSE antigen)

Test	Cases (Idt)	Controls (NHC)	Total Test Positive
Positive	55	-	55
Negative	20	75	95
Total	75	75	150

Cut off OD: 0.247

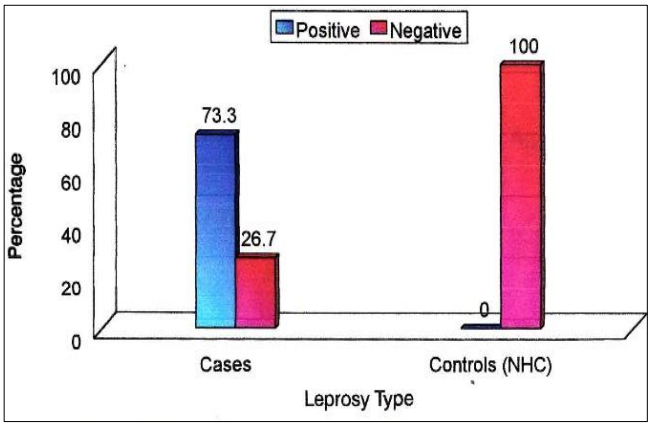


Fig 7: Experimental IgM ELISA (using MLSE-Ag) in Cases (IDT) and NHC

Out of 100 disease controls (TT-LL), 89 were positive while 11 cases were negative (Table 6).

Table 6: Experimental IgM ELISA for disease controls and normal healthy controls using MLSE Ag

Test	Cases (Disease control)	Controls (Normal healthy)	Total Test Positive
Positive	89	-	89
Negative	11	75	86
Total	100	75	175

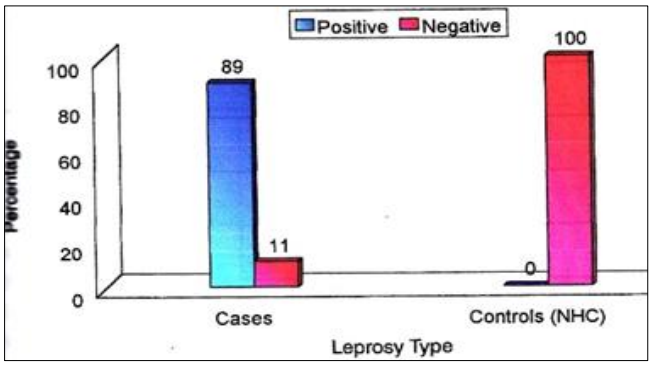


Fig 8: Experimental IgM ELISA (using MLSE-Ag) in Cases (IDT) and NHC

Sensitivity
True positive/Total diseased case × 100; 89/100 × 100 = 89%

Specificity
True negative/Total negative × 100; 75/75 × 100 = 100%

Positive predictive value (PPV)
True positive/Total test positive × 100; 89/89 × 100 = 100%

Negative predictive value (NPV)
True negative/Total test negative × 100; 75/86 × 100 = 87.2%

Table 7: Combined results of IgM ELISA in cases (Idt) and disease controls & normal healthy controls using MLSE antigen against IgM antibody

Study group	Number	Positive Number		Negative Number		Mean OD ± S.D.
		No.	%	No.	%	
Cases						
Indeterminate(Idt)	75	55	73	20	26.6	0.458±0.299
Disease Controls						
Tuberculoid leprosy (TL)	20	14	70	6	30	0.365±0.172
Borderline tuberculoid (BT)	20	16	80	4	20	0.407±0.139
Borderline borderline (BB)	20	19	95	1	5	0.517±0.171
Borderline lepromatous (BL)	20	20	100	-	-	0.7±0.146
Lepromatous leprosy (LL)	20	20	100	-	-	0.818±0.179
Normal Healthy Controls (NHC)	75	-	-	75	100	0.148±0.036

Cut off OD: 0.221

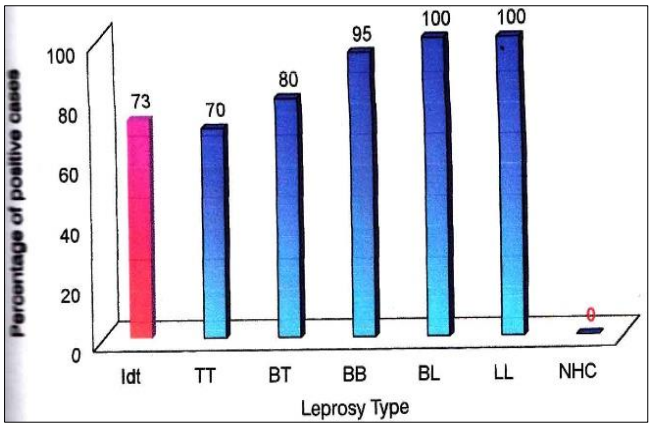


Fig 9: combined results of IgM ELISA in cases (Idt) and disease control and normal healthy control using MLSE antigen

Reproductivity
The test were repeated twice on two different occasions. Reproducibility was found to be 100% in all cases and controls.

We observed MLSE ELISA had a higher sensitity in cases of Idt (87%) whiel specificity was 100%. ELISA for detection of IgM antibodies to PGL-1 antigen had a lower sensitivity of 62.6% and specificity was 100% (Table 8).

Table 8: Comparative sensitivity, specificity and predictive values in indeterminate cases serum ELISA using PGL-1 and MLSE Antigens against IgM antibody

Antigens	PGL-1 Ag	MLSE Ag
Antibodies	**IgM**	**Igm**
True positive	47	55
True negative	28	20
Sensitivity	62.6	87
Specificity	100	100
PPV	100	100
NPV	72.8	85.2

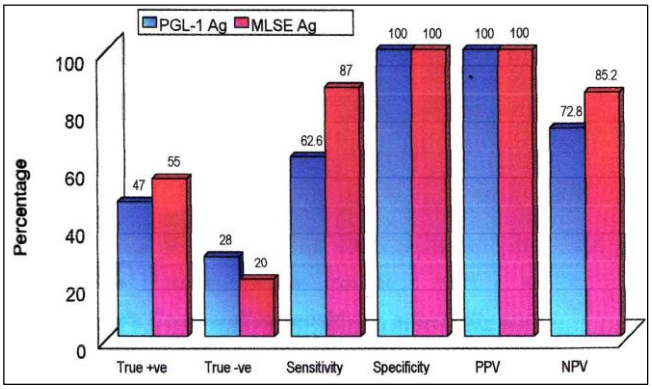


Fig 10: Comparative sensitivity specificity and predictive values in Idt cases, ELISA using PGl-1 and MLSE antigen

We observed in total leprosy cases of spectral group MLSE ELISA had a higher sensitivity 89% than PGL-1 ELISA sensitivity was 87% against IgM antibody. The specificity of both antigens was 100%.

Table 9: Comparative sensitivity, specificity and predictive value total leprosy cases of spectral groups, serum ELISA using PGL-1 and MLSE Antigens against IgM antibody

Antigens	PGL-1 Ag	MLSE Ag
Antibodies	IgM Ab	Igm Ab
True positive	87	89
True negative	13	11
Sensitivity	87	89
Specificity	100	100
PPV	100	100
NPV	85.22	87.2

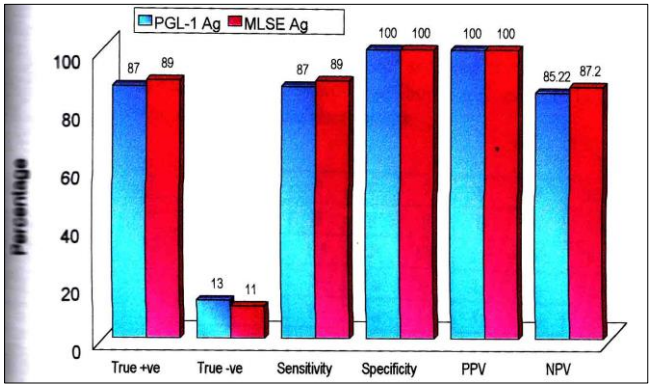


Fig 11: Comparative sensitivity, specificity and predictive value in disease controls, ELISA using PGL-1 and MLSE antigen

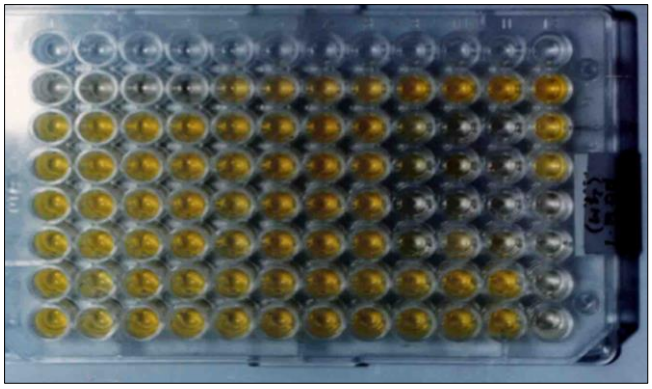


Fig 12: ELISA (IgM) plate: showing the positivity for different grades of Leprosy antibody and normal healthy serum as shown in the chart below

Table 10

	1	2	3	4	5	6	7	8	9	10	11	12
A	B	B	B	B	B	B	B	B	B	B	B	B
B	NC	NC	NC	NC	LP	LP	LP	LP	HP	HP	HP	HP
C	T_1	T_4	T_7	T_{10}	T_{13}	T_{16}	T_{19}	T_{22}	T_{25}	C_3	C_6	C_9
D	T_1	T_4	T_7	T_{10}	T_{13}	T_{16}	T_{19}	T_{22}	T_{25}	C_3	C_6	C_9
E	T_2	T_5	T_8	T_{11}	T_{14}	T_{17}	T_{20}	T_{23}	C_1	C_4	C_7	C_{10}
F	T_2	T_5	T_8	T_{11}	T_{14}	T_{17}	T_{20}	T_{23}	C_1	C_4	C_7	C_{10}
G	T_3	T_6	T_9	T_{12}	T_{15}	T_{18}	T_{21}	T_{24}	C_2	C_5	C_8	C_{11}
H	T_3	T_6	T_9	T_{12}	T_{15}	T_{18}	T_{21}	T_{24}	C_2	C_5	C_8	C_{11}

B: Blank, T_1 – T_{25}: Cases, C_1 –C_{11}: Controls NC: Negative control LP: Low positive controls H: High positive controls

8. Discussion

Study of leprosy is very fascinating to immunologist, interested in understanding the basic mechanisms involved in immune- deviation, tolerance and immunological enhancement.

Conventional ELISA has been carried out by several workers (Bos *et al*., 1976; Yang and Kennedy, 1979; Speiser, 1980 and Jyotsna *et al*., 1985) for the sero- deagnosis of infections diseases. We have also chosen this test for sero diagnosis of indeterminate leprosy from other leprosy groups of spectrum. In ELISA system back ground reaction is an important factor which can be overcome by the use of Tween-20 and Bovine serum albumin (BSA) (Voller, 1974, 1975 and Bullock and Walls, 1977). We used successfully BSA to alleviate the nonspecific binding which increased the flexibility of the test. Several workers (Miller *et al*., 1983; Young and Buchanan, 1983, Douglas *et al*., 1984 and Abreu & Abreu & Gonzalez, 1987) have used wet-method system for the coating of ELISA plates with M. leprae and phenolic glycolipid antigen and have obtained reproducible results for the sero-diagnosis of leprosy. The wet method for coating was also used by us and it has worked well in our system. In this study, Mycobacterium leprae sonicated extrate in sonicated state has lost some of its property in detecting M. leprate antibodies in indeterminate. TT, BT and BB cases at mean + 2SD cut off point. It could detect 100% antibodies in only BL and LL disease control sera which are known to contain high concentration of antibodies. MLSE ELISA were positive in 55 of 75 (73%) cases of indeterminate leprosy, it was capable of detecting considerably high percentage of positive leprosy cases particularly from the upgrading side of the spectrum group of the Ridley-Jopling (1966) scale of leprosy. MLSE atigen gave positivity in 14 of 20 (70%) cases of TT, 16 of 20 (80%) cases of BT, 19 of 20 (95%) cases of BB and 20 of 20 (100%) cases of BL and LL respectively. In our result TT, BT, BB, BL and LL disease control group and 55 of 75 Idt cases had raised antibody levels (ELISA values 0.221). Detection of raised values in these cases and disease control groups suggested activation of The2 pathway, resulting in antibody formation. However, 20 of 75 Idt cases, 6 of 20 TT, 4 of 20 BT and 1 of 20 BB disease control group failed to produce antibodies (ELISA value mean + 2SD OF healthy controls) suggesting that in this case and disease control group activation of The2 a pathway did not occur. One BB subject had low level of antibody and this patient was being treated for the last one year. Two LL cases had very high levels of antibodies 9ELISA values = 1.019 & 1.32), production of such a high level of antibody in LL is difficult to explain. Similarly, two other Idt patients produced antibody level as high as 1.186 and 1.471 (ELISA values) posed difficulty in interpretation. It may be explained on the basis of presence of bacilli/increased antigenic load in these patients. These patients had a single macular lesions with thicked nerve and did not have atrophic epidermis. Possible the bacilli might be harbouring at extra- cutaneous sites (nerves). These cases should be observed longitudinally for possible downgrading of the disease.

The sensitivity, specificity, positive predictive value and negative predictive value of MLSE ELISA in indeterminate cases was 73.3%, 100%, 100% and 78.9%. The MLSE ELISA showed 100% reproducibility.

PGL-1 ELISA could detect 100% antibodies in BB, BL, and LL disease control sera which are known to contain high concentration of antibodies. PGL-1 ELISA were positive in 47 of 75 (62.6%) cases of indeterminate leprosy, it was also capable of detecting considerably high percentage of positive

leprosy case & particularly from the upgrading side of the spectrum group of the Ridley- Jopling (1966) scale of leprosy. PGL-1 ELISA antigen gave positivity in 13 of 20 (65%) TT cases, 14 of 20 (70%) BT cases and 20 of 20 (100%) BB, BL and LL cases respectively. In our results TT, BT, BB, BL and LL disease control group and 47 of 75 Idt cases had raised antibody levels (ELISA values 0.228). Detection of raised values in these cases and disease control groups suggested activation of The2 pathway, resulting in antibody formation. However 28 of 75 Idt cases 7 of 20 TT and 6 of BT disease control group failed to produce antibodies (ELISA values mean + 2SD of healthy controls) suggesting that in this case and disease control group activation of Th2 pathway did not occur.

The sensitivity specificity, positive predictive value and negative predictive value of PGL-1 ELISA was 62.6% 100%, 100% and 72.8%. The PGL-1 ELISA also showed 100% reproducibility.

In comparison, the two antigens namely PGL-1 and MLSE seem to have closer relationship in detecting M. leprae antibodies. MLSE antigen appears to be more sensitive technique for screening of the cases of leprosy. In our study we observed MLSE ELISA had a higher sensitivity in cases of indeterminate (87%) while specificity was 100% ELISA for detection of IgM antibodies to PGL-1 antigen had a lower sensitivity of 62.6% and specificity was 100%. Detection of IgM antibody against MLSE antigen of total case serum of spectral group had higher sensitivity 89% than sensitivity of PGL-1 antigen 87% specificity of both antigen were same.

Detection of anti PGL-1 antibody level appeared to be of little value in the spectral diagnosis of leprosy. Twenty eight of 75 (37.3%) Idt patients did not produce antibodies, suggesting a little role of antibody assay in seroepidemology. Detection of anti PGL-1 antibody by ELISA technique is presently the most sensitive test available to measure the humoral response.

Stefani *et al*. (1998) have reported that the anti-phenolic glycolipid-1 (PGL-1) assay as currently applied for leprosy is conceived as an early marker of asymptomatic infection, early disease diagnosis and cure monitoring.

Desforges *et al*. (1989) [6] measured IgM anti-PGL-1 antibodies in multibacillary (MB) patients (100% positive), and in paucibacillary patients (21% positive).

Serum IgG, IgA and IgM in BB, BL & LL were found to be normal by Bullock *et al*., (1970) and Young Chaiyud *et al*., (1975) while Lim and Fusaro (1968) found only serum IgM to be normal in BB, BL & LL. Serum IgM in (LL & BL) have been documented to have significantly decreased values after prolonged treatment while serum IgA have increased significantly after treatment (Bullock *et al*., 1970) our present results coincide with the results of Bullock *et al*. (1970) who had tried to correlate serum immunoglobulins level with treatment.

In follow through cases (n=10), surprisingly in our study each patients serum IgM protein has been found to decrease after MDT treatment. The possible decrease of the level of IgM antibodies in cases after treatment seem to have a direct link with the decrease in the antigenic load due to prolonged treatment, which lessens the number of bacteria due to stoppage of multiplication of the bacilli.

In only two indeterminate case the lesion persisted and a new lesions was also seen. This case gave positive result in IgM ELISA (Post treatment). Cho *et al*. (2001) reported already that PGL-1 antigen may be useful in the assessment of leprosy patients at the time of diagnosis and possibly in monitoring patients following chemotherapy.

It was concluded that correct diagnosis of indeterminate leprosy from other leprosy groups of spectrum could be made if results of clinical, histopathological, bacteriological and immunological were interpreted together. Detection of antibody levels appeared to be useful in the diagnosis and to confirm the indeterminate leprosy from other groups of spectrum. The early diagnosis and treatment of leprosy at indeterminate stage should be beneficial to reduce and to eradicate the leprosy from the community.

9. References

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