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Significance of Lepromin test (DTH) in diagnosis of indeterminate Leprosy

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Abstract

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. 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. Early response was measured after 48 hours of intradermal injection only 28 of 75 (37.3%) indeterminate leprosy cases gave positive response in which 20 of 75 (26.7%) cases gave 1^+ response, 5 of 75 (6.7%) cases gave 2^+ response and only 1 of 75 (1.3%) indeterminate cases gave 4^+ DTH response. In diseases control groups 19 of 20 (95%) TT, 17 of 20 (85%) BT, 18 of 20 (90%) BB and 5 of 20 (25%) BL cases gave positive DTH response but DTH response is negative in all cases of lepromatous leprosy. Late reaction (Mitsuda response) was measured after 3 weeks of intradermal injection only 23 of 75 (30%) indeterminate leprosy cases gave positive response. Eight of 20 (40%) TT, 6 of 20 (30%) BT, 3 of 20 (15%) BB and 1 of 20 (5%) BL, disease control group gave positive response. Mitsuda response is negative in LL case. Most interesting observation was the detection of DTH of lepromin in 28 of 75 indeterminate cases, suggesting the usefulness of delayed reaction (in duration diameter measured after 48 hrs) in early diagnosis of in determinate leprosy.

Key- words: Indeterminate Leprosy, lepromin test, delayed reaction

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.

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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.

The preventive health hygiene and socio-economic status of society of developing countries has not well improved so this disease is still prevalent in the community. 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. Present research work will be helpful in differentiating the indeterminate (Idt) cases from other paucibacillary cases and the work will help in confirming an early cases to be 'leprosy' or 'not leprosy'.

The present study has been undertaken to determine the diagnostic significance of delayed reaction of lepromin test in early detection of indeterminate leprosy.

Material 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 :

(a) Clinical Study; early single or two-three hypopigmented or erythematous asymptomatic macular patch on skin with vague margins and sensory impairment.

(b) 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

1- Disease controls were selected from multi-bacillary type, borderline, borderline lepromatous & lepromatous leprosy and paucibacillary type tuberculoid leprosy & borderline tuberculoid of leprosy subjects.

2- 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 2: LEPROMIN TEST

It was done using Dharmendra Lepromin antigen. M-leprae antigen prepared from armadillo was supplied by Dr. U. Sengupta (Director), Central Jalma Institute of Leprosy, Agra. The test was performed using 0.1 ml of Dharmendra antigen injected intradermally (i.d.i.) on the volar surface of left forearm. Early and late response was recorded. Early reaction in the form of redness and induration was read after 48 hrs. An induration diameter <6 mm was recorded as negative. Induration diameter between 6 mm to 10 mm was graded as positive (1+). The reaction was considered to be moderately positive (2+) when diameter of induration varied from 11 mm to 15 mm in diameter. It was graded as strongly positive (3+) when diameter of induration was between 16 to 20 mm (Bates et al., 1979).

Late (Mitsuda) reaction was read after 3 weeks and graded as follows (Hyashi, 1933).

1+ = diameter of induration 5-8 mm,
2+ = diameter of induration 9-12 mm,
3+ = diameter of induration 13-16 mm, and
Negative = diameter of induration was <5 mm.

Lepromin skin test was also done in positive controls and results were similarly interpreted.

Results and Discussion

LEPROMIN TEST

Dharmendera lepromin antigen skin test were done in all cases of indeterminate leprosy and disease control i.e., other leprosy groups of spectrum (Tuberculoid leprosy to lepromatous leprosy).

Thee early (Farnandez reaction probably elicits pre-existing, delayed type hyper sensitivity (DTH), while the late (Mitsuda reaction) reflects cell mediated immunity (CMI).

Results of Mitsuda skin test revealed gradual increase in diameter of induration from LL of TL patients. Similar results were obtained in early reaction.

Early response (delayed type hypersensitivity) was measured after 48 hours of intradermal injection only 28 of 75 (37.3%) indeterminate leprosy cases gave positive response in which 20 of 75 (26.7%) cases gave 1^+ response, 5 of 75 (6.7%) cases gave 2^+ response, 2 of 75 (2.7%) cases gave 3^+ response and

only 1 of 75 (1.3%) patients gave 4^+ DTH response. The test were negative in 47 of 75 (62.7%) indeterminate cases (Table 1).

In disease control group nineteen of 20 (95%) tuberculoid leprosy cases had positive DTH response in which 15 of 20 (75%) cases gave 1^+ response, 2 of 20 (10%) cases gave 2^+ response, one of 20 (5%) cases gave 3^+ response and only one of 20 (5%) cases gave 4^+ delayed type hypersensitivity response (Table 1).

Seventeen of 20 (85%) borderline tuberculoid showed positive DTH response in which 15 of 20 (75%) cases gave 1^+ response and 2 of 20 (10%) cases gave 2^+ response (Table 1).

18 of 20 (90%) borderline leprosy cases gave only 1^+ DTH response (Table 23a).

5 of 20 (25%) borderline lepromatous leprosy cases gave 1^+ delayed type hypersensitivity response and early reaction for lepromatous leprosy was negative in all 20 of 20 (100%) cases (Table 1).

Table 1: Results of Dharmendra Lepromin Skin Test (delayed type hypersensitivity) after 48 hrs. in cases (Idt) and disease controls

Leprosy types	DTH skin response							
	1+	2+	3+	4+	Total No. of positive		Total No. of Negative	
					No.	%	No.	%
CASES:								
Indeterminate leprosy (Idt) (n=75)	20	5	2	1	28	37.3	47	62.7
DISEASE CONTROLS:								
Tuberculoid leprosy (TL) (n=20)	15	2	1	1	19	95	1	5
Borderline tuberculoid (BT) (n=20)	15	2	-	-	17	85	3	15
Borderline borderline (BB) (n=20)	18	-	-	-	18	90	2	10
Borderline lepromatous (BL) (n=20)	5	-	-	-	5	25	15	75
Lepromatous leprosy (LL) (n=20)	-	-	-	-	-	-	20	100

Abbreviation

1^+ = induration between 6 to 10 mm diameter.
2^+ = induration between 11 to 15 mm diameter.
3^+ = induration between 16 to 20 mm diameter.
4^+ = induration between 21 to 25 mm diameter.
-ve = induration < 6 mm diameter.

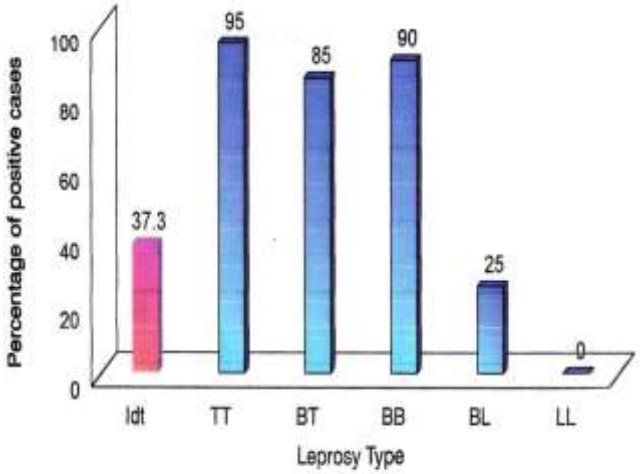

Fig. 18: Results of Dharmendra lepromin skin test (delayed type hypersensitivity) after 48 hrs in cases (Idt) and disease controls

Late reaction (Mitsuda response) was measured after 3 weeks of intradermal injection. Only 23 to 75 (30%) indeterminate leprosy patients gave 1^+ mitsuda response (Table 2).

In disease control group eight of 20 (40%) tuberculoid leprosy case gave 1^+ mitsuda response, 6 of 20 (30%) borderline tuberculoid gave 1^+ mitsuda response 3 of 20 (15%) borderline borderline leprosy cases gave 1^+ mitsuda response and only one of 20 (5%) case of borderline lepromatous gave 1^+ positive response. Mitsuda response was negative in all 100% cases of lepromatous leprosy (Table 2).

Table 2: Mitsuda reaction in cases (Idt) and disease controls after 3 weeks

Leprosy types	DTH skin response							
	1+	2+	3+	4+	Total No. of positive		Total No. of Negative	
					No.	%	No.	%
CASES:								
Indetermina	2	-	-	-	23	3	52	69

te leprosy (Idt) (n=75)	3					0		
DISEASE CONTROLS:								
Tuberculoid leprosy (TL) (n=20)	8	-	-	-	8	40	12	60
Borderline tuberculoid (BT) (n=20)	6	-	-	-	6	30	14	70
Borderline borderline (BB) (n=20)	3	-	-	-	3	15	17	85
Borderline lepromatous (BL) (n=20)	1	-	-	-	1	5	19	95
Lepromatous leprosy (LL) (n=20)	-	-	-	-	-	-	20	100

Abbreviation

1^+ = induration between 5 to 8 mm diameter.
2^+ = induration between 9 to 12 mm diameter.
3^+ = induration between 13 to 16 mm diameter.
4^+ = induration between 17 to 20 mm diameter.
-ve = induration < 5 mm diameter.

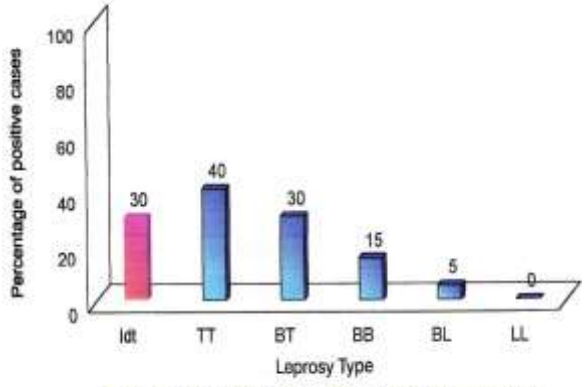

Fig. 19: Results of Mitsuda reaction in cases (Idt) and disease controls

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

It is exiciting to observe association of failure to clear infection along with lepromin non-reactivity and enhancement of antibody formation in lepromatous leprosy patients. Conversely, tuberculoid patients develop lepromin-mediated delayed hypersensitivity (DH) , clear the myco-bacteria and may or may not be associated with antibody formation. Other spectral groups, eg. borderline lepromatous (BL), borderline borderline (BB) and borderline tuberculoid (BT) develop balance of both T and B cell mediated immune responses in various combinations and may fail to develop immunity. However, BT patients may succeed to clear the bacilli (Fig. 30 to 34). In indeterminate leprosy patients we observed lepromin positivity in some cases. Antigens of M. Leprae may inhibit adherence of M leprae by macrophages of M. Leprae intigens to T-cells (Birdi et al & Salgame et al., 1983).

Results of recent studies clearly suggest existence of two populations of T helper (Th) cells- The1 and Th2. The1 subset is primarily concerned in generation of delayed hypersensitivity T (TDH) cells, while The2 cells are involved in antibody formation. Suppressor T (Ts) cells are mainly concerned in inhibition of the The1 cells thus their proliferation results in suppression of DH. In LL patients activation of The2 and Ts pathways occur, resulting in enhancement of antients formation and failure to clear infection and develops lepromin reactivity. (Fig. 30).

Among BL patients it appears that activation of all the three pathways The1 The2 and Ts occur but The2 and Ts pathways are relatively more activated as compared to The1 cell stimulation. This results in development of moderate amount of antibody formation lepromin anergy and development of little immunity (Fig. 31).

Among BB patients both The1 and The2 cells get equally activated and this results in formation of mild antibody response. Moreover, BB patients above to hav e relatively more immunity as compared with BL patients (Fig. 32).

BT cases develop activation of all the three pathways mentioned above. However, The1 cell stimulation and proliferation is more marked. This type of reactivity drifts the response in favour of clearance of bcilli and DH reactivity (Fig. 33).

TT patients primarily develop the activation of The1 pathway, resulting in high grade Mitsuda reactivity and clearance of bacilli and little or no antibody formation (Fig. 34).

In this study Dharmendra lepromin antigen was used to detect delayed cutaneous hypersensitivity - DH to M. leprae. Both early and late (Mitsuda) responses were measured. Early reaction was measured as an area of in duration 48 hours after intradermal injection while late response were read after

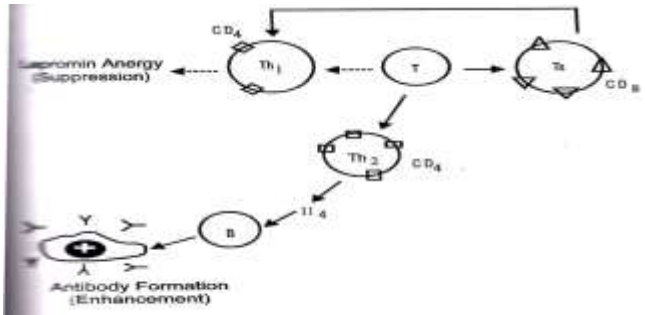

Fig. 30 : Immunological Pathways in Lepromatous Leprosy (LL) Patients

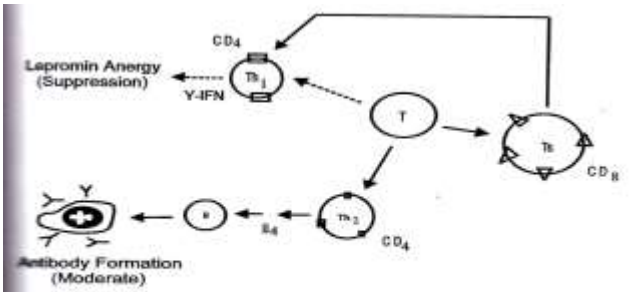

Fig. 31 : Immunological Pathways in Borderline Lepromatous (BL) Patients

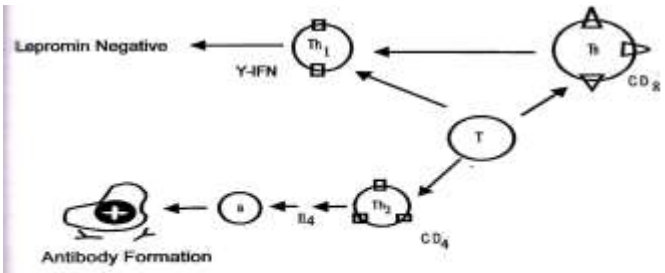

Fig. 32: Immunological Pathways in Borderline Borderline (BB) Patients

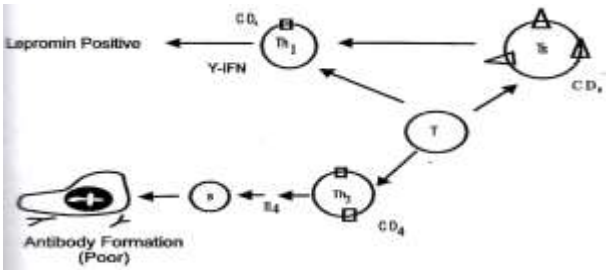

Fig. 33 : Immunological Pathways in Borderline Tuberculoid (BT) Patients

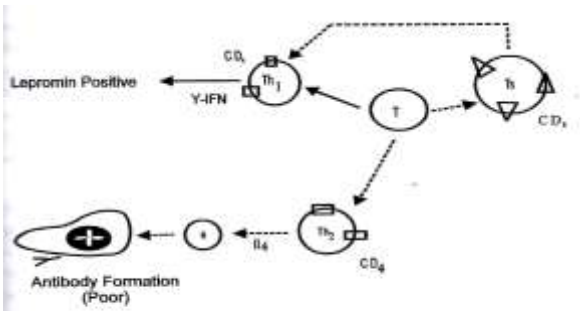

Fig. 34 : Immunological Pathways in Tuberculoid Leprosy (TL) Patients

3 weeks. Appearance of early DH suggested presence of pr-existing effector TDH cells and their recruitment at the site of antigen inoculation. Appearance of late (Mitsuda) reaction suggested capacity of the patients to mount a DH immune response against lepromin. Results of this study revealed gradual increase in Mitsuda DH reactivity from lepromatous to tuberculoid poles. It was not surprising to have obtained this type of reaction in different spectral groups. In addition, bacilli were detected in LL and BL groups of patients, suggesting direct relationship between DH and immunity. Both DH as well as immunity against M. Leprae appears to be T-cell mediated. Parobably both are generated simultaneously. Lymphokines released as a result of T- cell stimulation appear to be involved in development of DH as well as in macrophage-mediated intracellular killing of the organis. One of the lymphokines (gmma- INE) is known to activate the macrophages and may be responsible for clearance of bacilli.

Lepromin skin test

Results of lepromin test revealed gradual increase in DTH from LL to TT poles. Moreover, bacilli were not detected in BB, BT and TT cases, suggesting a direct relationship between DTH and immunity. Lymphokines released as a result of stimulation of Th_1 cells appeared to be involved in generation of lepromin reactivity as well as macrophage-mediated intracellular killing of the organism. One of the lymphokines secreted by Th_1 cells (IFN-□) is known to activate the macrophages and may be responsible for clearance of bacilli.

- Early response was measured after 48 hours of intradermal injection only 28 of 75 (37.3%) indeterminate leprosy cases gave positive response in which 20 of 75 (26.7%) cases gave 1^+ response, 5 of 75 (6.7%) cases gave 2^+ response and only 1 of 75 (1.3%) indeterminate cases gave 4^+ DTH response.

- In diseases control groups 19 of 20 (95%) TT, 17 of 20 (85%) BT, 18 of 20 (90%) BB and 5 of 20 (25%) BL cases gave positive DTH response but DTH response is negative in all cases of lepromatous leprosy.
- Late reaction (Mitsuda response) was measured after 3 weeks of intradermal injection only 23 of 75 (30%) indeterminate leprosy cases gave positive response. Eight of 20 (40%) TT, 6 of 20 (30%) BT, 3 of 20 (15%) BB and 1 of 20 (5%) BL, disease control group gave positive response. Mitsuda response is negative in LL case.

Most interesting observation was the detection of DTH of lepromin in 28 of 75 indeterminate cases, suggesting the usefulness of delayed reaction (in duration diameter measured after 48 hrs) in early diagnosis of in determinate leprosy.

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