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Histopathological studies for diagnosis of indeterminate leprosy in Uttar Pradesh, India

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Abstract
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 bacilli and occur in the human host intracellularly. The present case control study was carried out with aim to study the suspected cases of indeterminate leprosy in clinically diagnosed patients in out patients department (OPD) of Gandhi memorial and associated hospitals. Department of Medicine at King George's Medical College, Lucknow. Study group consisting of 75 cases of indeterminate leprosy, 100 subjects of other groups of leprosy spectrum, i.e., tuberculoid leprosy to lepromatous leprosy (TT - LL), taken as disease control in this study. This study emphasizes the importance of performing histopathological examinations on leprosy patients undergoing research studies for the confirmation of diagnosis and for proper classification of the diagnosis. The study help in effective use of the clinical and histopathological methods in finding out the indeterminate leprosy.

Keywords: *Mycobacterium leprae*, indeterminate leprosy, histopathology

Introduction
About 85% of Leprosy reported are in Asia and it is found that the majority (50% or more) of these cases are being detected at the stage when the only visible sign of the disease is a single lesion (Gupte, 1996; Peat *et al*., 1995; WHO, 1996) [6, 8]. Although, it is well known that most of the single lesion paucibacillary (PB) cases may heal spontaneously without any specific treatment (Ekambaram & Sithambaran, 1977) [4], a significant proportion of such cases may develop more severe disease and be at risk of developing nerve damage. Indeterminate leprosy is a benign form, relatively unstable, seldom bacteriologically positive, presenting flat skin lesions which may be hypopigmented or erythematous; the reaction to lepromin is either negative or positive. Neurotic manifestations, more or less extensive, may develop in cases that have persisted for long period. Ebenezer (1997) studied in 37 clinically diagnosed borderline tuberculoid (BT) leprosy patients skin biopsies were done prior to starting MDT and at the end of 6 months therapy. Only 13 biopsies showed features of IDT leprosy. After 6 months of MDT, 13 histopathologically diagnosed IDT cases all were clinically inactive but histological activity persisted in 3 cases (23%). This study emphasizes the importance of performing histopathological examinations on leprosy patients undergoing research studies for the confirmation of diagnosis and for proper classification of the diagnosis.
The present case control study was carried out with aim to study the suspected cases of indeterminate leprosy in clinically diagnosed patients. We have collected all suspected subjects from the skin out patients department (OPD) of Gandhi memorial and associated hospitals. Department of Medicine at King George's Medical College, Lucknow.

Material and Methods
Study group consisting of 75 cases of indeterminate leprosy, 100 subjects of other groups of leprosy spectrum, i.e., tuberculoid leprosy to lepromatous leprosy (TT - LL), taken as disease control in our study. The disease control group consist of 20 cases of each tuberculoid leprosy, borderline tuberculoid, borderline borderline, borderline lepromatous and lepromatous leprosy and 5 healthy skin biopsies. Skin biopsies of 75 cases with indeterminate leprosy and 100 disease controls of other leprosy groups were collected and fixed in 10% formalin. Sections were cut from the paraffin blocks and stained by hematoxylin – eosin and Fite-Faraco stains; the avidin biotin peroxidase techniques was used with primary antibodies rabbit anti-mycobacterium bovis (BCG) to detect bacillary antigens and bacilli. Dharmendra lepromin skin test was done in all cases and controls. Sera form all 75 cases (IDT) and 100 disease controls as well as 75 normal healthy controls were evaluated in ELISA using PGI-1 antigen and MLSE antigen for the detection of IgM antibody in cases of indeterminate leprosy and controls (disease controls and normal healthy controls).

Study group
Group A: Cases (indeterminate leprosy) (n = 75)
Group B: Controls
B.I. Disease control (n = 100)
i. Tuberculoid leprosy (n = 20)
ii. Borderline tuberculoid (n = 20)
iii. Borderline borderline (n = 20)
iv. Borderline lepromatous (n = 20)
v. Lepromatous leprosy (n = 20)
B.II. Normal healthy control
i. Skin biopsy (n = 5)
ii. Serum (n = 75)

Procedure
1. **Skin Biopsy:** Histopathological evaluation is essential for accurate classification of leprosy lesions (Binford, 1982).

In the case of paucibacillary (especially in early tuberculoid or indeterminate) leprosy, if sensory impairment is not marked. Skin biopsy may be of diagnostic value in children in whom sensory deficit cannot be verified with certainty.

2. **Sweat Function Tests:** In 1889, Father Joseph Damien de Veuster first observed that perspiration was absent on the macules of leprosy which had developed on his skin. It is a most common sign of autonomic nerve damage. Pilocarpine nitrate and acetylcholine sweat function tests are some of the sudomotor tests employed for the diagnosis of leprosy (Yawalkar, 1974 and Parikh, 1966).
3. **Lepromin Test:** The lepromin test is a guide to cell-mediated immunity (CMI) of the patient against leprosy. Lepromin is a suspension of autoclaved *M. leprae* obtained from armadillos 0.1 ml injected intradermally into the forearm and the early reaction after 48-72 hours (Fernadez reaction) and 3-4 weeks (Mitsuda reaction) reading are recorded (Bates, 1979). The Fernandez reaction probably elicits pre-existing, delaying type hypersensitivity, while Mitsuda reaction reflects CMI. The results are variable in indeterminate leprosy.

Observations and Findings

Part I: Clinical finding: The age of indeterminate leprosy patients (n=75) ranged from 6 to 70 years (median age 23 years). Over all male female ratio was 3.41.1

- The age distribution of other leprosy types (disease controls) ranged from TL (7 yrs to 60 yrs, median 25 years), BT (12 years to 70 yrs, median 21 yrs), BB (6 yrs to 50 yrs, median 26 years), BL (7 yrs to 58 yrs, median 26 years), and in LL (10 yrs to 65 yrs, median 32 years). Over all male female ratio of disease control group was TT (1.8:1), BT (2.3:1). BB (1:1), BL (2.3:1) and in LL (3:1).
- Duration of symptoms in indeterminate leprosy cases, ranged from 1 month of 4 years (median, 12.09 months).
- In 100 disease controls group duration of symptoms ranged from 2 months to 4 years (median, 12.9 months). All patients and disease control groups had insidious onset and progressive course. Twelve of 75 (16%) indeterminate cases gave history of close contact with leprosy patients.
- Forty two of 75 (56%) indeterminate patients had macules. Twenty four of 75 (32%) indeterminate cases had papules. Three of 75 (4%) IDT patients had both maculopapular lesions and patches. Two of 75 (2.7%) patients had macule with maculopapular lesions and only one patient had macule with papule, except macule other finding were interesting in IDT case.
- In other 100 disease control group 9 had macule, 5 had both papule and maculopapular lesion, 3 had plaques, 7 had macule with nodules, 34 had both patches and patches with macules and only 3 cases had macules with popular lesions.
- The most interesting finding was the detection of neural involvement in 34 of 75 (45.3%) indeterminate patients and 78% disease control group, suggesting tropism of *M. leprae* for neural tissue.
- Sixty-seven of 75 (89.3%) indeterminate cases and 29% disease control group had hypopigmentation, suggesting involvement of melanocytes.
- Presence of anhidrosis in 16 of 75 (21.3%) IDT cases and 73% disease control group suggests involvement of sweat glands i.e. sign of autonomic nerve damage.
- Development of claw hand deformity suggested collagenization and fibrosis of subcutaneous tissue.

Part II: Histopathological finding

In this study histopathological investigations of paraffin embedded tissue sections was done in all cases and controls of part I by the H&E staining and modified Fite's staining. Slit skin smear was prepared from infective sites of all cases and controls for bacteriological examination.

Lymphocytic infiltration were observed in all the patients of indeterminate leprosy. Forty two of 75 (56%) patients had histiocytes inflammation. Eighteen of 75 (24%) patients had epidermal thickening (hyperkeratinization), 31 of 75 (41.3%) cases had rate pages flattening, 66 of 75 (88%) patients showed appendageal inflammation (Fig. 4). Twenty seven of 75 (36%) patients showed nerve ending destruction while 55 of 75 (73.3%) cases had nerve inflammation. Seventy of 75 (93.3 %) patients had perivascular inflammation, 69 of 75 (98.6%) patients had papillary dermis inflammation and 66 of 75 (88%) patients had reticular dermis inflammation. there was no finding of granuloma and clear zone in case of indeterminate leprosy (Table 1). Section were positive for *Mycobacterium leprae* according to Fite's staining in 9 of 75 (12%) patients (Table 2, Fig. 7).

Tuberculoid leprosy cases had 100% epithelioid cell granuloma, lymphocyte around granuloma and intervening subepidermal zone (Fig.1). Twelve of 20 (60%) cases had Langhans giant cells. Nerve bundles were not observed in 16 of 20 (80%) cases (Table 1). Fite's staining for *Mycobacterium leprae* were positive in 12 of 20 (60%) cases (Table 2, Fig. 7).

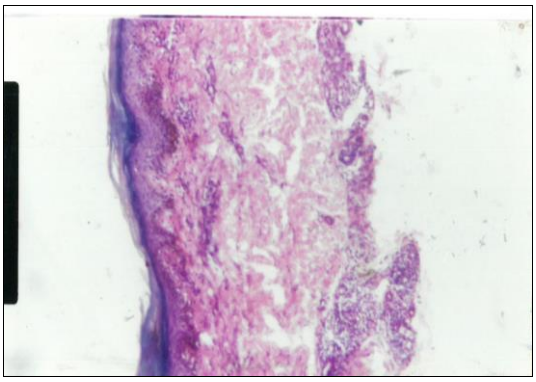

Fig 1: Indeterminate leprosy: photomicrograph of lesion show infiltration of mononuclear cell in dermis (HE *100)

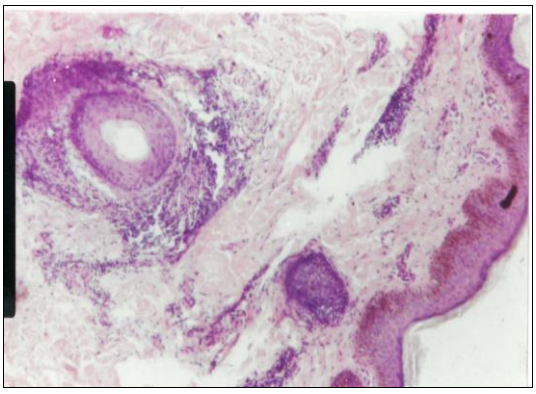

Fig 2: Indeterminate leprosy shows lymphocytic collection around hair follicle (HE x 100).

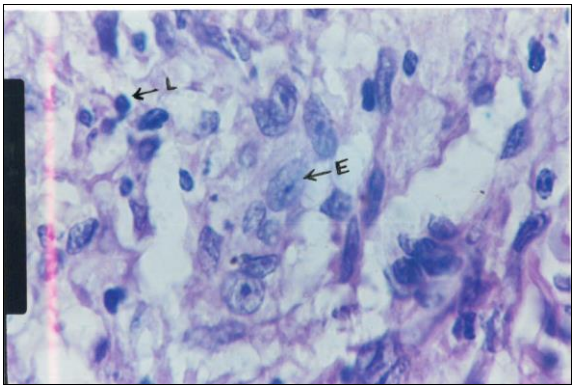

Fig 3: Borderline-tuberculoid leprosy: Photomicrograph shows epithelioid cells and lymphocytes (HE x 1000)

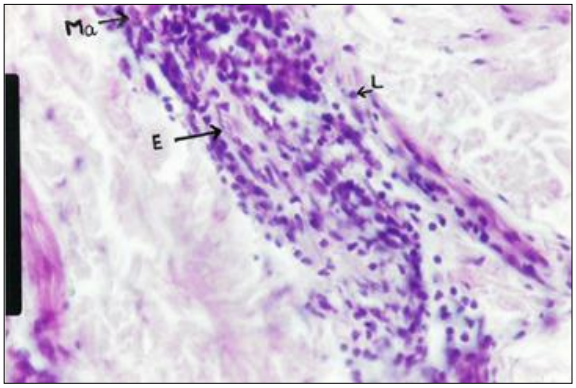

Fig 4: Borderline tuberculoid leprosy: Leprous neuropathy shows nerve bundle, heavily infiltrated by lymphocytes with occasional epithelioid cells and macrophages (HE x 100).

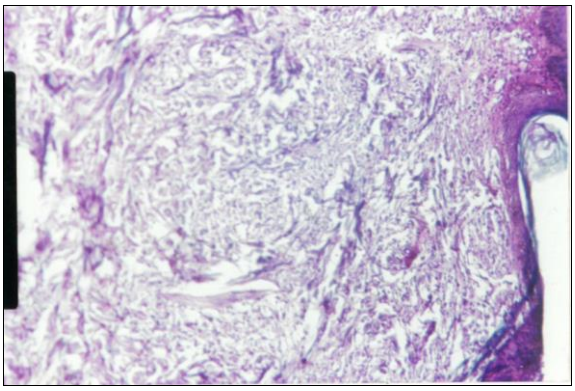

Fig 5: %4: Lepromatous leprosy shows destruction of cells (HE x 100).

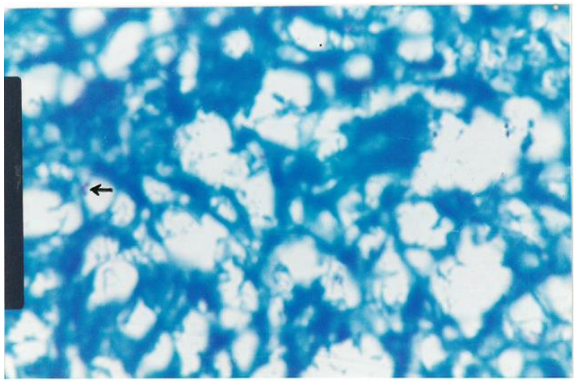

Fig 6: e Indeterminate leprosy scanty *Mycobacterium leprae* (Modified Fite's Stain x 1000)

Borderline tubeculoid cases had 100% clear subepidermal zone and epithelioid granuloma focalized by lymphocytes (Fig.1). Twelve of 20 (60%) cases had langhan's giant cells and 16 of 20 (80%) cases showed nerve bundles swollen and infiltrated (Table 1). Fite's staining for *Mycobacterium leprae* were positive in 17 of 20 (85%) cases (Table 2, Fig. 7).

Borderline cases had 100% clear subepidermal Zone, lymphocytes scanty and diffuse and Schwann cell proliferation in nerve bundles. Ten of 20 (50%) cases had epithelioid granuloma and langhan's giant cells were seen in 8 of 20 (40%) cases (Table 1). Fite's staining for *Mycobacterium leprae* were positive in all 20 (100%) cases (Table 2, Fig.7).

Borderline lepromatous cases hed 100% clear subepidermal zone and foamy histiocytes. Epidermal atrophy, scanty lymphocytes and perineural cuffing were observed in 16 of 20 (80%) cases (Fig. 2). Fifteen of 20 (75%) cases had histiocytic collections. Nerve bundles were not seen in 5 of 20 (25%) cases (Table 1). Fite's staining for *Mycobacterium leprae* were positive for 16 of 20 (80%) cases (Table 2, Fig.7).

Lepromatous leprosy cases had 100% epidermal atrophy, foamy histiocytes and pernieural inflammation. Sixteen of 20 (80%) cases showed diffuse scanty lymphocytes. Nerve bundles were not seen in 12 of 20 (60%) cases (Table 1). Fite's staining for *Mycobacterium leprae* were positive in all cases. The bacilli wee present in globi (Table 2, Fig.1, 7, 2, 3, 4, 5, 6).

Table 1: Histopathological findings of cases and disease controls (1.) CASES: (Indeterminate Leprosy) (n=75)

	Histopathological features	Number	Percentage
(ldt n=70**)**	Epidermal Thickening (Hyperkeratinizatoin)	18	24.0
	Rate pegs flattening	31	41.3
	Appendageal inflammation	66	88.0
	Granulomas	0	0.0
	Clear zone	0	0.0
	Nerve ending destruction	27	36.0
	Nerve inflammation	55	73.3
	Perivascular inflammation	70	93.3
	Papillary dermis nflammation	69	98.6
	Reticular dermis inflammation	66	88.0
	Lymphocytes	75	100.00
	Histiocytes	42	56.0
	Acid Fast Bacilli (AFB)	9	12.0

(2.) Disease controls (n=100)

Leprosy Types (n=100)	Histopathological features	Number	Percentage
Tuberculoid leprosy (TL) (n=20)	▪ Epithelioid cell granulomas	20	100.00
	▪ Langhan's giant cells	12	60.00
	▪ Lymphocyte around granuloma	20	100.00
	▪ Intervening subepidermal zone	20	100.00
	▪ Nerve bundles not seen	16	80.00
	▪ AFB (1+)	12	60.00
Border line tuberculoid (BT) (n=20)	▪ Clear Subeidermal zone	20	100.00
	▪ Epithelioid granuloma focalized by lymphocytes	20	100.00
	▪ Langhan's giant cells	12	60.00
	▪ Nerve bundles swollen and infiltrated	16	80.00
	▪ AFB (1+, 2+)	17	100.00
Borderline borderline (BB) (n=20)	▪ Clear subepidermal zone	20	100.00
	▪ Epithelioid granulomas	10	50.00
	▪ Langhan's giant cells	8	40.00
	▪ Lymphocytes santy and diffuse	20	100.00
	▪ Nerve bundles: Schwann cell proliferation	20	100.00
	▪ AFB (2+, 3+)	20	100.00
Borderline lepromatous (BL) (n=20)	▪ Clear Subepidermal Zone	20	100.00
	▪ Epithelioid granulomas	16	80.00
	▪ Histiocyte collections	15	75.00
	▪ Scanty lymphocytes	16	80.00
	▪ Foamy histiocytes	20	100.00
	▪ Perineural cuffing	16	80.00
	▪ Nerve Bundles not seen	5	25.00
	▪ AFB (3+,4+)	16	80.00
Lepromatous Leprosy (LL) (n=20)	▪ Epidermal atrophy	20	100.00
	▪ Foamy histocytes	20	100.00
	▪ Diffuse scanty lymphocytes	16	80.00
	▪ Perinural inflammation	20	100.00
	▪ Nerve bundles not seen	12	60.00
	▪ AFB (5+) in Globi	20	100.00

Table 2: Results of modified FITE'S staining.

S. No.	Types of leprosy	Number of AFB in tissue section						Total no of Positive		Total No. of Negative	
		Scanty	1+	2+	3+	4+	5+	No.	%	No.	%
1.	Cases										
	Indeterminate leprosy (ldt) (n=75)	9	-	-	-	-	-	9	12	66	88
2.	Disease Controls										
	Tuberculoid leprosy (TL) (n=20)	5	7	-	-	-	-	12	60	8	40
	Borderline tuberculoid (BT) (n=20)	-	9	8	-	-	-	17	85	3	15
	Borderline borderline (BB) (n=20)	-	-	20	-	-	-	20	100	-	-
	Borderline lepromatous (BL) (n=20)	-	-	4	12	-	-	16	80	4	20
	Lepromatous leprosy (LL) (n=20)	-	-	-	6	5	9	20	100	-	-

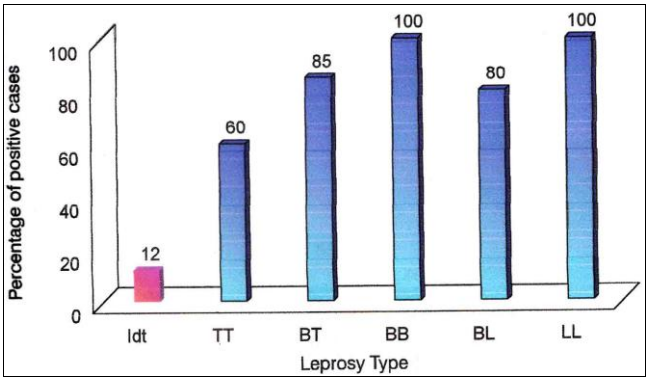

Fig 7: results of modified FITE's staining in cases (IDT) and disease controls

Lepromatous leprosy cases had 100% epidermal atrophy, foamy histiocytes and pernieural inflammation. Sixteen of 20 (80%) cases showed diffuse scanty lymphocytes. Nerve bundles were not seen in 12 of 20 (60%) cases (Table 1). Fite's staining for *Mycobacterium leprae* were positive in all cases. The bacilli wee present in globi (Table 2, Fig. 7).

Conclusion

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. According to Sadeghi *et al*. (2000) [7] lack of clinical suspicion and unfamiliarity with the histology of IDT leprosy delayed diagnosis and treatment. Leprosy should be considered in the differential diagnosis of patients presenting with unusual rheumatic and persistent cutaneous manifestations. The 75 indeterminate leprosy patients had following major histopathological features. Lymphocytic infiltration 100%, rete pegs flattening 31 of 75 (41.3%) cases, 42 of 75 (56%) IDT cases had histiocytes, 18 of 75 (24%) patients had epiderma thickening, 66 of 75 (88%) patients showed appendageal inflammation, 27 of 75 (36%) patients showed

nerve ending destruction while 55 of 75 (73.3%) cases had nerve inflammation. 70 of 75 (93.3%) patients had perivascular inflammation, 69 of 75 (98.6%) patients had papillary dermis inflammation and 66 of 75 (88%) patients had reticular dermis inflammation. Absence of granulomas and clear zone, suggesting that indeterminate leprosy is a early stage of leprosy. Section were positive for *M. leprae* in 9 of 75 (12%) cases. In case of IDT (n=75) absence of epithelioid cells in these lesions suggested role of nonmacrophage-mediated mechanisms in clearance of *Mycobacterium leprae* infection. The early diagnosis and treatment of leprosy at indeterminate stage should be beneficial to reduce and to eradicate the leprosy from the community.

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