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Effectiveness of Modified FITE's staining in 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 our 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 find the effectiveness of Effectiveness of FITE's Staining in diagnosis of Indeterminate Leprosy.

Keywords: *Mycobacterium leprae*, indeterminate leprosy, FITE's Staining, 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]. Importance of histopathological studies were established by earlier workers viz. Ebenezer (1997) but efficacy of various methods are still need to be established. 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-Farraco 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.

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. Borderlien borderline (n = 20),
- iv. Borderline lepromatous (n = 20) &
- v. Lepromatous leprosy (n = 20)

Modified Fite's Staining (Job and Chacko, 1986) were done foe demonstration of acid fast bacilli of leprosy.
Fixation: 10% formalin
Section: Paraffin

Reagents Required
a) Carbol - Fuchsin (Ziehl - Neelsen) (Qualigens Fine Chemicals, India)
b) i) Malachite Green (Qualigens Fine Chemicals, India) 0.5 gm malachite green dissolve in 100ml distilled water
   ii) Methylene Blue (Qualigens Fine Chemicals, India)

Procedure
1. The section was deparaffinized and treated with xylene and peanut oil mixture (2 parts of xylene and 1 part of peanut oil). Two changes of 12 minutes each were done.
2. The excess oil was drained and blotted to opacity.
3. Staining was done in Ziehl-Neelsen carbol fuchsin at room temperature for 30 minutes. Washing was done in tap water for 3 minutes.
4. Decolorization: Section was deceolourized in 5% H2SO4 in 25% ethyl alcohol for 2 changes of 2 minute each till sections were left with a light pink colour. Washing was done in tap water for 5 minutes.
5. Counterstain: Sections were counterstained with malachite green and methylene blue for 30 seconds.
6. Sections were washed in running tap water for 5 minutes, blotted and dried in an oven at 50 C.
7. Sections were cleared in xylol and mounted with Canada balsam.

The sections were examined using oil immersion objective (magnification x 1000).

Interpretation

- Acid fast bacilli showed red staining
- Background showed green staining when stained with malachite green and blue staining when stained with methylene blue.

Observations and Findings

In all the patients observed in this study of indeterminate leprosy section were positive for *Mycobacterium leprae* according to Fite's staining in 9 of 75 (12%) patients. Fite's staining for *Mycobacterium leprae* were positive in 12 of 20 (60%) cases (Table 1, Fig.2).

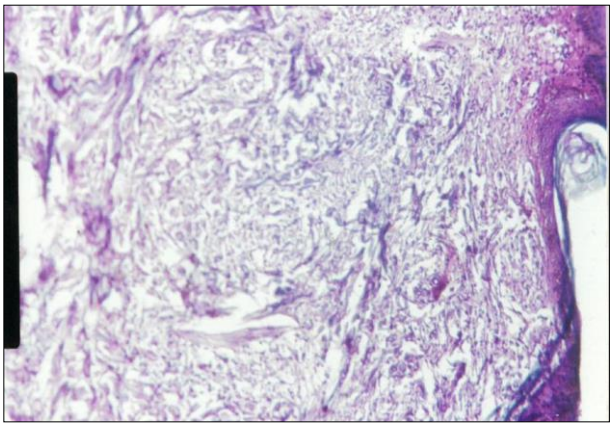


Fig A: *Mycobacterium leprae* Acid fact bacilli are present separately (Modified Fite's Stain x 1000)

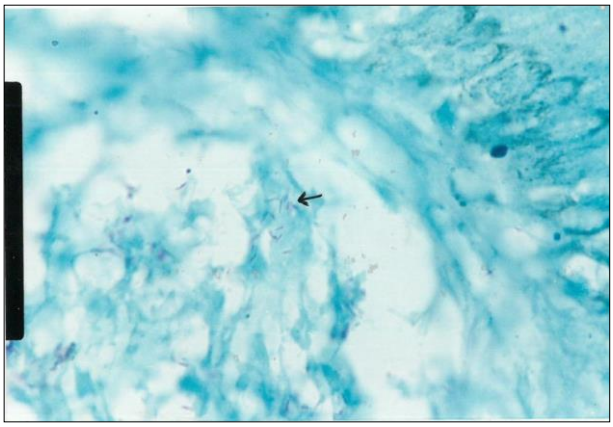


Fig B: Lepromatous leprosy: shows destruction of cells (HE x 100),

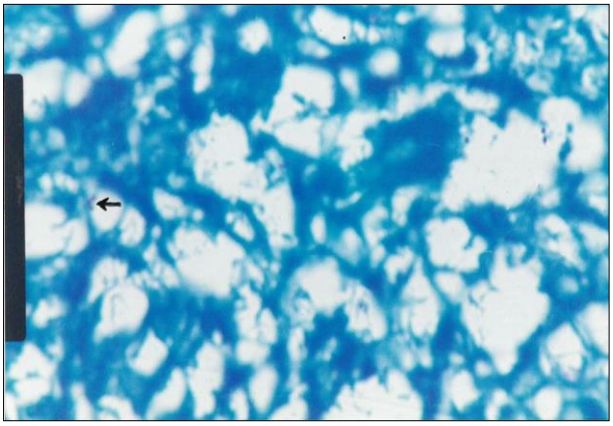


Fig C: Indeterminate leprosy: scanty mycobacterium leprae (Modified Fite's Stain x 1000)

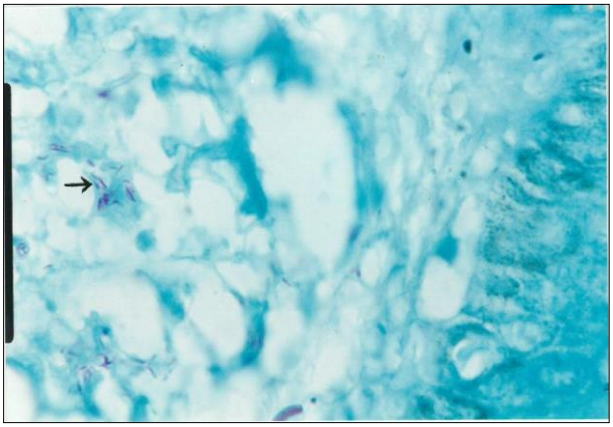


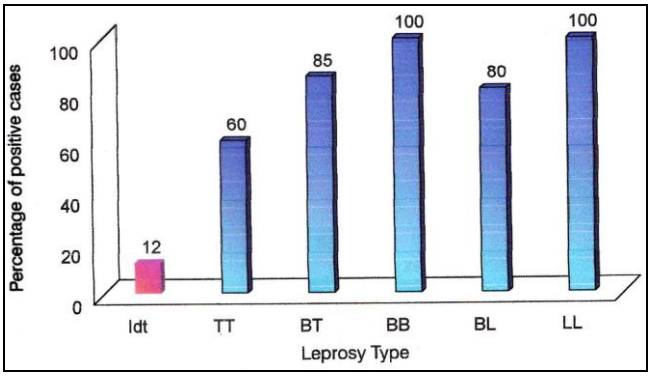
Fig D: *Mycobacterium leprae* : Discretely lying acid fast bacilli (Modified Fite's Stain x 1000).

Fig 1: Modified Fite's Staining in different cases of Leprosy

Fite's staining for *Mycobacterium leprae* in Borderline tubeculoid were positive in 17 of 20 (85%) cases. In Borderline cases Fite's staining for *Mycobacterium leprae* were positive in all 20 (100%) cases. In Borderline lepromatous cases Fite's staining for *Mycobacterium leprae* were positive for 16 of 20 (80%) cases. In Lepromatous leprosy cases Fite's staining for *Mycobacterium leprae* were positive in all cases. The bacilli were present in globi (Table 1, Fig.1).

Table 1: 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	-	-



Fite's staining for *Mycobacterium leprae* in Lepromatous leprosy were positive in all cases. The bacilli wee present in globi (Table 1, Fig.2).

Fig 2: Results of modified FITE's staining in cases (IDT) and disease controls

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) 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 Fite's Staining in Borderline borderlie (BB) cases and Lepromatous leprosy (LL) cases show 100 % positive result. Other cases also show the 60 – 85 % result. It also successfully show positive result in 12 % of Indeterminate leprosy. This method of staining act as the primary method of Diagnosis and useful in determining the cases for further study.

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