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Original Research Article

Histopathological and Fluorescence-Based Detection of Mycobacterium leprae in Leprosy: A Comparative Study

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Abstract:

Background: Leprosy remains a public health concern, with early diagnosis essential to prevent deformities and transmission. Modified Fite-Faraco (FF) staining is standard for detecting Mycobacterium leprae but is laborious and less sensitive in paucibacillary cases. Auramine Rhodamine (AR) fluorescence staining may enhance detection.

Methods: A cross-sectional study was conducted at a tertiary care hospital over 4.5 years on 100 clinically diagnosed leprosy patients. Skin biopsies were processed for Hematoxylin–Eosin, Fite Faraco and Auramine Rhodamine staining. Histopathological types, bacillary load and stain sensitivity and specificity were compared.

Results: Tuberculoid leprosy (TT) was the most common type (22%), followed by borderline tuberculoid (BT) 19%. Male predominance was observed (71%). Auramine Rhodamine staining detected bacilli in 53 cases, including 13 Fite Faraco negative cases, demonstrating higher sensitivity. Auramine Rhodmaine showed superior specificity across most leprosy types compared to Fite Faraco staining.

Conclusion: Auamine Rhodamine fluorescence staining is a sensitive and specific adjunct to Fite Faraco for detecting Mycobacterium leprae, particularly in paucibacillary cases. Its use can improve early diagnosis and treatment, supporting NLEP objectives.

Keywords: Leprosy, Auramine Rhodamine, Fite-Faraco, Histopathology, Paucibacillary.

Introduction

Leprosy is one of the oldest diseases known, likely originating in India, where it was historically termed "Kushtha" and associated with social stigma. The disease primarily affects the skin and peripheral nerves, often mimicking other conditions, making early diagnosis challenging [1].

According to the National Leprosy Eradication Programme (NLEP) 2011–2012 report, India had 0.83 lakh cases with a prevalence of 0.69/10,000. Karnataka reported child leprosy cases at 10.76%. Globally, prevalence remains significant, with 189018 cases registered in 2013 [5,6,7]

Early diagnosis and effective treatment are critical for reducing transmission. While clinical evaluation and skin smears are routine, some borderline or early cases require histopathological confirmation. The Modified Fite-Faraco (FF) stain is standard but time-consuming and subject to observer fatigue [10]. Fluorescent microscopy using Auramine Rhodamine (AR) improves detection, reduces fatigue, and aids early intervention.

Materials and Methods

This study was conducted at a tertiary care hospital over 4.5 years. Skin biopsies from 100 clinically diagnosed leprosy patients were collected in 10% formalin. During grossing the shape, size, and cut sections were recorded. Sections were processed for:

- Hematoxylin–Eosin (H&E) for histopathology
- Modified Fite-Faraco (FF) stain
- Auramine Rhodamine (AR) fluorescence stain using fluorescence microscope

Exclusion: Patients not diagnosed as leprosy

Data on age, sex, clinical type, and bacillary load were recorded. Sensitivity and specificity of Fite Faraco and Auamine Rhodamine staining were calculated.

Results

Demographics and Clinical Types

Table 1: Sex distribution of different cases of leprosy

	Male	Female	Total
TT	17	5	22
BT	13	6	19
BB	2	0	2
BL	12	7	19
LL	9	4	13
IL	10	7	17
Histioid	5	0	5
ENL	3	0	3
Total	**71**	**29**	**100**

TT- Tuberculoid, BT- Borderline Tuberculoid, BB- Mid borderline, BL- Borderline Lepromatous, LL- Lepromatous leprosy, ENL- Erythema nodosum leprosum

The maximum cases were seen in males compared to females.

Table 2: Pathological types of leprosy

	Number of Cases
TT	22
BT	19
BB	2
BL	19
LL	13
IL	17
Histioid	5
ENL	3
Total	**100**

TT- Tuberculoid, BT- Borderline Tuberculoid, BB- Mid borderline, BL- Borderline Lepromatous, LL- Lepromatous leprosy, ENL- Erythema nodosum leprosum

Among the pathological types of leprosy maximum cases were diagnosed as TT and minimum number of cases were BB.

Table 3: Comparison of Sensitivity between Fite Faraco and Auramine Rhodamine of the present study

Types of Leprosy	FF	AR
TT	0%	9%
BT	0%	42%
BB	0%	0%
BL	73.7%	89.4%
LL	100%	100%
IL	11.8%	23.5%
Histoid	80%	80%
ENL	100%	100%

In our study we have compared the sensitivity between the two stains Fite Faraco and Auramine Rhodamine. It was seen that the sensitivity of Auramine Rhodamine was more compared to Fite Faraco. The sensitivity of Auramine Rhodamine was 9% for TT and 42% for BT whereas it was 0% for BB.

Table 4: Stains of Lepra bacilli

	Number of cases
FF+	40
AR+	53
FF+, AR+	40
FF-, AR+	13

FF-Fite Faraco, AR- Auramine Rhodamine

Among the staining techniques combined positivity for Fite Faraco and Auramine Rhodamine was 53 cases but in 13 cases though Fite Faraco showed negative staining the Auramine Rhodmaine staining was detected positive were. This highlights the importance of early detection of bacillary load and better sensitivity and thus helps in doubtful cases of leprosy where demonstration of bacilli is important for diagnosis

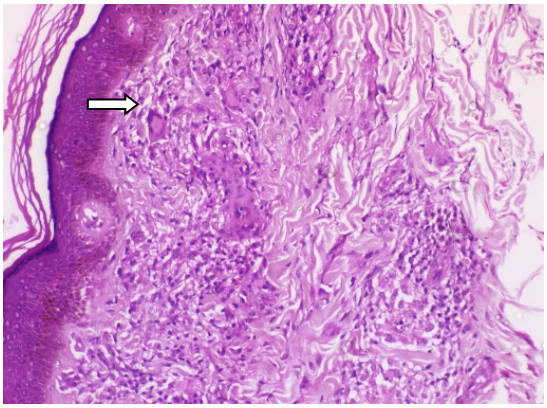

Fig1- BT-Microscopy shows granuloma with Langhans giant cell (marked with arrow) hugging the normal epidermis (x20, H&E)

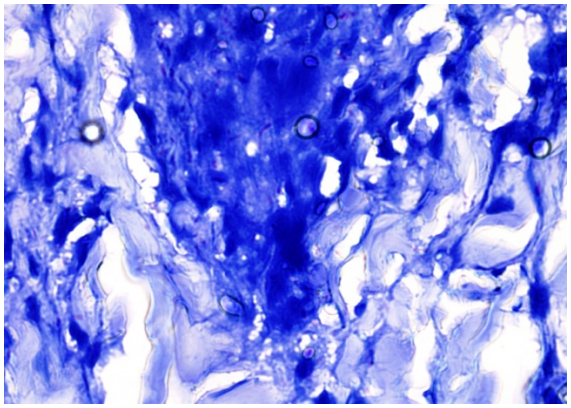

Fig 2- BT- Microscopy shows no bacilli with Modified Fite Faraco staining, Bacillary index is 0+(x100)

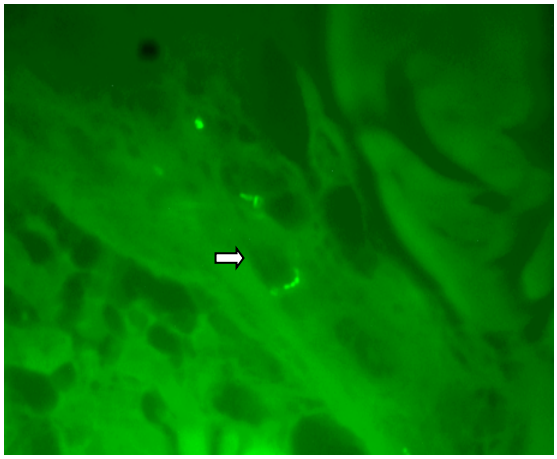

Fig 3- BT-The biopsy above when stained with Auramine Rhodamine showed bacilli [marked with arrow] using blue light fluorescence below 530nm

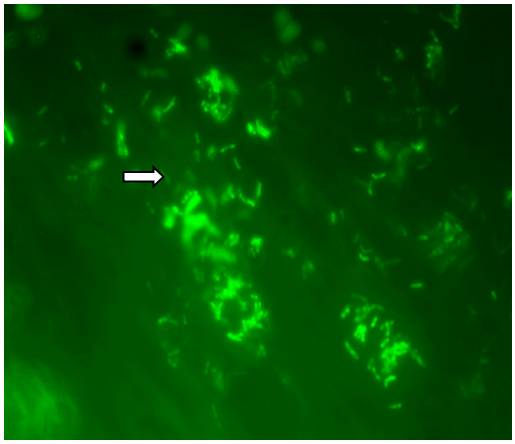

Fig 4- BL- Auramine Rhodamine stain showing bacilli (marked with arrow) in a multibacillary case (using blue light fluorescence below 530nm)

Discussion

The study confirms that TT is the most common type in our cohort, consistent with local epidemiology, while BB remains the least frequent. Male predominance aligns with prior studies, likely due to greater exposure and mobility.

Table 5: Comparison of Positivity Rate of Auramine Rhodamine in the present study with other studies

Types of Leprosy	Present Study	Nayak et al [15]	Nagarajappa et al [10]
TT	100%	77.7%	16.7%
BT	100%	83.3%	7.1%
BB	0%	-	30%
BL	54.8%	-	-
LL	100%	100%	-
IL	66.7%	52%	8.3%
Histoid	50%	-	-
ENL	50%	-	-

In the present study the positivity rate of Auramine Rhodamine is higher compared to the study conducted by Nayak et al. [15] The positivity rate of TT and BT was 100% compared to the study conducted by Nayak et al in which the positivity rate was 77.7% for TT and 83.3% for BT. The positivity rate in our study of BL was 54.8%, 50% of Histoid and 50 % of ENL. The positivity rate of LL in our study was 100% which was the same in Nayak et al. The positivity rate of BB in our study was 0% and in Nagarappa et al it was 30%. However we were not able to compare these with the study conducted by Nayak et al as these parameters were not assessed in their study.

Auramine Rhodamine fluorescence demonstrates higher sensitivity and specificity than Fite Faraco, particularly in paucibacillary forms (TT, BT, IL). This supports its use as a complementary diagnostic tool, facilitating early intervention and reducing deformity risk. Previous studies have similarly shown AR's utility in rapid and accurate detection. Child leprosy remains a concern, emphasizing the need for early detection and intervention under NLEP guidelines.

Conclusion

Auramine Rhodamine staining is a sensitive and specific adjunct to Modified Fite-Faraco in detecting Mycobacterium leprae, particularly in paucibacillary cases which can't be detected by Fite Faraco staining. Its routine use can improve early diagnosis, guide treatment and support leprosy eradication programs.

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