Indian J Lepr 2021, 93 : 15-27

http://www.ijl.org.in

Original Article

Clinicopathological Diagnosis of Leprosy: Comparative Evaluation of Three Staining Methods for Acid Fast bacilli in Slit Skin Smears and Biopsy Specimens

A Kamle[1], P Awasthi[2], N Rawat[3], S Parikh[4], P Jadhav[5]

Received : 22.02.2020 Accepted : 23.10.2020

Detection of acid fast bacilli (AFB) in slit smears and histopathological specimens is of paramount importance for diagnosis and classification of leprosy. In this study the results of conventional staining technique for AFB has been compared with modified rapid AFB and Fite Faraco stain in slit skin smears and punch biopsy specimens from clinically diagnosed cases of leprosy. Processed skin biopsies and slit skin smears of 42 patients attending outdoor clinic of a tertiary care centre were stained with three stains viz Fite Faraco, modified Rapid AFB and conventional Ziehl Neelsen staining. According to clinical diagnosis the maximum number of patients belonged to Borderline Tuberculoid leprosy which correlated with histopathological diagnosis of skin biopsy. Clinical and histopathological correlation was not observed in 14/42 cases - histopathological categorization of biopsies from these cases revealed Indeterminate leprosy (9 cases), Tuberculoid Leprosy (2 cases), Borderline Lepromatous (1 case), Histoid Leprosy (1 case) and Mid- Borderline Leprosy (1 case). Maximum positivity for AFB was seen with Fite Faraco staining followed by modified Rapid AFB both in the biopsy specimens and slit skin smears. Fite Faraco staining showed highest sensitivity in both paucibacillary and multibacillary cases followed by modified rapid AFB and conventional AFB staining. Though biopsy and slit skin smears have their individual diagnostic advantages and limitations, biopsy deserves to be viewed as gold standard in case of difficulty in arriving at a confirmed diagnosis. Findings of this study need to be validated in a larger number of leprosy cases at community level studies and correlated with classification currently recommended by WHO and NLEP.

Keywords : Granuloma, Slit Skin Smears, Acid Fast Bacilli, Leprosy, Fite Faraco, Modified AFB staining

Introduction

Despite the efforts of the National Leprosy Eradication Programme (NLEP) strategies and plans the fact remains that India continues to account for 60% of new cases reported globally each year and is among the 22 "global priority countries" that contribute 95% of world numbers of leprosy warranting a sustained effort to bring the numbers down (Lockwood 2002, Gurung et al 2019, Srinivas et al 2002). Clinically, leprosy is

[1] Mr. Apurv Kamle, MBBS, 3rd Professional Student
[2] Dr. Pragati Awasthi, MBBS, MD, Associate Professor, Department of Pathology
[3] Dr. Niharika Rawat, MBBS, MD, Assistant Professor, Department of Pathology
[4] Dr. Shrini Parikh, MBBS, III[rd] year Post Graduate, Department of Dermatology
[5] Dr. Pradeep Jadhav, MD, Professor and Head, Department of Pathology
Departments of Pathology & Dermatology, People's Medical College and Research Center, Bhopal-462038, Madhya Pradesh, India.
Correspondence : Dr Pragati Awasthi; **Email:** dr.pragatisharma19@gmail.com

diagnosed when patient shows two out of three cardinal signs and known cardinal signs of leprosy are: i) Loss of sensation in skin lesion. ii) Enlarged peripheral nerve iii) Positive skin smears (Walker & Lockwood 2006). Conventional procedures, slit skin smear and skin biopsy have individual diagnostic advantages and limitations (Rao & Suneetha 2018).

Demonstration of acid fast bacilli (AFB) has immense diagnostic significance with and without histopathology. While conventional Ziehl Neelsen staining (smears) and Fite Faraco staining (histopathology) have been extensively used over a period of several decades, a modified staining method for AFB (Nayak et al 2003) has not been investigated adequately. In the present study we describe our experience with conventional AFB, Fite Faraco staining and a modified Rapid AFB method in estimating the bacillary load in slit skin smears and skin biopsy specimens. Secondly, the histopathological classification of skin biopsies has been correlated with clinical diagnosis. Thirdly, bacillary index of slit skin smear (BI) and bacterial index of granuloma (BG) in untreated leprosy patients has been compared and correlated with s multibacillary and paucibacillary classification of WHO 1988 (WHO 1988, Parkash 2009) so as to explore the clinical relevance of findings of present study.

Material and Methods

The present study is a hospital based study, slit skin smears and punch biopsy were taken from clinically suspected untreated patients of leprosy attending out-patient Department of Dermatology of People's Medical College Hospital and Research Centre, Bhopal (MP) over a period of 6 months from April 2018 to October 2018. In present study cases were decided on the basis of clinical findings in form of presenting complaints like loss of sensation in skin lesions or the hands or feet, aches and pains in face or limbs, numbness, sleepy or dead feelings in the affected areas.

On examination skin lesions which showed: hypopigmented or erythematous macules or papules or nodules and plaques which were skin colored or slightly red and cases with enlarged peripheral nerve (Walker & Lockwood 2006).

Slit skin smear and punch biopsies were taken after the informed consent of patient from the same active lesion for easy comparison and biopsies were routinely processed and Haematoxylin eosin stained sections were classified into Tuberculoid (TT), Borderline Tuberculoid (BT), mid Borderline (BB), Borderline Lepromatous (BL) and Lepromatous Leprosy (LL) according to Ridley & Jopling scale (Ridley & Jopling 1966).

In present study microscopic examination of punch biopsies and slit skin smear slides was done by single observer. Slit skin smears for both modified rapid AFB and Fite Faraco stains were fixed in 90% Iso Propyl Alcohol. Slit skin smears and skin biopsy sections were stained by conventional Ziehl Neelsen staining (Bancroft et al 2013), modified rapid AFB (Nayak et al 2003) as well as Fite Faraco staining for histopathological specimens (Bancroft et al 2013).

Modified Rapid AFB method (Nayak et al 2003) involved deparaffinization of the slide in xylene, dipping the slide in 10% periodic acid for 30 minutes, next dipping it in water for 2-3 times, followed by flooding the slide with para-rosanaline stain and keep it in the incubator at 70 Degrees for 8-10 minutes and then wash it. Further the slides were decolorized with 1% hydrochloric acid in 70% ethanol, washed and counter stained with 1% methylene blue for 30 seconds. Finally, these were dehydrated in absolute alcohol (1-2 dips).

Bacterial index (BI) of slit skin smear and skin biopsy (BG) were compared correlated with classification as multibacillary and paucibacillary clinical types as classified by WHO earlier (WHO 1988, Parkash 2009).

Results

Initially the present study could enroll 45 cases, of them, 3 were diagnosed as Hansen's clinically but due to inadequate skin biopsy histopathological correlation could not be done, and thus were excluded from the study. The rest 42 cases are analyzed as follows:

On Clinical Analysis

In our present study we found that out of 42 cases 20 cases (47.6%) were from age group 15-35 yrs, 15 cases (35.7%) from age group 36-55 yrs and 7 cases (16.7%) from age group 55-75 yrs with mean age of 40 years. Out of 42 patients, 30 patients were male and 12 were female with M:F ratio of 5:2. About 95.2% of cases presented with skin lesions with predominant complaint of multiple red raised lesions, 90.4% of cases had nerve involvement with common complaint of paraesthesia and patches of altered hot and cold senses and 4.7% cases showed type 1 lepra reaction.

Out of total 42 cases, 28 cases showed clinical and histopathological correlation. Histopathological categorization of biopsies was done as follows - Borderline Tuberculoid leprosy in (14 cases - 33.33%) followed by ENL (6 cases - 14.2%), lepromatous leprosy (4 cases - 9.5%), Borderline Leprosy (2 cases - 4.7%), Tuberculoid Leprosy (1 case - 2.3%) and Histoid Leprosy (1 case - 2.3%). (Table 1).

In present study, out of total 42 cases, clinical and histopathological correlation was not observed in 14 cases. Histopathological categorization of biopsies revealed Indeterminate leprosy (9 cases), Tuberculoid Leprosy (2 cases), Borderline Lepromatous (1 case), Histoid Leprosy (1 case) and Mid-Borderline Leprosy (1 case). Kappa statistics was applied to assess the level of agreement between clinical and Histopathological diagnosis which revealed moderate level of agreement between clinical and Histopathological findings (κ=0.59; p=0.001).

In present study, out of 17 cases who were clinically diagnosed as Borderline Tuberculoid Leprosy, slit smear was positive in 17.6% cases with AFB stain and Rapid AFB stain, whereas it was positive in 23.5% cases on Fite Farco stain. Similarly, on biopsy, AFB positivity was seen in 47.1%, 52.9% and 64.7% cases on AFB, Modified AFB and Fite Farco staining, respectively. Out of 9 cases of Lepromatous Leprosy, AFB and modified AFB stain on slit smear and biopsy was

Table 1 : Correlation of clinical and histopathological diagnosis of leprosy (Number or figures in parenthesis represent percentage)

Clinical	Histopathological diagnosis							
diagnosis	I	TT	BT	BB	BL	LL	Histoid	ENL
TT	0 (0)	1 (33.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
BT	1 (11.1)	2 (66.7)	14 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
BL	5 (55.6)	0 (0)	0 (0)	1 (100)	2 (66.7)	0 (0)	0 (0)	0 (0)
LL	3 (33.3)	0 (0)	0 (0)	0 (0)	1 (33.3)	4 (100)	1 (50)	0 (0)
HL	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (50)	0 (0)
ENL	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	6 (100)
Total	9	3	14	1	3	4	2	6

κ=0.59; p=0.001

positive in 66.7% cases each whereas it was positive in 77.8% and 88.9% cases on Fite Farco staining on slit skin smear and biopsy, respectively. However, the association of clinical diagnosis with slit skin smear and skin biopsy was not statistically significant (p>0.05) using chi square test (Table 2).

The slit skin smears were positive in 13 cases (30.9%) with AFB stain. Similarly, with modified rapid AFB stain 14 cases and with Fite Faraco stain 16 cases were positive. There was one case (100%) of mid Borderline Leprosy in which slit skin smear with modified rapid AFB and Fite Faraco stain was positive but negative with AFB stain. Similarly, Fite Faraco stain was positive for AFB in 28.6% of Borderline Tuberculoid whereas 21.4% cases each with Borderline Tuberculoid leprosy were positive for AFB stain and modified rapid AFB stain. All smears were negative in Indeterminate and Tuberculoid Leprosy. The observed association (using chi square test) of histopathological diagnosis with findings of AFB stain, modified rapid AFB stain and Fite Farco stain was statistically highly significant (p<0.01). Overall, 19 cases (45.2%) showed skin biopsy positivity for AFB. Similarly, 21 cases (50%) showed BIG positivity with Modified rapid AFB stain and 26 cases (61.9%) showed BG positivity with Fite Faraco stain. In cases with Borderline Tuberculoid leprosy, 78.6%, 64.3% and 57.1% cases were positive on Fite Faraco, Modified rapid AFB and AFB staining respectively. Similarly, in cases with ENL, Fite Faraco staining was positive in 66.7% cases whereas Modified AFB was positive in 50% and AFB stain was positive in 33.3% cases. Significant AFB could not be seen in Indeterminate and Tuberculoid leprosy. Scanty bacilli were detected in Indeterminate leprosy with Fite Faraco stain. The observed association of histopathological diagnosis with findings of AFB, Modified AFB and Fite Faraco staining was statistically highly significant (p<0.01). The mean BG with AFB stain is 1.07, with modified rapid AFB stain is 1.33 and with Fite Faraco is 1.60. The BI and BG in different types of leprosy is shown in Table 3.

Table 2 : Depicting Clinical diagnosis and positivity in slit skin smear (Number or figures in parenthesis represent percentage)

Clinical Diagnosis	Number of Patients	Slit skin smear			Skin biopsy		
		Conventional AFB staining	Modified Rapid AFB Staining	Fite Faraco staining	Conventional AFB staining	Modified Rapid AFB Staining	Fite Faraco staining
TT	1	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
BT	17	3 (17.6)	3 (17.6)	4 (23.5)	8 (47.1)	9 (52.9)	11 (64.7)
BL	8	2 (25)	3 (37.5)	3 (37.5)	2 (25)	2 (25)	3 (37.5)
LL	9	6 (66.7)	6 (66.7)	7 (77.8)	6 (66.7)	6 (66.7)	8 (88.9)
HISTOID	1	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)
ENL	6	1 (16.7)	1 (16.7)	2 (33.3)	2 (33.3)	3 (50)	4 (66.7)
TOTAL	42	13 (31)	14 (33.3)	17 (40.5)	19 (45.2)	21 (50)	27 (64.3)
Chi square value		10.16	9.69	9.53	5.39	5.1	7.25
P value		0.07	0.084	0.09	0.37	0.41	0.20

Table 3 : Depicting Positivity in Slit skin smears and skin biopsy (Number or figures in parenthesis represent percentage)

Histopathological Diagnosis	Number of Patients	Slit skin smear			Skin biopsy		
		Conventional AFB staining	Modified Rapid AFB Staining	Fite Faraco staining	Conventional AFB staining	Modified Rapid AFB Staining	Fite Faraco staining
I	9	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (11.1)
TT	3	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
BT	14	3 (21.4)	3 (21.4)	4 (28.6)	8 (57.1)	9 (64.3)	11 (78.6)
BB	1	0 (0)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)
BL	3	3 (100)	3 (100)	3 (100)	2 (66.7)	2 (66.7)	3 (100)
LL	4	4 (100)	4 (100)	4 (100)	4 (100)	4 (100)	4 (100)
HISTOID	2	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)
ENL	6	1 (16.7)	1 (16.7)	2 (33.3)	2 (33.3)	3 (50)	4 (66.7)
TOTAL	42	13(30.9)	14 (33.3)	16 (38.1)	19 (45.2)	21 (50)	26 (61.9)
Chi square value		27.07	27.64	24.2	20.09	20.48	22.59
P value		0.001	0.001	0.001	0.005	0.005	0.002

Bacterial index of Skin biopsy as well as skin smear on AFB stain was negative in 100% cases of indeterminate and tuberculoid leprosy. As compared to SSS (13 cases i.e 31%) the BG identified significantly more number of positivity (19 cases i.e 45.2%) with AFB stain. Bacillary index of 1+ was noted in 3 (21.4%) of BT cases on SSS and 6 (42.9%) cases on biopsy. About 100% and 15% cases of Histoid type were reported to be 5+ in BI and BG respectively (Table 4). Kappa statistics was applied to assess the level of agreement between skin smear and biopsy techniques. The present study observed moderate level of agreement (κ=0.61) between the findings of SSS and biopsy for biopsy and the observed agreement was statistically highly significant ($p<0.01$).

As compared to SSS (14 cases i.e 33.33%) the BG identified significantly more number of positivity (21 cases i.e 50%) with modified rapid AFB stain. In all the cases of Indeterminate and Tuberculoid leprosy, bacillary index was 0 on Modified AFB stain. About 78.6% and 35.7% cases respectively had BI of 1+ on slit smear and biopsy. There were 8 cases which were positive in biopsies but not in SSS including 6 cases of BT and 2 cases of ENL. The present study observed minimal level of agreement (κ=0.36) between the findings of SSS and biopsy and the observed agreement was statistically highly significant ($p<0.01$). (Table 5)

As compared to SSS (16 cases i.e 38.1%) the BG identified significantly more number of positivity (26 cases i.e 61.9%) with Fite Faraco stain. There were 10 cases which were positive in biopsies but not in SSS including 7 cases of BT, 2 cases of ENL and 1 case of indeterminate leprosy. All TT and 8 cases out of 9 cases of indeterminate leprosy were negative on both SSS and biopsy for AFB. About 100% cases of Borderline Lepromatous leprosy were 5+ on SSS whereas only 33.3% cases were 5+ on biopsy. All the cases of Histoid leprosy showed bacillary index of 6+ on both SSS and

Table 4 : Comparative evaluation of slit skin smears for bacterial index (BI) and skin Biopsy for bacterial index of granuloma (BG) in AFB stain. (Number or figures in parenthesis represent percentage)

Histo-patho-logical	AFB															
	I		TT		BT		BB		BL		LL		Histoid		ENL	
N	9		3		14		1		3		4		2		6	
Value	SSS	BG	SSS	BG	SSS	BG	SSS	BG	SSS	BG	SSS	BG	SSS	BG	SSS	BG
0+	9 (100)	9 (100)	3 (100)	3 (100)	11 (78.6)	6 (42.9)	1 (100)	0 (0)	0 (0)	1 (33.3)	0 (0)	0 (0)	0 (0)	0 (0)	5 (83.3)	4 (66.7)
1+	0 (0)	0 (0)	0 (0)	0 (0)	3 (21.4)	6 (42.9)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0) (33.3)	1 (16.7)	2
2+	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (14.3)	0 (0)	1 (100)	0 (0)	0 (0)	1 (25)	1 (25)	0 (0)	0 (0)	0 (0)	0 (0)
3+	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (66.7)	2 (66.7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
4+	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (33.3)	0 (0)	0 (0)	1 (25)	0 (0)	1 (50)	0 (0)	0 (0)
5+	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	3 (75)	2 (50)	2 (100)	1 (50)	0 (0)	0 (0)

Chi sq=16.84; p=0.001; κ=0.61; p=0.001

Table 5 : Comparative evaluation of slit skin smears for bacterial index (BI) and skin biopsy for bacterial index of granuloma (BG) in modified rapid AFB stain. (Number or figures in parenthesis represent percentage)

	Modified Rapid AFB staining															
	I		TT		BT		BB		BL		LL		Histoid		ENL	
N	9		3		14		1		3		4		2		6	
Value	SSS	BG	SSS	BG	SSS	BG	SSS	BG	SSS	BG	SSS	BG	SSS	BG	SSS	BG
0+	9 (100)	9 (100)	3 (100)	3 (100)	11 (78.6)	5 (35.7)	0 (0)	0 (0)	0 (0)	1 (33.3)	0 (0)	0 (0)	0 (0)	0 (0)	5 (83.3)	3 (50)
1+	0 (0)	0 (0)	0 (0)	0 (0)	3 (21.4)	6 (42.9)	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (16.7)	2 (33.3)
2+	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	3 (21.4)	0 (0)	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (16.7)
3+	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (66.7)	1 (33.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
4+	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (33.3)	1 (33.3)	0 (0)	1(25)	0 (0)	0 (0)	0 (0)	0 (0)
5+	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (50)	2 (50)	2 (100)	1 (50)	0 (0)	0 (0)
6+	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (50)	1(25)	0 (0)	1 (50)	0 (0)	0 (0)

Chi sq=125; p=0.001; κ=0.36; p=0.001

Table 6 : Comparative evaluation of slit skin smears for bacterial index (BI) and skin biopsy for bacterial index of granuloma (BG) by Fite Faraco staining. (Number or figures in parenthesis represent percentage)

	Fite Faraco staining															
Leprosy	**I**		**TT**		**BT**		**BB**		**BL**		**LL**		**Histoid**		**ENL**	
N	**9**		**3**		**14**		**1**		**3**		**4**		**2**		**6**	
Value	**SSS**	**BG**	**SSS**	**BG**	**SSS**	**BG**	**SSS**	**BG**	**SSS**	**BG**	**SSS**	**BG**	**SSS**	**BG**	**SSS**	**BG**
0+	9 (100)	8 (88.9)	3 (100)	3 (100)	10 (71.4)	3 (21.4)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	4 (66.7)	2 (33.3)
1+	0 (0)	1 (11.1)	0 (0)	0 (0)	4 (28.6)	8 (57.1)	1 (100)	0 (0)	0 (0)	1 (33.3)	0 (0)	0 (0)	0 (0)	0 (0)	2 (33.3)	2 (33.3)
2+	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	3 (21.4)	0 (0)	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (33.3)
3+	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
4+	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0	0 (0)	0 (0)	0 (0)	1 (33.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
5+	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0	0 (0)	0 (0)	3 (100)	1 (33.3)	1 (25)	2 (50)	0 (0)	0 (0)	0 (0)	0 (0)
6+	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0	0 (0)	0 (0)	0 (0)	0 (0)	3 (75)	2 (50)	2 (100)	2 (100)	0 (0)	0 (0)

Chi sq=95.54; p=0.001; κ=0.35; p=0.001

Table 7 : Reliability Analysis by Chronbach's Alpha

	Chronbach's Alpha	Interpretation
AFB BG vs. Modified AFB BG	0.976	Excellent Internal Consistency
AFB BG vs. FF BG	0.943	Excellent Internal Consistency
AFB SSS vs. Modified AFB SSS	0.970	Excellent Internal Consistency
AFB SSS vs. FF SSS	0.980	Excellent Internal Consistency

Cochran's Q= 30.52, p=0.001

Table 8 : Comparison of Paucibacillary and Multibacillary leprosy (WHO 1988) with staining (Number or figures in parenthesis represent percentage)

			Paucibacillary	Multibacillary	P value
AFB	BG	Positive	10 (38.5)	9 (56.2)	0.26
		Negative	16 (61.5)	7 (43.8)	
	SSS	Positive	4 (15.4)	9 (56.2)	0.005
		Negative	22 (84.6)	7 (43.8)	
Modified AFB	BG	Positive	12 (46.2)	9 (56.2)	0.53
		Negative	14 (53.8)	7 (43.8)	
	SSS	Positive	4 (15.4)	10 (62.5)	0.002
		Negative	22 (84.6)	6 (37.5)	
Fite Farco	BG	Positive	15 (57.7)	12 (75)	0.26
		Negative	11 (42.3)	4 (25)	
	SSS	Positive	6 (23.1)	11 (68.8)	0.003
		Negative	20 (76.9)	5 (31.2)	

biopsy. The kappa statistics documented minimal level of agreement (κ=0.35) between the findings of SSS and biopsy and the observed agreement was statistically highly significant (p<0.01). (Table 6)

The difference between the values (bacterial yield) of BI and BG was statistically analyzed with paired – t-test and was found to be highly significant (p<0.0001). The Cronbach's Alpha was calculated and compared with different stains which showed excellent internal consistency as shown in (Table 7).

The cases were grouped on the basis of bacillary index into multibacillary and paucibacillary. It was observed, which can be noted that there is a consistent difference between positivity of biopsy and SSS. On Biopsy, 10 cases were paucibacillary whereas 9 were multibacillary on AFB. Similarly, on SSS, 4 cases were paucibacillary and 9 were multibacillary. Also, difference was noted in BG and SSS findings of modified AFB and Fite farco stain as shown above in (Table 8 and in Figs 1 & 2). Test of significance (chi square test) showed statistically significant association of split skin

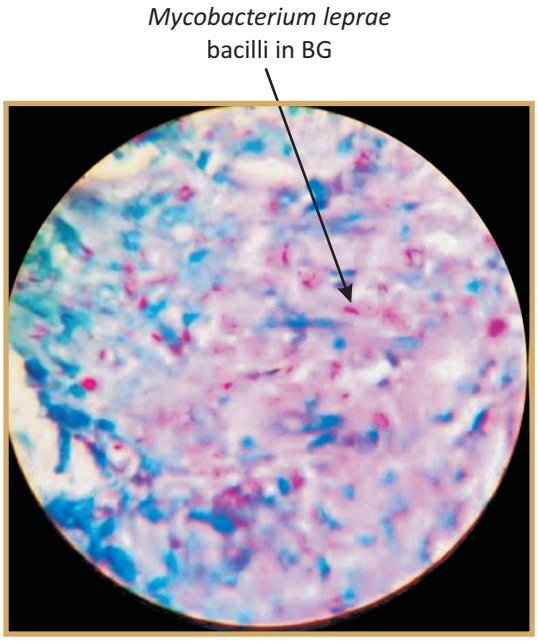

Fig. 1 : Section shows *Mycobacterium leprae* bacilli with BI 5^{+} [100x view Fite Faraco stain]

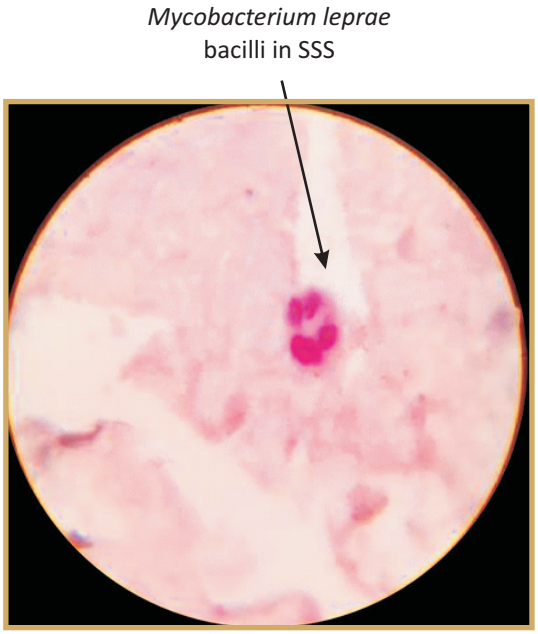

Fig 2 : Slit skin smear shows *Mycobacterium leprae* bacilli with BI-5^{+} [100x view AFB stain]

smear with paucibacillary and multibacillary leprosy ($p<0.05$), whereas no such association was observed with finding of biopsy ($p>0.05$).

Discussion

In our present study we found that most of the patients were found to be in the mean age of 40 years with M:F ratio of 5:2 which correlated with the other studies (Lucas & Ridley 1989, Bhushan et al 2008). Clinical correlation was carried out on all the cases. Out of 42 cases diagnosed histopathologicaly, in 28 cases clinical and histopathological correlation was established. The remaining 14 cases mostly belonging to paucibacillary type, no histopathological or clinical correlation was established.

Slit skin smears though specific have poor sensitivity. On the other side, skin biopsy has stood well in its sensitivity and AFB is better demonstrated in biopsies. In our study we evaluated whether additional information from BG would increase the diagnostic accuracy in identifying multibacillary and paucibacillary cases.

As biopsy is an invasive procedure and requires trained personnel. A biopsy specimen has to undergo fixing, grossing and staining, while slit skin smear though invasive need not to go through such cumbersome procedure. Microscopic examination of slit skin smear was earlier included in the World Health Organization (WHO) case definition (WHO 1988, Parkash 2009). It has been included again in the recent guidelines of WHO (2018) and also NLEP (website). It is thus the appropriate time to compare existing techniques for AFB staining for their application at clinical and public health levels.

In our study more patients were identified as multibacillary (as shown in Fig. 1 & 2) with BG (26% with AFB stain, 30.9% with modified rapid AFB stain & 33.33% with Fite Faraco stain) as compared to BI (21.4% with all three stain) which shows that slit skin smear had lower sensitivity for demonstrating AFB, the reason being as proposed by Bhushan et al (2008) could be the presence of bacilli in deep reticular dermis where they remain inaccessible to SSS and also bacilli are usually obscured by the excess blood in slit skin smears and thus affects the BI. To avoid this discrepancy procedure to prepare slit skin smears should be followed precisely so that false negativity could be avoided.

We have demonstrated that positive BG is also significantly seen in pauci lesional Borderline Tuberculoid cases which were slit skin smear negative. Shrinivas et al (2002) also reported few cases with AFB negative smears yet positive with biopsy. Our analysis reconfirmed earlier findings that in highly bacillated cases, SSS is quite sensitive but not in cases with low tissue density of AFB. Therefore, SSS has significant under diagnosis of paucibacillary cases. This highlights that skin smears taken to detect intradermal AFB have high specificity but low sensitivity. Though, skin smears are important in diagnosing most infectious patients and those at high risk of relapse (Kumaran et al 2015). Histopathological diagnosis, when available is considered as gold standard for diagnosis of leprosy.

In the course of our study it was observe that higher values of bacillary index in skin biopsies was seen as compared to Bacillary index in slit skin. Therefore, higher BG in skin biopsies indicates that BI in slit skin smear reflects density of bacilli in a given foci, while BG takes into account both the size of foci and bacterial density (Kumaran et al 2015) which was also observed by Ridley (1977) and Ridley & Jopling (1966) in his study.

Bhushan et al (2008) and Groren et al (1995) also reported few cases (11.63% & 15%) of BI positive but BG negative. A similar finding in our study was also seen in a single case of Borderline Lepromatous leprosy. The justification for this observation could be the improper biopsy technique or low bacillary load at the site from where biopsy was taken.

Demonstration of *Mycobacterium leprae* in the lesions of slit skin smears and skin biopsies with special stains is the method of diagnosis. The present study demonstrates that Fite Faraco showed a higher positivity rate in detecting the bacilli as compared to modified rapid AFB and AFB stain which correlated with the studies done by Bhatia et al (1987).

Also, in our present study AFB (Z-N) stain showed lower positivity compared to modified rapid AFB & Fite Faraco stain. Modified rapid AFB showed better positivity then AFB stain but lesser than Fite Faraco stain.

In our study the difference in rate of positivity between different stains in skin biopsy and slit skin smears was higher in Borderline Tuberculoid cases as compared to other studies done by Adiga et al (2016), which showed difference in rate of positivity was higher in indeterminate cases. This could be because of fewer indeterminate cases in our study.

Considering Fite Faraco (FF) method to be the standard test, we compared the performance of AFB (Z-N) stain and modified rapid AFB stain methods. In our study Fite Farco stain showed better positivity as compared to other stains as it was observed in one case of Indeterminate leprosy in which AFB and modified rapid AFB stain showed AFB negative with slit skin and skin biopsy but Fite Faraco stain was positive in skin biopsy

with BG 1+ in which bacilli could be seen around nerve which shows that Fite Faraco is a better stain for *Mycobacterium leprae bacilli* and has stood the test of time (DDG 2017).

Modified Rapid AFB stain in which combination of periodic acid and pararosanaline stain is used as a supplement showed better positivity than routine AFB stain in detecting AFB. It showed better BI and BG than AFB stain in pauci-lesional cases of Borderline Tuberculoid leprosy this highlights the superiority of Fite Faraco stain. This is variance with the findings of Nayak et al (2003) who observed better sensitivity with Modified rapid AFB as compared to AFB and Fite Faraco stain. The reason could be the technical errors during the procedure of staining such as sections being detached from slide or errors in decolorisation.

If we compare the BG in one case each of ENL and mid Borderline Leprosy, there was a shift of BG from 1^+ to 2^+ with Fite Faraco stain as compared AFB and modified rapid AFB stain. Hence this can shift a paucibacillary case to multibacillary category, which in turn has implications in therapy, prognosis, possibility of relapse and complications. Therefore, special stains which are used in detecting Lepra bacilli can play a very important role in classification of leprosy cases as paucibacillary or multibacillary cases.

Our study had limitations that false positive, false negative, true negative and specificity of various stains could not be calculated since, the data involve multiple variables. Hence, only true positive cases or sensitivity was represented. However, Kappa Statistics was applied to show the level of agreement between two methods. Further sampling bias of self reporting cases to a tertiary care centre may not represent the distribution at community level.

Conclusion

For effective management and control, diagnosis should be definite. Clinical features along with Bacterial index is useful in making accurate diagnosis so that appropriate treatment could be started and hence deformity and disability can be prevented. In our study Fite Faraco was found to be a better stain in detecting AFB as compared to Modified rapid AFB and conventional AFB staining. Multicentric community based studies will be required to draw firm conclusions and validate our findings for eventual application at public health level.

References

1. Adiga DSA, Hippargi SB, Rao G et al (2016). Evaluation of Fluorescent Staining for Diagnosis of Leprosy and its Impact on Grading of the Disease: Comparison with Conventional Staining. *J Clin Diagno Res*. **10(10)** : EC23–EC26.
2. Bancroft JD, Layton C, Suvarna SK (2013). Bancroft's Theory and Practice of histological techniques. (6^{th} edition)
3. Bhatia VN, Rao S, Saraswathi G (1987). Auramine staining in histopathological sections. *Indian J Lepr*. **59(4):** 386-389.
4. Bhushan P, Sardana K, Koranne R V et al (2008). Diagnosing multibacillary leprosy: A comparative evaluation of diagnostic accuracy of slit-skin smear, bacterial index of granuloma and WHO operational classification. *Indian J Dermatol Venereol Leprol*. **74**: 322-6.
5. Deutsche Dermatologische Gesellschaft (DDG) (2017). Leprosy - an overview of clinical features, diagnosis and treatment. *J German Soc Dermatol.* Vol ?? ISSN: 1610-038.
6. Groenen G, Saha NG, Rashid MA (1995). Classification of leprosy cases under field conditions in Bangladesh. I. Usefulness of skin-smear examinations. *Lepr Rev*. **66**: 126–33.
7. Kumaran SM, Bhat IP, Madhukara J et al (2015). Comparison of Bacillary Index on Slit Skin Smear with Bacillary Index of Granuloma in Leprosy and

its Relevance to Present Therapeutic Regimens. *Indian J Dermatol*. **60(1**): 51-54.

8. Lockwood DNJ (2002). Leprosy elimination - a virtual phenomenon or a reality?. *BMJ Clin Res Ed*. **324**: 1516–8.
9. Lucas SB, Ridley DS (1989). The use of histo-pathology in leprosy diagnosis and research. *Lepr Rev*. **60**: 257–62.
10. Nayak SV, Shivarudrappa AS, Nagarajappa AH et al (2003). Role of modified rapid AFB method in histopathological sections of Hansen's disease. *Indian J Dermatol Venereol Leprol.* **69**: 173-4.
11. National Leprosy Eradication Programme (NLEP), Directorate General of Health Services, Ministry of Health & Family Welfare, Govt of India (2019). Training Manual for health supervisors. (cltri.gov. in)
12. Prakash O (2009). Classification of leprosy into multibacillary and paucibacillary groups: an analysis. *FEMS Immunol Med Microbiol*. **55** : 1–5.
13. Gurung P, Gomes CM, Vernal S (2019). Diagnostic accuracy of tests of leprosy: A systematic review and meta-analysis. *Clin Microbiol Infect*. **25(11):** 1315-1327.
14. Rao PN, Suneetha S (2018). Current situation of leprosy in India and its future implications. *Indian Dermatol Online J*. **9(2):** 83–89.
15. Ridley DS, Jopling WH (1966). Classification of leprosy according to immunity: A five group system. *Int J Lepr Other Mycobact Dis*. **34**: 255-73.
16. Ridley DS (1977). Skin biopsy in leprosy. Histo-logical interpretation and clinical application. Documenta Geigy. Basle, Switzerland: CIBA-GEIGY Ltd.
17. Srinivas D, Rao PN, Lakshmi TS et al (2002). Bacterial index of granuloma and its relevance compared to BI of skin smears. *Lepr Rev*. **73**: 79-80.
18. Walker SL, Lockwood DN (2006). The clinical and immunological features of leprosy. *Br Med Bull*. **78**: 103-21.
19. World Health Organization (1988). WHO expert committee on Leprosy : 6th report. World Health Organization Technical Series Report. **768**.
20. World Health Organization (2018). Guidelines for the diagnosis, treatment and prevention of leprosy. WHO, Regional Office for South-East Asia. Available from https://apps.who.int/iris/handle/10665/274127.

How to cite this article : Kamle A, Awasthi P, Rawat N et al (2021). Clinicopathological Diagnosis of Leprosy: Comparative Evaluation of Three Staining Methods for Acid Fast bacilli in Slit Skin Smears and Biopsy Specimens. *Indian J Lepr.* **93**: 15-27.