Indian J Lepr 2020, 92 : 1-9

http://www.ijl.org.in

Original Article

A Comparative Evaluation of Acid Fast Bacilli Positivity by Slit Skin Smears, Bacterial Index of Granuloma in Paucibacillary and Multibacillary Leprosy Types as per WHO Operational Classification

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Received : 18.01.2018 Accepted : 17.10.2019

The diagnosis and classification of leprosy still remains a challenge. Less than desired specificity and sensitivity of operational WHO classification may result in administering inadequate/ over treatment in some cases. The aim of this study was to analyse Paucibacillary (PB) and Multibacillary (MB) classified by WHO operational criteria for positivity for acid fast bacilli (AFB) by Slit Skin Smears (SSS) and Bacterial Index of Granuloma (BIG) in the tissue sections from these leprosy cases using Job-Chacko modification of Fite Faraco (FF) staining and modified Ziehl Neelsen (ZN) staining. In case of 30 cases classified as PB by clinical criteria, AFB could be detected in 2/30 (6.6%) by SSS, 5/30 (16.7%) by BIG - modified ZN and 8/30 (26.67%) by BIF-FF. On the other in clinically MB patients, AFB positivity was 42/70 (60%) by SSS, 56/70 (80%) by BIG-modified ZN and 63 (90%) by BIG-FF. Clinical relevance of these findings needs to be determined by therapeutic outcomes of present regimens being used for these PB/MB types. The Inter-rater agreement (kappa) is 0.64. BIG by ZN stain showed no bacilli in 39 patients among which 10 patients showed bacilli in histopathology stained by FF stain. It may be worthwhile to consider adding estimation of BIG in the diagnostic work up of Paucibacillary (PB) cases classified by WHO clinical criteria and study its implications in terms of therapeutic outcomes. If found useful therapeutically, such services can be made available in Tertiary Care Institutions.

Key words : Leprosy, Hansen's Disease, WHO's Operational Classification, Slit Skin Smears (SSS), Bacterial Index of Granuloma (BIG)

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Introduction

Leprosy is a chronic disease caused by *Mycobacterium leprae* (*M. leprae*) affecting skin and peripheral nervous system and several others tissues (Jopling 1995). In the year 1873, a young Leprosy Medical Officer of Norway, Gerhardt Henrick Armauer Hansen discovered a type of rod shaped bacilli from lesions of leprosy patient (Trautman 1994) The bacterium discovered was later named as *M. leprae*. Leprosy is chronic granulomatous disease usually presents with hypopigmented skin lesion with or without loss of sensation and involvement of the feeding peripheral nerves (Park 2009). According to Ridley Jopling's classification leprosy has been divided into Tuberculoid (TT), Borderline Tuberculoid (BT), midborderline (BB), Borderline Lepromatous (BL) and Lepromatous Leprosy (LL) (Ridley & Jopling 1966). This classification was based primarily on immunological basis and then the correlation of clinical, bacteriological and histopathological findings (Ridley & Jopling 1966). The various diagnostic modalities available for leprosy are slit skin smear (SSS), histo-pathological examination and polymerase chain reaction (Banerjee et al 2011). Slit skin smear examination include demonstration and also semi-quantification of acid fast bacilli (AFB) by modified Ziehl Neelsen (ZN) stain method in smears prepared taking tissue fluid from skin lesion. AFB bacilli are found in slit skin smear in more numbers in case of lepromatous side of spectrum and LL pole (Charles & Joyce 2010). Slit skin smear examination (SSS) is outdoor based procedure thereby needs less time and is widely available, however, not widely used. SSS is done to calculate Bacterial Index (BI) (Charles & Joyce 2010) by Ridley or Dharmendra scales to monitor the bacillary load for monitoring the progress of disease under treatment. Histopathological examination using Haemotoxylin and Eosin (H&E) stain shows epitheloid granuloma and Langhans' giant cell in the TT and BT part of spectrum whereas diffuse macrophage infiltration with leprae cells or Virchow cell in case of LLpole (Lucas 2009).

Histopathological examination using ZN stain and Fite Faraco (FF) can demonstrate AFB bacilli in tissue sections (Job & Chacko 1986). ZN stain is more commonly done as it is readily available and is of low cost. Using this stain acid fast bacilli can be demonstrated and quantified in tissue section which is termed as Bacteriological Index of Granuloma - BIG (Job & Chacko 1986).

Although it is widely known that AFB are more frequently and abundantly found in skin biopsies compared to skin smears, few studies have been done to compare these readings. Ridley (1955) had observed higher values of BIG in skin biopsies compared to BI of slit skin smear in a study of patients of leprosy and he opined that the BI using SSS only reflect density of the bacilli in a foci, while BIG takes in to account both size of foci and bacterial density. In other words, BIG was more sensitive in assessing the bacterial status of the tissue specimen.

For field workers, WHO has classified leprosy based on number of skin lesions for treatment purpose (WHO 1998). This study has been carried out to find out the relative sensitivity of the bacteriological positivity by SSS vs. BIG using ZN and FF staining in PB and MB Hansen's Disease cases classified by WHO clinical criteria.

Materials and Methods

This institution based cross-sectional study was conducted in the Hansen's Clinic of Medical College, Kolkata, West Bengal. All new cases of leprosy, classified purely on clinical grounds (WHO 1998) attending the Hansen's Clinic over one year period (duration from June 2011 to May 2012), were included in the study after obtaining

their informed consent. The study was approved by Institutional Ethics Committee.

The inclusion criteria was based on the presence of any of the following (WHO 1998):

1. Hypopigmented or erythematous skin lesion(s) with definite loss/impairment of sensations.
2. Involvement of peripheral nerves, as demonstrated by definite thickening with sensory impairments.
3. Skin smears positive for acid fast bacilli.

Non consenting patients or any patient who had already received treatment/on treatment for leprosy, were excluded. After initial screening, thorough clinical evaluation was done, slit skin smear examination (from seven sites), and punch biopsy from anaesthetic or hypoaesthetic hypopigmented patch/erythematous plaques/ nodules were taken. In cases of Pure Neuritic Hansen's disease (IAL 1982), biopsy was taken from the dorsal aspect of 2nd finger of left hand. Slit skin smear (SSS) were taken from both ear lobules, nasal blow-out and four skin lesions; stained by modified Ziehl Neelsen (ZN) stain and Bacteriologial Index (BI) was calculated.

Tissue sections were stained by Haematoxylin and Eosin stain (H&E) to study the histopathological correlation with clinical diagnosis. For determining the Bacterial Index of Granuloma (BIG), the tissue sections were stained both by Job-Chacko modification of Fite Faraco (FF) stain (Job & Chacko 1986) and modified ZN stain (Charles & Joyce 2010).

Job-Chacko modification of Fite Faraco (FF) stain: Tissue sections were deparaffinized in a mixture of xylene and peanut oil (2 parts of xylene, 1 part of peanut oil), two changes of 6 minute each, then drained, wiped off the excess oil and blotted with filter paper and washed in running tap water for 4 minutes. Staining with Ziehl-Neelsen's Carbol Fuschin solution for 30 minutes at room temperature was done followed by washing in tap water for 2 minutes then differentiation of sections in 5% Sulfuric acid in 25% Alcohol for two changes of 2 minutes each was done again followed by washing in running tap water for 5 minutes. Harris Haematoxylin stain was used as counterstain. The excess water drained and dried with a filter paper and finally mounted. This staining method has two modifications from the standard FF stain for AFB. Firstly, use of alcohol was minimized to prevent excessive decolorization. Secondly, the counter staining was done using Hematoxylin instead of Methylene Blue. AFB stained red in colour and nuclei stained dark blue giving the localization of AFB in relation to the cells.

Modified ZN stain: After placing the slide on the staining rack, the whole slide was covered with Carbol Fuchsin and heated with a spirit lamp till it caused steam to rise from all parts of the slide, but boiling was avoided. The slide was left for 10 min without further heating. Then wash the slides gently in running water. The slides were decolourized by adding 5% of Sulphuric acid for 20 seconds or until the smear became light pink in colour and was again washed with gentle running water and counter stained with 0.2% Methylene Blue for about 1 minute and washed in running water and allowed to dry.

Bacterial Index of Granuloma (BIG) : BIG was used to demonstrate and quantify AFB in tissue section by both modified FF and modified ZN stains. BIG was assessed according to number of AFB found in tissue section seen under oil immersion field and the scoring was done as per Ridley's scale of bacillary positivity in granuloma (Job & Chacko 1986).

Statistical analysis: The descriptive statistics were expressed as frequency, percentage, mean ± standard deviation, and range. For analytical

statistics, the numerical data were analyzed by using unpaired t-test and categorical data were analyzed by using chi-square test. Medcalc 11.5.0 version was used for statistical analysis and p value $\leq$ 0.05 was considered significant.

Results

Among 106 leprosy patients attending the leprosy clinic during the study period, 2 did not give consent and 4 of them did not agree for skin biopsy. Thus 100 patients were included in the study.

Demographic and Disease profiles

Among the 100 recruited patients most had Borderline Tuberculoid (BT) leprosy (46%) followed by Borderline Lepromatous (BL, 21%), Lepromatous Leprosy (LL, 20%), Pure Neuritic (5%), Typical Tuberculoid (TT, 4%), Histoid (3%) and one case of midborderline (BB) leprosy. Males

Table 1 : Clinico-demographic profile of study population

Parameters	TT (n=4)	BT (n=46)	BB (n=1)	BL (n=21)	LL (n=20)	Histoid (n=3)	Pure neuritic (n=5)	Total (N=100)
Age of Onset								
Mean	40.5	33.93	40	34.47	28.15	34	38.4	32.6
±SD	±13.3	±11.85	40	±14.07	±16.61	±2.64	±5.85	±13.03
Median	35	32	0	28	21.5	35	41	30
Range	32-60	10-68		11-62	30-35	31-36	28-42	10-67
Age at Presentation								
Mean	41.5	33.93	41	34.47	28.15	34	38.4	33.49
±SD	±13.3	±11.84	41	±14.07	±16.61	±2.64	±5.85	±13.19
Median	36	32	0	28	21.5	35	41	31
Range	33-61	10-68		11-62	10-65	31-36	28-42	10-68
Sex								
Male:Female	2 : 2	31 : 15	1 : 0	16 : 5	19 : 1	2: 1	2: 3	73: 27
Residence								
Rural: Urban	3:1	29:17	0:1	16:5	9:11	2:1	1:4	60:40
Literacy								
Illiterate: Literate	1:4	15:31	0:1	12:9	7:13	0:3	2:3	37:63
Income group								
APL:BPL	2:2	20:26	1:0	7:14	9:11	2:1	3:2	44:56
Number of skin lesions								
No lesion	0	0	0	0	0	0	5	5
< 2lesion	4	5	0	0	0	0	5	9
2 -5lesion	0	16	0	0	0	0	0	16
>5 lesion	0	25	1	21	20	3	0	70
Number of Peripheral nerves involved								
<2 nerve trunk	4	22	0	0	0	3	1	30
$\geq$ 2 nerve trunk	0	26	1	21	20	0	4	70

(n=73) outnumbered female (n=27) in our study population. Male: Female ratio was found to be maximum for LL (19:1) followed by BL (3.2:1) and BT (2.06:1). The patients belonged to both rich (Above poverty level - APL, 56%) and poor (Below poverty level BPL, 44%) socio-economic status and were found to be mostly literate (63%). It was noted that majority of patients of study population were either student or involved in small scale business (36%) followed by manual labourers (35%) and home-makers (27%). Majority of Pure-Neuritic patients (80%) were manual labourers. All patients of LL, BL and Histoid presented with more than 5 skin lesions. Among BT patients 21% presented with 2-5 and 20% with more than 5 skin lesions. Majority of the patients had more than two nerves involvement (70%). (Table 1)

Majority of the patients (73%) were from districts with high Annual New Case Detection Rate (ANCDR) rate (Bankura, Purulia) and were mostly BL (80%) followed by BT (76%) and LL (65%) (Table 2). It needs to be highlighted that 11% of our patient population was from low ANCDR districts (NLEP Annual Report 2009-10).

Skin slit smears

SSS were negative for AFB in 56 patients and bacilli were seen in 44 patients. All patients of TT, Pure Neuritic were negative for bacilli, most of the BT patients were negative by SSS (86.96%) and 7 (33.33%) patients of BL were also negative but all patients of LL, BB and Histoid leprosy were showing bacilli. 2/30 (6.6%) of PB and 42/70% (60%) MB were positive for AFB by this SSS examination.

Histological examination

Grenz zone was seen in 44 (44%) cases in which 20 cases were of LL leprosy, 13 cases of BL leprosy and 3 cases of Histoid leprosy (p=<0.0001, Chi-square test). Dermal changes such as epithelioid granuloma was seen in 41% in which 39 cases were of BT leprosy, 2 cases of TT leprosy (p<0.0001, Chi-square test). Foamy cell granuloma was seen in 39% cases in which 20 cases were of LL leprosy, 16 were BL leprosy and 3 were Histoid leprosy (p<0.0001, Chi-square test). 20% were with well defined well formed granuloma, 17 of which were BT leprosy, 3 were of TT leprosy (p=0.0006, Chi-square test). Giant cells of Langhans type were seen in 20% cases in which 18 cases were of BT leprosy, 2 were of TT leprosy (p=0.0971, Chi-square test). Perineural infiltration was the next common infiltration with 42 (56%) cases in which 29 were of BT leprosy, 9 of TT leprosy, 2 of LL leprosy,1 of BL and Histoid leprosy each (p<0.0001, Chi-square test). The clinico histopathological correlation was seen in 81% of patients.

Table 2 : Residence of the study population in relation to ANCDR and Prevalence rate*

	TT (n=4)	BT (n=46)	BB (n=1)	BL (n=21)	LL (n=20)	Histoid (n=3)	Pure neuritic (n=5)	Total
ANCDR RATE (per 100,000 population)								
LOW < 10	1	4	0	3	1	0	2	11
MEDIUM 10 TO <20	0	7	1	1	6	0	1	16
HIGH 20 TO >30	3	35 (76%)	0	17 (80%)	13 (65%)	3	2	73

*NLEP Progress report 2009-2010

AFB positivity by SSS and BIG vis a vis WHO classification

In this study population, as per clinical criteria PB and MB types (WHO 1998) were 30 and 70 patients respectively. Acid fast positivity by SSS and positivity cum grading in the tissues by BIG using ZN / Fite Faraco stains in both clinically PB and MB cases were compared and shown in Fig. 1.

SSS demonstrated AFB in 2 (6.67%) of clinically PB patients and 42 (60%) of clinically MB patients (kappa 0.43, p value <0.0001). By BIG analysis AFB by ZN stain were seen in 5(16.67%) of clinically PB patients and 56 (80%) of clinically MB patients but it was negative in most (25/30) of clinically PB and 14 (20%) of clinically MB patients. (kappa is 0.58). AFB by FF stain were seen in 8(26.67%) of clinically PB patients and 63(90%) of clinically MB patients but it was negative in 22(73.33%) of clinically PB and 7 (10%) of clinically MB patients (kappa is 0.64).

Comparison of AFB positivity by SSS and BIG

Comparative positivity of AFB positivity by SSS and BIG is presented in Table 3. SSS showed no AFB in 56 patients among which 27 (48.21%) patients showed bacilli in histopathology stained by FF. SSS showed presence of bacilli in 44 patients which all showed AFB in tissue. Thus inter-rater agreement between SSS and BIG using FF was also found to be poor (kappa 0.48, p value <0.0001).

SSS showed no AFB in 56 patients among which 17 (30.35%) patient showed bacilli in histopathology stained by ZN stain. SSS showed presence of bacilli in 44 patients which all showed AFB in tissue. The inter-rater agreement between SSS and BIG using ZN was strong (kappa 0.63, p value <0.0001).

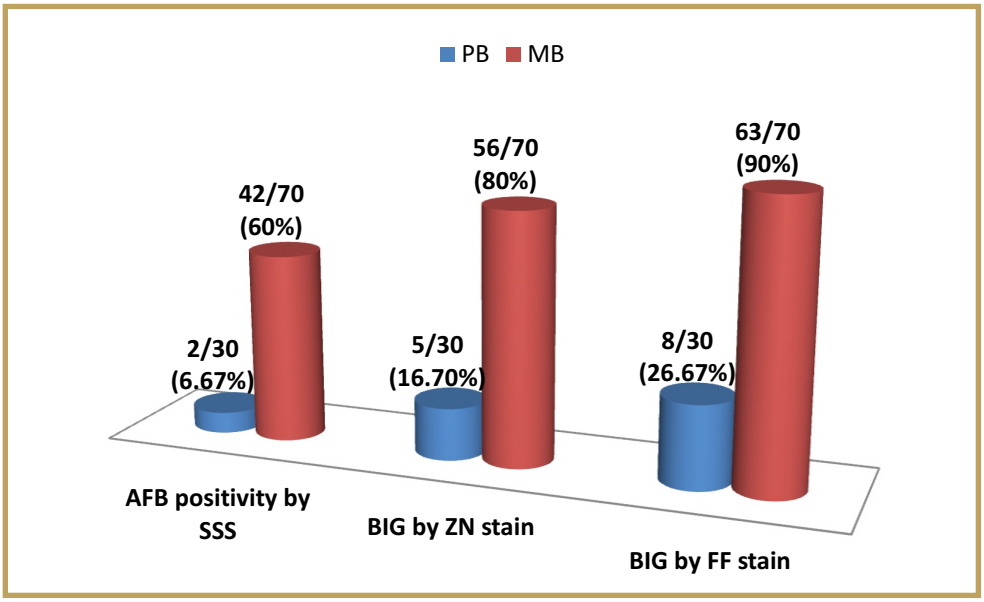

Fig 1 : Comparative performance of SSS and BIG by ZN & FF stain in clinically PB and MB types

Table 3 : Comparative performance of AFB positivity by BIG both by FF and ZN stain

	BIG by ZN(AFB absent) n = 39	BIG by ZN (AFB present) n = 61
BIG by FF(AFB absent) n=29	29	0
BIG by FF (AFB present) n=71	10	61

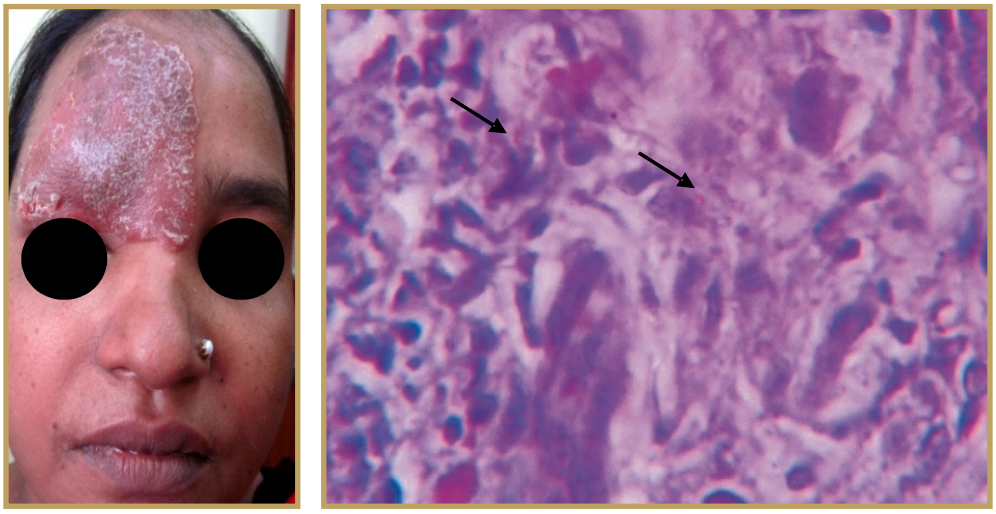

Fig 2 : Bacillary positivity in tissue by FF stain in a leprosy patient with single borderline tuberculoid plaque. (x100 immersion oil)

BIG by FF stain showed AFB in 71 cases among which were all cases of LL, Histoid, BB and 18 (85.71%) patients of BL. AFB could be demonstrated in majority of patients of BT (54.34%) and Pure Neuritic (80%). BIG by ZN stain showed AFB in 61 cases among which were all cases of LL, Histoid, BB and 17(80.95%) patients of BL. No cases of TT, Pure Neuritic and majority of patients of BT (56.52%) showed any bacilli in tissue. Detection of AFB by ZN stain showed no bacilli in 39 patients among which 10 (25.64%) patients showed bacilli in histopathology stained by FF stain. Bacillary positivity in tissue by FF stain in a leprosy patient with single borderline tuberculoid plaque showed its usefulness for demonstration of AFB in such cases (Fig. 2). Both stains ZN and FF showed presence of bacilli in 61 patients. Thus we found that detection and quantification of AFB positivity by BIG using two different methods (ZN and FF) showed strong inter-rater agreement (kappa 0.70, p value <0.0001).

Discussion

Leprosy is a chronic debilitating disease. Early diagnosis and treatment by MDT has contributed to the reduction in disease burden particularly the cases with disabilities. Thus early diagnosis

remains a priority. The diagnostic modalities include clinical examination, SSS, histopathological examination and polymerase chain reaction methods. The detection of acid fast bacilli *Mycobacterium leprae* either in smear or in tissue section(s) is a simple method and can play a major role in definitive diagnosis of leprosy in case of patients having suspicious lesions. If established to be of therapeutic value by well designed follow up studies, this may be useful in improving the easy to use clinical classification recommended by WHO and followed by our National Leprosy Eradication Programme (NLEP).

In our study among the 100 recruited cases, WHO operational classification identified 70% cases as MB as concordant to the results of Bhushan et al (2008) where 56 of 76 (73.68%) were MB patients.

In our study SSS identified 44 MB cases and BIG by FF stain revealed 71 MB cases and the association was significant ($p<0.0001$) compared to the results of Bhushan et al (2008). SSS showed no AFB in 56 patients among which 27 (48.21%) patients showed bacilli in histopathology stained by FF stain. SSS showed presence of bacilli in 44 patients which all showed AFB in tissue. The difference was statistically significant (p value <0.0001, Chi-square test). The Inter-rater agreement (kappa) is 0.48, these results were comparable to those reported by Srinivas et al (2002).

Detection of AFB by ZN stain showed no bacilli in 39 patients among which 10 patients showed bacilli in histopathology stained by FF stain as summarized in Table 3. Both stains ZN and FF showed presence of bacilli in 61 patients. The difference was statistically significant (p value <0.0001, Chi-square test). The Inter-rater agreement (kappa) is 0.70. BIG by FF stain and BIG by ZN stain were 5-6+ in 34 patients and for 37 of BIG by FF stain 1-4+ only 27 (75.67%) were positive by BIG by ZN stain. The above results denotes that there was no such difference of the diagnostic efficacy of ZN and FF stain in highly bacillated cases but the difference may be important in cases with single or few lesions (Fig. 2) and in Pure Neuritic leprosy cases. BIG by FF stain was more effective in these cases.

The poor inter-rater agreement between SSS by modified ZN and clinical classification (kappa 0.43) suggest that modified ZN may not be a sensitive tool for determination of bacillary load. Similarly poor correlation was found between SSS and BIG by modified FF (kappa 0.48), with SSS capable of detecting AFB in 56.58% whereas and BIG by modified FF detected AFB in 85.53%; highlighting that SSS by modified ZN can be replaced by BIG by FF for improving the clinical classification after carefully conducted studies on such classification(s) versus therapeutic outcomes. This finding is further corroborated by the strong correlation between BIG using FF and clinical classification (kappa 0.64).

Our study thus shows that correlation of clinical and histopathological features along with bacteriological examination of tissue specimens appears to be more useful for accurate typing of leprosy than considering any one of the single parameters alone. It shows AFB can be demonstrated in the biopsy specimens of many Hansen's Disease patients which were otherwise negative by SSS, hence it can be well concluded that the probability of detecting AFB in biopsy specimen stained by ZN and FF stain is more than that of detection by SSS. However, the significance of finding of AFB in histopathology of patients grouped as PB should be determined by analyzing their response to therapy (PB/MB regimens) so that the patients could be treated with proper drug regimen as it might prevent under treatment and further complications. If found useful therapeutically, such services can be made available in tertiary care institutions.

References

1. Banerjee S, Biswas N, Das NK et al (2011). Diagnosing leprosy: revisiting the role of slit skin smear with critical analysis of applicability of polymerase chain reaction (PCR) as diagnostic tool. *Int J Dermatol.* **50**: 1522-7.
2. Bhushan P, Sardana K, Koranne RV 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.
3. Charles K J, Joyce P (2010). Laboratory Diagnosis. In: IAL Textbook of Leprosy, (Kar KH, Kumar B eds.). Jaypee Brothers Medical Publishers, New Delhi, pp.176-96.
4. Indian Association of Leprologists (1982). Clinical, histopathological and immunological features of the five type classification approved by the Indian Association of Leprologists. *Lepr India.* **54**: 22-32.
5. Jopling WH (1995). Definition, Epidemiology and World Distribution. In: Handbook of Leprosy, 5th Ed., (Jopling WH, McDougall AC Eds.), CBS Publishers, New Delhi, pp.1-9.
6. Job CK, Chacko CJG (1986). A modification of Fite's stain for demonstration of *M. leprae* in tissue section. *Indian J Lepr.* **58**: 17-8.
7. Lucas S (2009). Bacterial Diseases. In: Lever's histopathology of the skin, 10th Edition (Elder ED, Ed.). Lippincot Williams and Wilkins, New Delhi, pp. 560-5.
8. NLEP Progress report for the year 2009-2010 ending on 31 March 2010. Central leprosy division, Directorate General of Health Services Government of India, New Delhi.
9. Park K (2009). Epidemiology of communicable diseases. In: Park's Text Book of Preventive and Social Medicine, 20th ed., (Park K Ed.), Banarsidas Bhanot Publishers, Jabalpur, pp.275-88.
10. Ridley DS (1955). The bacteriological interpretation of skin smears and biopsies in leprosy. *Trans R Soc Trop Med Hyg.* **49**: 449-52.
11. Ridley DS, Jopling WH (1966). Classification of leprosy according to immunity: A five group system. *Int J Lepr Other Mycobact Dis.* **34**: 255-73.
12. 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.
13. Trautman JR (1994). The history of leprosy. In: Leprosy, 2nd ed., (Hastings RC Ed.), Churchill Livingstone, New York, pp.11-25.
14. World Health Organization (1998). Expert Committee on Leprosy. 7th Report. Techn Rep Ser.874, World Health Organization, Geneva.

How to cite this article : Roy S, Patra AC, Bandhyopadhyay D et al (2019). A Comparative Evaluation of Acid Fast Bacilli Positivity by Slit Skin Smears, Bacterial Index of Granuloma in Paucibacillary and Multibacillary Leprosy Types as per WHO Operational Classification. *Indian J Lepr.* **92**: 1-9.