INDIAN J DERMATOL VENEREOL LEPROL 1989; 55 : 370-372

BACILLEMIA AND ITS RELATIONSHIP WITH BACTERIOLOGICAL INDEX IN LEPROSY

C N Parvathi, Srikara Mallya, J N Shetty and C N Nagesha

Detection of bacillemia in 40 untreated cases of leprosy was carried out by buffy coat, haemolysis and Petroff's concentration methods. Bacillemia was detected in 17 (42.5%) cases by Petroff's method. Out of 20 LL-BL cases, it was positive in 16 (80%) patients. Petroff's and haemolysis methods revealed bacillemia in 100% and 90% of LL cases respectively The Petroff's method of concentration was found superior over the other techniques for better detection and quantitation of bacillemia. A significant relationship between the bacillary load (BI) in the skin and the degree of bacillemia was observed, especially in the lepromatous part of the spectrum of the disease.

Key words : Bacillemia and BI, Leprosy.

Fite[1] observed leprosy lesions in blood vessels and suggested continuous discharge of bacilli into the circulation. Lowe[2] seems to be the first worker to observe bacillemia in leprosy, and recently many workers[3-7] have demonstrated bacillemia in various clinical types of leprosy, especially in the lepromatous spectrum of the disease. Bacillemia has been detected by different concentration methods.[3-5] In the present communication, an attempt has been made to determine a relationship between the degree of bacillemia and the bacteriological index in the different clinical types of leprosy.

Materials and Methods

Forty untreated cases of leprosy consisting of 10 LL, 10 BL, 10 BT and 10 TT cases were investigated. Skin smears were taken from 5 sites (2 ear-lobes and 3 skin lesions) for bacteriological index (BI). Skin tests with dinitrochlorobenzene (DNCB) and lepromin were performed for grading the cell-mediated immunity (CMI), to classify the leprosy patients according to Ridley-Jopling[8] clinical scale.

From the Departments of Microbiology and Skin and STD, Kasturba Medical College, Mangalore-575001, India.

Address correspondence to : C N Nagesha, Department of Microbiology, Dr B R Ambadkar, Medical College, Bangalore-560045, India.

To detect and quantitate bacillemia, 10 ml blood was collected from the median cubital vein by double syringe technique in a heparinised (10 iu/ml blood) container to minimise skin contamination of blood samples with *M. leprae*. Concentration methods used were buffy coat,[3] haemolysis[5] and modified Petroff's methods. The bacillary load/ml blood was determined by the haemolysis and Petroff's methods, while the crude buffy coat technique was used only for detection of bacillemia. In the buffy coat method aliquots of 1 ml blood were centrifuged in Wintrobe haematocrit tube at 3000 rpm for 30 minutes and smears of the leukocyte layer were stained with ZN method and scanned for 30 minutes for detection of acid fast bacilli.

Counting of AFB was done by the haemolysis and Petroff's methods, detailed as follows : Aliquot of 5 ml blood was transferred to a conical centrifuge tube containing 10 ml sterile distilled water and the mixture was frequently stirred using a sterile glass rod for 30 minutes to lyse the red cells and disrupt the white cells. The sample thus lysed was centrifuged at 3000 rpm for 30 minutes. The supernatant was discarded, 15 ml distilled water was added to the sediment, stirred well with the glass rod and centrifuged again as before. The process was repeated until the supernatant was clear. The

final deposit was suspended in 0.1 ml (100 μl) saline and 2 smears each of 10 μl were prepared from 0.02 ml saline suspension for counting the AFB. The number of AFB counted in 2 ZN smears gives the bacillary count/ml blood.

In the modified Petroff's method of concentration employed by us, 0.5 ml 1% NaOH was added to the remaining 0.08 ml saline suspension obtained by the haemolysis technique and homogenised for 5 minutes at 37°C. The material thus processed was centrifuged as before and the deposit was washed twice with 5 ml distilled water. The recentrifuged sediment was suspended in 0.08 saline and 2 smears of 10 μl each were prepared. The bacilli counted in 2 ZN smears would indicate the number of bacilli/ml blood.

Results

Table I reveals comparison of the 3 concentration methods for detection of bacillemia in various clinical types of leprosy. Out of 20 cases of leprosy in the lepromatous part of the spectrum (LL-BL), bacillemia was detected in 16 (80%) cases by the Petroff's method, 13 (65%) cases by the haemolysis method and 11 (55%) cases by the buffy coat method. Bacillemia was detected in 1 (5%) of 20 cases in the tuberculoid spectrum (BT-TT) of infection by the Petroff's method only.

Table II reveals the relationship between the BI and the degree of bacillemia. All 10 LL cases with BI 5-6 showed bacillary counts in the blood in the range of 2415 ± 472 and 1752 ± 123 by Petroff and haemolysis methods respectively. Out of 10 BL cases with BI 3-4, 6 cases revealed bacillemia with the bacillary counts of 1382 ± 93 and 1204 ± 56 by these techniques. Of 10 BT cases, 1 case with BI 2 showed 412 bacilli/ml blood by the Petroff's method only.

Comments

Bacillemia in LL cases by the buffy coat method has been reported to be 81.8%,[3] 100%[5] and 16%[9] compared to our results of 55%. Various studies[5-7] with the haemolysis method have revealed positivity rates of 52%, 85.7% and

Table I. Comparison of the 3 methods for detection of bacillemia in different clinical types of leprosy.

Type of leprosy	Number of cases	Number (%) of cases showing bacillemia by		
		Buffy coat	Haemolysis	Petroff
LL	10	8 (80)	9 (90)	10 (100)
BL	10	3 (30)	4 (40)	6 (60)
BT	10	0	0	1 (10)
TT	10	0	0	0

Table II. Degree of bacillemia by the haemolysis and Petroff's methods in relation to BI of skin smears.

Type of leprosy	Number of cases studied	Number of cases showing bacillemia	BI	Bacillary count/ml blood	
				Haemolysis Mean $\pm$ SD	Petroff's Mean $\pm$ SD
LL	10	10	5—6	1752 ± 123	2415 ± 472
BL	10	6	3—4	1204 ± 56	1382 ± 93
BT	10	1	2	0	412
TT	10	0	2	0	0

100% respectively compared to 65% in our study. Significantly, Petroff's method of concentration in the present investigation has shown bacillemia in 100% of LL cases thus confirming its superiority over other concentration methods for detection and quantitation of bacillemia.

Some workers[4-6] have reported correlation between a high bacterial index and bacillary load of blood, while others[10-11] have not observed any relationship between BI and the degree of bacillemia. In the present study, the results clearly indicate that the degree of bacillary load of the skin definitely influences and bears relationship with the degree of bacillemia.

One BT case with consequent bacillemia detected by the Petroff's method, revealed positivity in the smears from 3 skin lesions with a mean BI of 2. There were 8 dry, macular skin lesions of varying size and shape, asymmetrical in distribution, with well-defined edges in some parts and ill-defined in some areas. There was impairment of hair growth with markedly diminished sensation in all the lesions and lost in some parts of 3 lesions.

Acknowledgements

We are grateful to Dr K V Desikan, Director, Central JALMA Institute for Leprosy for kindly supplying the lepromin.

References

1. Fite GL : The vascular lesions in leprosy, Internat J Leprosy, 1941; 9 : 193-202.
2. Lowe J : Bacillemia in leprosy, Ind Med Gaz, 1933; 68 : 503-506.
3. Drutz DJ, Chen TSN and Lu WH : The continuous bacillemia of lepromatous leprosy, New Eng J Med, 1972; 287 : 159-164.
4. Shankara Manja KS, Bedi BMS Kasturi G et al : Demonstration of *M. leprae* and its viability in peripheral blood of leprosy patients, Leprosy Rev, 1972; 43 : 181-187.
5. Sreevatsa, Sengupta U, Ramu G et al : Evaluation of bacteraemia in leprosy patients, Leprosy India, 1978; 50 : 381-387.
6. Raval Saibhaktiben N, Sengupta U, Ramu G et al : A study of continuous bacillemia in borderline and lepromatous type of leprosy, Leprosy India, 1982; 54 : 623-632.
7. Sengupta U, Ramu G and Desikan KV : Further modification of the haemolysis method in determining bacteraemia in leprosy patients, Leprosy India, 1983; 55 : 670-674.
8. Ridley DS and Jopling WH : Classification of leprosy according to immunity. A five group system, Internat J Leprosy, 1966; 34 : 255-273.
9. Ganapati R and Chulawala RG : Bacteraemia in leprosy and its relation to distribution of *M. leprae* in skin, Leprosy India, 1976; 48 : 42-47.
10. Padma MN and Desikan KV : Bacillemia in leprosy, Ind J Med Res, 1975; 63 : 888-895.
11. Balvinder K and Handa F : Correlation of bacillemia with clinical types of leprosy, Ind J Dermatol Venereol Leprol, 1986; 52 : 272-274.