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A concentration method for detection and quantitation of bacillaemia in leprosy and its comparison with other techniques

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Summary Detection and quantitation of bacillaemia in 50 untreated cases of leprosy were evaluated by the buffy coat method, the haemolysis method and the present Petroff's method. Bacillaemia was detected in 29 (58%) out of 50 cases and in 32 LL–BL cases it was detected in 28 patients with a success rate of 87·5%. Both the haemolysis method and Petroff's method were found useful in estimating the bacillary load per millilitre of blood. Importantly, the smears of concentrated deposit obtained by Petroff's method revealed only AFB free from any artefacts and also yielded high bacterial counts. In conclusion, Petroff's method of concentration was found superior over other methods for detection and quantitation of bacillaemia in the lepromatous spectrum (LL–BL) of the disease.

Introduction

Lowe[1] appears to be the first worker to observe bacillaemia in leprosy. Subsequently many workers[2–6] have demonstrated bacillaemia in various types of leprosy, especially in the lepromatous spectrum of the disease. Fite[7] has found leprosy lesions of blood vessels and suggested a continuous shedding of bacilli into circulation. The bacilli have been found in the endothelial cells of blood vessels in the skin and subcutaneous veins of leprosy patients.[8–10] Bacillaemia has been detected by various concentration methods.[2–4]

In the present communication, we have used Petroff's method of concentration for detection as quantitation of bacillaemia in different clinical types of leprosy and the results are compared with those obtained by other concentration methods to evaluate the usefulness of our technique in determining the bacillary load in the blood of a leprosy patient.

Material and methods

Fifty untreated cases of leprosy consisting of 22 LL, 10 BL, 10 BT and 8 TT patients were investigated. Skin smears from 5 different sites were taken for BI and skin tests using DNCB and

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lepromin were done for cell-mediated immunity (CMI) to select only such cases which were definite and unequivocally classifiable according to the Ridley–Jopling clinical scale.[11]

Specimens of venous blood were drawn twice (double syringe technique[2]) at the antecubital fossa where the lepra bacilli are sparse in the skin with negligible contamination of specimens. The first 8 ml of blood was collected in a plain sterile bottle and the serum separated was used for the estimation of serum proteins and immunoglobulins. The second 13 ml specimen was obtained in a fresh syringe and collected in a heparinized container (10 IU/ml blood) for detection and quantitation of bacillaemia. Alliquots of 3 ml, 5 ml and 5 ml of heparinized blood were used for the concentration of AFB by buffy coat[2] haemolysis[4] and the present modified Petroff's technique respectively.

Since Petroff's method of concentration for AFB in the sputum and other exudates using 4% NaOH is a well known technique, we have used this method with slight modification. In the modified Petroff's method of concentration, the sediment obtained from 5 ml of blood by haemolysis technique was homogenized with 0·5 ml 1% NaOH for 5 min, mixed with 10 ml distilled water and centrifuged at 3000 rpm for 30 min. The concentrated deposit thus obtained was suspended in 0·1 ml (100 μl) saline. In each specimen, 2 smears of 10 μl were prepared from saline suspension obtained by the haemolysis method and modified Petroff's method separately. Bacilli counted in 2 ZN stained smears (20 μl) would indicate the number of bacilli/ml of blood as calculated by $5 \times B/5$, where numerator 5 represents 100 μl (ie 5×20 μl) saline suspension of the deposit, B total number of bacilli in 2 smears (2×10 μl) and denominator 5 represents 5 ml of blood used for concentration.

Results

The data of this study are presented in Tables 1 and 2. Table 1 shows the comparison of 3 concentration methods for detection of bacillaemia in different clinical types of leprosy revealing positive skin smears. Bacillaemia was frequently detected in the lepromatous spectrum of the disease, especially in clinically LL cases. Out of 22 LL cases with positive skin smears, bacillaemia was demonstrated in all patients by Petroff's method, in 20 patients by the haemolysis method and in 17 patients by the buffy coat method achieving a success rate of 100%, 91% and 77·3% respectively. In 10 BL cases with positive skin smears, bacillaemia was detected in 6 cases, 4 cases and 3 cases by the respective methods thus revealing detection rate of 60%, 40% and 30% in that order. All the 3 concentration methods did not help in the detection of bacillaemia across the tuberculoid spectrum of the disease and only in 1 out of 10 BT cases was bacillaemia demonstrated by Petroff's method.

Out of 32 cases of leprosy in the lepromatous spectrum of the disease, bacillaemia was seen in 28 cases (87·5%) by Petroff's method, 24 cases (75%) by the haemolysis method and 20 cases (62·5%)

Table 1. Detection of bacillaemia by different concentration methods in various clinical types of leprosy

Type of leprosy	No. of cases	No. of cases showing positive skin smears	Detection of bacillaemia by: Buffy coat No. (%)	Haemolysis No. (%)	Petroff's No. (%)
LL	22	22	17 (77·3)	20 (91)	22 (100)
BL	10	10	3 (30)	4 (40)	6 (60)
BT	10	2	0	0	1 (10)
TT	8	0	0	0	0

Table 2. Bacillaemia detected by concentration methods in the lepromatous and tuberculoid spectrums of the disease

Disease spectrum	No. of cases	No. of cases with positive skin smears	Detection of bacillaemia by		
			Buffy coat No. (%)	Haemolysis No. (%)	Petroff's No. (%)
Lepromatous (22 LL + 10 BL)	32	32	20 (62·5)	24 (75)	28 (87·5)
Tuberculoid (10 BT + 8 TT)	18	2	0	0	1 (5·5)

Table 3. Bacillary load by the haemolysis method and Petroff's method

Type of leprosy	No. of cases	No. of cases with bacillaemia	Bacillary load/ml blood by	
			Haemolysis method Mean ± SD	Petroff's method Mean ± SD
LL	22	22	1955 ± 150	2550 ± 585
BL	10	6	1308 ± 75	1415 ± 95
BT	10	1	0	560
TT	8	0	0	0

by the buffy coat method (Table 2). Bacillaemia was detected in 1 (5·5%) out of 18 cases in the tuberculoid spectrum of the disease only by the Petroff's method.

Table 3 shows the bacillary load/ml blood as revealed by the haemolysis method and Petroff's method, which were useful to make bacterial counts in the smears of concentrated deposits. Bacterial counts of 2550 ± 585 by Petroff's method as against counts of 1955 ± 150 by the haemolysis method per ml blood were observed in 22 LL cases with bacillaemia. Out of 6 BL cases with bacillaemia, bacillary load of 1415 ± 95 and 1308 ± 75/ml blood was observed by Petroff's and haemolysis methods respectively, whereas bacterial counts of 560 and 0/ml blood were recorded by the respective methods in 1 BT case associated with bacillaemia.

Discussion

In the present study bacillaemia was observed in 29 out of 50 cases of leprosy with a detection rate of 58%. On the other hand the positivity rate of bacillaemia was 85·5% (28 cases) in the lepromatous spectrum of 32 LL–BL cases. Bacillaemia in LL cases by the buffy coat method has been reported by different workers[2,4,9] with success rates of 81·8%, 100%, and 16%, respectively as against our results of 77·3%. Various studies[4-6] with the haemolysis method have shown a positivity rate of 52%, 85·7% and 100% in that order as compared to 91% success achieved in the present study. Importantly, the present work has shown bacillaemia in 100% of LL cases by Petroff's method.

A success rate of 42·8% and 55·5% by the haemolysis method was observed by some workers[4,6] in demonstrating bacillaemia in BL to BT spectrum of the disease. A positivity rate of 60% by Petroff's method was observed in BL cases in our hands. These findings have clearly revealed the superiority of Petroff's method over other methods in the detection of bacillaemia in the lepromatous (LL & BL) spectrum. None of the 3 concentration methods were helpful in demonstrating AFB in the blood of TT cases.

Both the haemolysis method and Petroff's method could be used to assess the bacillary load per millilitre of blood. The smears of concentrated deposit obtained by Petroff's technique revealed only AFB free from artefacts as against the AFB smears of deposit by the haemolysis method. On the other hand, Petroff's method yielded high bacterial counts when compared with the haemolysis method.

In the present study Petroff's methods proved superior to other methods for demonstration and quantitation of bacillaemia in lepromatous spectrum (LL & BL) of the disease, especially in BL cases.

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