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BCG immunotherapy as an adjunct to chemotherapy in BL-LL patients – its effect on clinical regression, reaction severity, nerve function, lepromin conversion, bacterial/antigen clearance and 'persister' *M. leprae*

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Summary

Background and Objective: Multidrug therapy in leprosy has failed to eliminate the problem of persister bacilli. Clearance of bacterial antigens is extremely slow which could predispose to continued nerve damage even after release from treatment. In the present study the immunomodulatory efficacy of BCG vaccine administered post-MDT in BL-LL leprosy patients was investigated in depth with a view to determining if augmenting chemotherapy with immunotherapy would help in faster clearance of *M. leprae*/antigens, bring down the level of persisters and minimise the occurrence/severity of reaction and nerve damage.

Methods: This is a placebo-controlled study in treated BL-LL patients. The patients are matched with respect to age, sex, bacteriological index and history of reaction, stratified and allocated to the two groups. One group (Gr A) received two doses of BCG-MOSCOW (3-33x10^5 cells) and the other (Gr B) normal saline (0·85%), injected intra-dermally at 3 month intervals. The Primary outcomes assessed at the end of 6 months were bacterial/antigen clearance, lepromin conversion, granuloma clearance and the occurrence of persisters. The secondary outcomes were clinical regression, occurrence and severity of reaction and changes in nerve functions.

Material: A total of 107 BL-LL patients comprised of 49 in Gr A and 58 in Gr B; of which 36 and 42 respectively completed the study as per protocol, and are included in the final analysis.

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Findings: The study findings show that both the primary and the secondary out comes were comparable in the two groups. Two doses of BCG administered post-MDT (Gr A) did not significantly alter the level of persisters or help in hastening the bacterial/antigen clearance, clinical regression of lesions and granuloma clearance. Lepromin conversion rates were also comparable. While the frequency of lepra reaction/neuritis following the intervention was comparable, the severity of reactions was significantly higher in Gr A. On the positive side neural functions assessed by nerve conduction studies showed that deterioration of motor nerve conduction was significantly lower in the BCG arm. Since all patients developing moderate to severe reactions, immediately received a course of corticosteroids, it is possible that timely use of it might have helped.

Introduction

The immune system plays an important role in protection, susceptibility and the clinical manifestations of leprosy. Epidemiological studies have shown that while most individuals are resistant to leprosy, those who suffer from the disease exhibit specific immunological deficits.[1]

Currently, Bacillus Calmette-Guerin (BCG) vaccination given soon after birth or in childhood is thought to be responsible, in part, for the decline in leprosy incidence observed in several populations. Data from Malawi,[2] South India[3] and Brazil[4] show significant protection afforded by BCG to the later development of Multibacillary (MB) (BL-LL) as well as Pauci-bacillary (PB) (BT-TT) forms of the disease. However, meta-analysis indicated that the effect of BCG in prevention does not exceed 26% for leprosy compared to 50% protection against tuberculosis.[5,6]

Many candidate mycobacterial vaccines against leprosy viz, BCG, *Mycobacterium welchii* (Mw), *M ICRC, Mycobacterium vaccae* have been injected singly or in combination as experimental immunotherapeutic agents. When administered during the initial stage of chemotherapy, success has been claimed in terms of lepromin conversion and accelerated rate of decline in bacteriological index (BI).[7–11] It has been the experience of clinicians that combination therapy [immunotherapy and chemotherapy] effects changes in Bacteriological Index (BI), Morphological Index (MI), lepromin conversion, and reversal reactions for extended periods after vaccination.[9–11] A combination of heat-killed *M.leprae* and BCG was reported to activate granuloma formation and accelerate bacillary clearance in skin biopsies.[12] However, epidemiological studies reported little beneficial effect of a combination of *M. leprae* with BCG over BCG alone.[13]

Evidence suggests that the immunotherapeutic approach may need to be combined (not necessarily simultaneously) with Multi Drug Therapy (MDT) to produce synergistic anti-leprosy immunity, in analogy with the anti-Schistosome drug Praziquantel.[14]

In leprosy after release from treatment (RFT) it takes from 5 to 10 years for relapse to manifest, suggesting that more fully treated cases may relapse over a longer period of time post-MDT.[15,16] Augmenting chemotherapy with immunotherapy to determine whether the clearance of antigens was enhanced and the level of persister bacilli reduced would appear to be a reasonable approach. A preliminary study of BCG immunotherapy in MB cases along side MDT, hinted at a possible role of the vaccine in enhancing bacterial killing and bacterial clearance.[12] Other than this study there is scant information on the effect of such

immunomodulation on 'persister' organisms which are implicated in relapse and incomplete antigen clearance implicated in continuing nerve damage in leprosy.

The working hypothesis for the current study is that BCG used as an adjunct to MDT boosts cellular immunity, thereby hastening antigen clearance, reduces the population of 'persister' bacilli, thus reducing the chance of relapse and further nerve damage.

Materials and Methods

STUDY POPULATION AND INCLUSION CRITERIA

Only BL–LL patients in the age group 15-60 years, residing within and around Mumbai, released from WHO-MB-MDT regime. RFT duration of 6 to 8 months between the termination of MDT treatment and the initial evaluation of the patient for the trial was maintained.

EXCLUSION CRITERIA

1) Pregnancy or breast feeding; 2) Smear negative and lepromin positive [but smear negative and lepromin negative were included]; 3) History of pulmonary tuberculosis, and/or other systemic conditions such as cancer, diabetes, hypertension, renal, hepatic or cardiac diseases; 4) HIV positivity or symptoms suggestive of HIV infection such as recurrent infection.

SAMPLE SIZE AND RECRUITMENT

As per the THELEP study report regardless of the treatment regimen employed, around 10% of MB cases carry persister bacilli. To achieve 95% power of demonstrating a statistically significant difference with and without usage of BCG at the 1% level, required the recruitment of 100 patients each in groups A and B.[17] WHO-MB-MDT treated BL-LL patients, having RFT duration of 6 to 8 months were broadly matched with respect to age, sex, bacteriological index (BI) and history of reaction to adjust for any confounders or effect modifiers, were allocated into two groups (A & B) as follows.

INTERVENTION

BCG-MOSCOW containing 3 to 33 $x10^5$ cells in 0·1 mg (gifted by The Serum Institute of India, Pune) was injected intra-dermally in 0·1 ml volume in the right deltoid region at the onset of the study followed by a second dose after three months in the left deltoid region (Group A). The BCG-MOSCOW was chosen amongst other BCG strains, for its protective efficacy against TB was 83·85% as against BCG GUINDY 80·39% in trials carried out in New Delhi and Bangalore (personal communication Dr. S. S Jadhav, then the Medical Director, Serum institute). Similarly, patients in the placebo arm (Group B) received two doses of sterile normal saline (0·85%).

The criteria for discontinuation of the intervention were: anaphylactic reaction, exacerbating neuritis, severe lymphadenitis, persistent fever, splenomegaly, sever allergy, and hepatitis.

The primary outcomes assessed and compared in the two groups at two time points i.e. before the intervention (0m) and between 3 to 6 months after the second intervention were bacterial/antigen clearance, lepromin conversion, granuloma clearance and the occurrence of persisters.

Clearance of *M. leprae* scored through slit skin smear and antigens detected using *M. leprae* cross-reactive anti-BCG antibody. Granuloma index assessed through histopathology (through biopsy of one of the active lesion) and occurrence of viable bacteria assessed through growth in the foot pads of non-immunosuppressed Swiss White (S/W) mice. Lepromin conversion assessed through conversion of Mitsuda lepromin late reaction.

The secondary outcomes assessed were the host response in terms of Clinical regression of lesions, occurrence of lepra reaction, its type, and severity, Changes in deformity grades (using WHO grades), changes in nerve function assessed using electrophysiological methods The differences between the two groups were assessed using criteria as described previously.[18]

CLINICAL EXAMINATION

A detailed clinical examination was undertaken to record the patches, disability and its grading and presence of previous BCG vaccination scar, recorded by examination of the upper left arm for the presence of scar.

Clinical examinations were conducted at 3 monthly intervals for semi-quantitative assessment clinical regression, nerve function impairments using graded monofilament.

Occurrence and type of reaction both before and after the intervention and severity of reaction was recorded and graded as follows. The grade mentioned below is applied to both Type 1 (T1R) and Type 2 (T2R) reaction.

Mild: When there were one to five reaction skin lesions without neuritis (nerve pain) and /or no sensory and/or no motor impairment.

Moderate: If there were > five to ten reaction skin lesions with one or more tender nerves (mild neuritis) and/or sensory and/or motor impairment.

Severe: If one or more of the following symptoms were present, > 10 reaction skin lesions, or ulcerating skin lesions, sensory or motor impairment, edema impairing face or limb function, nerve tenderness/pain on gentle palpation, paraesthesia or pain disturbing sleep or impairing function or involvement of the organs such as eyes, joints, testis, etc (in case of T2R).

TREATMENT FOR REACTION

Any patient diagnosed of having severe or moderate reaction/neuritis during the course of the study were treated with corticosteroids (Prednisolone – 40 mg daily tapered to 5 mg daily over 12 weeks i.e. 40 mg, 30 mg, 20 mg, 15 mg, 10 mg, 5 mg for 2 weeks each (daily dose).[19] Patients with mild reaction, as far as possible, were managed with a non steroidal drug (Aspirin).

SLIT SKIN SMEAR (SSS)

These were obtained, using scalpel blade no. 11, from two-three sites at the onset of the study and at 6 months following the first intervention. Smears on glass slides were heat-fixed and stained by Ziehl-Neelsen's method and graded for bacteriological index (BI), using the Ridley-Jopling scale (0–6 +).[20]

NERVE FUNCTION ASSESSMENTS

Touch sensibility was tested with a standard set of five coloured Semmes-Weinstein monofilaments (MF). Voluntary muscle testing was done using the modified Medical Research Council (MRC) scale.[21]

Sensory charting for nerve involvement was done as per the standard protocol. Grading of nerve damage was performed using accepted semi- quantitative methods.[21]

NERVE CONDUCTION STUDIES

Electrophysiological investigation was performed (using Neurocare 2000 electromyograph, from Biotech Ltd, Mumbai), before the intervention and 3 months +4 weeks after the first and second dose of interventions respectively to ascertain the involvement of peripheral nerves and improvement or deterioration if any as a result of the interventions.

SENSORY NERVE CONDUCTION MEASUREMENTS (SNC)

Sensory action potential (SAP) parameters i.e. latency, peak to peak amplitude and sensory conduction velocity were measured bilaterally in four major sensory nerves i.e. ulnar, median, radial cutaneous and sural nerves at a fixed stimulation-recording distance of 14 cm. The ulnar and median nerves were stimulated at little and index fingers respectively with pick-up at wrist (orthodromic conduction); the radial cutaneous nerve action potential was recorded at the first web space following nerve stimulation over the radial side of the forearm (antidromic conduction). The sural nerve action potential was recorded behind the lateral malleolus following nerve stimulation at mid calf (antidromic conduction).

MOTOR NERVE CONDUCTION MEASUREMENTS (MNC)

Compound muscle action potential (CMAP) parameters i.e. latency, peak to peak amplitude and motor conduction velocity were measured bilaterally in four motor nerves i.e. ulnar, median, common peroneal and posterior tibial following stimulation at standard distal and proximal sites. The abductor digiti minimi muscle (ADM) served as the recording site for ulnar nerve; which was stimulated at wrist, below and above the sulcus; the distance between below and above sulcus being 10 cm. The Abductor pollicis brevis muscle (APB) was the recording site for the median nerve; the Extensor digitorum brevis muscle (EDB) was the recording site for the common peroneal nerve. The Abductor hallucis muscle (AH) was the recording site for posterior tibial nerve. The Windows driven software stored the evoked responses in a database for reference. The measured value for latency, amplitude, and conduction velocity were stored in a separate Access database. Skin temperatures were measured electronically at wrist and ankle and the measured latencies and velocities normalized for a temperature of 33°C at the time of analysis using standard formulae.[22]

NORMAL VALUES

Reference values were obtained from 25 normal subjects in the age group 23–52 years. Sensory conduction velocity for all the nerves sampled was $>$ 50 meters/sec (68·5 $\pm$ 14·2).

Peak to peak amplitude was > 10 microvolts (μV)(18·6 ± 6·7) for median, radial cutaneous and sural nerves and > 4 microvolts (7·1 ± 2·8) for the ulnar nerve. Motor conduction velocity for median and ulnar nerves was > 47 meters/sec (59·06 ± 11·3) while that for common peroneal and posterior tibial nerves it was > 40 meters/ sec (46·25 ± 6·5). Peak to peak CMAP Amplitude was > 10 millivolts (15·06 ± 3·75) for median and ulnar, >10 millivolts (17 ± 7·2) for posterior tibial and > 4 millivolts (6·8 ± 2·8) for common peroneal nerve.

BIOPSY AND ITS EXAMINATION

A deep incision skin biopsy from a skin patch was undertaken twice, once before the intervention (base line) and was repeated at 3 months (+3m) after the 2nd dose of intervention. Selection of lesion for biopsy was based on a) clinically most active in case of SSS negative patients or b) the one that scored highest in the SSS. Biopsies were performed under local anesthesia after obtaining the informed consent. Each biopsy was divided into three parts. One part of biopsy was utilised for bacterial viability studies in the mouse footpad. The second part was fixed for histopathology (electron microscopy in case of nerves only) and the third part of the skin biopsy was stored at −70°C.

HISTOPATHOLOGY

One part of the biopsy was fixed in Formal Zenker and embedded in paraffin; sections cut and stained with Trichrome modified Fite Faracco (TRIFF). They were used for Ridley-Jopling classification. Grading of cell types, tissue bacterial index and granuloma index were determined using a semi quantitative approach.[23] Special emphasis was given to document 1) reaction if any and its type, and 2) evidence suggestive of upgrading if any after the intervention that is between baseline and post intervention biopsies. Paraffin sections were also stained with Anti–Bacillus Calmette Guerin (BCG) by Peroxidase–Anti Peroxidase technique for determining the antigenic load.[24] This method of detecting antigen load has been found to be more sensitive than AFB detection. In the case of nerve biopsies one part of nerve was fixed in glutaraldehyde, stained with osmium tetraoxide and embedded in araldite for the ultra structural studies.

ASSESSMENT OF BACTERIAL LOAD/GRAM WEIGHT AND MOUSE FOOT PAD (MFP) TEST FOR VIABILITY DETERMINATION

Random bred Swiss white (S/W) mice, housed and bred at our institute were used for the study. Mice were fed with standard mouse diet and were kept in a 12 hr. light/dark cycle in an air-conditioned room. One part of the biopsy was homogenized within 24 h of collection and bacterial load per gram weight was determined using the standard protocol. The weight of the tissue available for homogenization ranged between 0·1 and 0·3 g. The final volume of the suspension was maintained at 1 ml per 0·1 g of tissue weight. The homogenate obtained, regardless of presence or absence of any AFB, was injected into both hind foot-pads (0·03 ml/foot pad and inocula size not exceeding 1 x 10^4 *M. leprae*), of a minimum of 8 non-immunosuppressed Swiss white mice. Foot-pad harvests were carried out at sixth, seventh and eighth and 12 months following inoculation using Shepard's method.[25] Briefly, a known volume of foot-pad tissue suspension was spread over the spot slide, fixed and stained.

Acid-fast bacilli were counted in a minimum of 200 microscopic fields per sample. The lower limit of detecting by this counting method is 1 x 10^4 *M. leprae*/ml. A minimum of two counts per foot-pad were obtained at sixth, seventh and eighth months. The foot-pads of the remaining mice were harvested at the 12^{th} month. One or more per foot pad counts showing >1 x 10^5 *M. leprae* in the harvests carried out at the sixth month or later were considered as positive yield.

LEPROMIN TESTING

Skin testing with Mitsuda lepromin was carried out at baseline and at three months (+3m) after the second dose of BCG/normal saline intervention to avoid any possibility of cross-sensitisation. It was also ensured that the patient was not taking any anti-inflammatory drug during the lepromin testing.

STATISTICAL ANALYSIS

Data entry and analysis was performed using SPSS version 19 and Epi-info. Significance of association was tested through the Chi square test with Yates continuity correction, *P* value and Fisher's exact test.

ETHICS COMMITTEE CLEARANCE

The study followed International Ethical Guidelines for Biomedical Research involving human subjects (CIOMS/WHO, 1993). This study received clearance from the Institutional Ethics Committee. This included permission for skin and/or nerve biopsies. Written consent was obtained from individual study subjects before inclusion in the study using a standard consent form. No financial incentives were given as actual to the patients. Travel expenses were reimbursed to the patients as actuals on each occasion.

The committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) cleared the use of animals for the study. The institute is registered with CPCSEA and has a valid registration number 423/01/1/CPCSEA.

Results

SUMMARY OF FINDINGS AT BASELINE (0M)

Of the 210 candidate RFT patients screened, 114 were found to be eligible. A total of 107 patients consented for the study, of which 78 completed the study. Of the remaining 29 (27%), 28 dropped out of the study at different time points for varying reasons and one died during the course of the study. Among the dropouts, 14 had received the first dose of injection (BCG or normal saline) and the remaining 14 had received both the doses but the post intervention biopsy was not done. One patient who died during the course of the study had received two doses of placebo. He developed malaria and died due to its complications. No patient was withdrawn from the study for medical reasons. Table 1 details the characteristics of patients in the two groups that show acceptable similarity.

Table 1. Characteristics of patients at baseline

Variables	Group A ($n = 49$)	Group B ($n = 58$)
Sex		
Male	38	50
Female	11	8
Mean Age	31·9	34·2
Previous BCG vaccination scar		
Scar present	12	14
Scar absent	34	39
Not recorded	3	4
Lepromin Status		
Negative	47	47
Positive	1	1
ND	1	10
Reaction History		
I) History of reaction	30 (61.%)	44 (76%)
a) T1R- single	4 (8.%)	8 (15.%)
b) T1R- multiple	3 (6.%)	5 (9%)
c) T2R −single	7 (14%)	9 (14%)
d) T2R-multiple	14 (29%)	14 (24.%)
e) T1R + T2R	2 (4.%)	3 (5%)
II) No history of reaction	19 (39%)	14 (24.%)
III) Neuritis only	0	5 (9%)
Grade of disability by WHO scale		
DG 0	28 (57%)	27 (47%)
DG 1	5 (10%)	3 (5%)
DG 2	16 (33%)	28 (48%)
BI assessed by SSS		
$<1+$	6	13
$1+$ -2 +	14	21
$3+$ -5 +	29	24
Histopathology		
Regressed	14 (28%)	15 (25%)
Regressing	29 (59%)	38 (66%)
Active	6 (12%)	4 (7%)
T1R	0	1
M leprae viability assessed in the mouse footpad		
Positive	8 (16%)	2 (3%)
Negative	41	56

SOCIO-DEMOGRAPHIC CHARACTERISTICS

The mean age of the group A was 31·9 years *vs* 34·2 years in group B. Recording of previous BCG vaccination scar was done in 99 patients of which only 26 (26/99 = 26%) patients including 12 in group A and 14 in group B had previous BCG scar.

Baseline lepromin status was recorded in 48 patients (97%) of group A and 48 patients (82%) of group B respectively. Amongst them, 97% patients were found to be lepromin negative in both the groups. A majority of them had a past history of reaction. Based on the symptoms described by the patients, the type of reaction and the frequency of episodes were estimated. Most patients provided a history of T2R.

The WHO scale was used for grading disability. A large proportion of patients in both the groups had deformity grade II. Patients with BI less than one were marginally higher (not significant).

Baseline histopathological findings suggested reasonable similarity between the two groups with respect to histopathological presentation.

M. LEPRAE VIABILITY SCORE USING MFP ASSAY

At baseline (0m), 10 samples from ten patients (9·3%) tested positive in the mouse foot pad proving the presence of viable bacteria in the tissue, of which eight (8/49 = 16%) belonged to group A and two (3%) belonged to group B.

The sensory and motor nerve impairment as assessed by NCV was also comparable in the two groups (Table 2).

FINDINGS DURING AND AT 3 + 3 MONTHS AFTER THE SECOND INTERVENTION

Reaction To Interventions at the Local Site

Size and intensity of local reaction at the injection site were documented in all the patients. All the patients in group A showed a positive reaction in the form of erythema, induration and few patients showed ulceration at the centre of the papule which crusted and healed over after 1-2 months. Local inflammation reaction usually developed within ~4-7 days, heightened at ~ 3 weeks. Some patients developed mild fever of 3-4 days which subsided with Paracetamol tablets. In most patients it was observed that when the second dose of BCG was given i.e. after 3 months, the first injection site also erupted with induration. Both sites then subsided as above in 1 to 2 months. The size of the papule ranged between 7 mm to 28·5 mm. The mean papule size was 12·8 mm. None of the patients from group B developed any such local reaction/inflammation.

The presence of a BCG scar, history of reaction, grade of disability, slit skin smear status, sensory and motor nerve status through NCV, lepromin status, and the nature of granuloma through histopathology were determined.

A total of 78, including 36 in group A and 42 in group B, who completed all the investigations at two time points i.e. zero and 6 months (i.e. 3 months after the second dose) were analysed (using semi quantitative grades) to study the difference.

REGRESSION OF LESIONS THROUGH CLINICAL ASSESSMENTS

On clinical examination, adjusted to 6 months the clinical regression was graded as a) Poor: when there is no change in the clinical condition or deterioration b) good: when there is

Table 2. Sensory and Motor nerve abnormalities assessed using SNCV and MNCV

	Sensory		Motor	
Level of conduction	Group A ($n = 41$)	Group B ($n = 57$)	Group A ($n = 41$)	Group B ($n = 57$)
Normal	109(33%)	96 (21%)	104(32%)	123(27%)
Abnormal	75	82	194	283
No conduction	144	278	30	50
Total	328	456	328	456

Table 3. Clinical regression of skin/nerve lesions

Range	Group A ($n = 36$)	Group B ($n = 42$)
Very good	0	0
Good	18 (50%)	12 (28%)
Poor	18 (50%)	30 (71%)

P value: 0·08

partial improvement defined as any improvement less than complete regression of skin/nerve lesions. c) Very good improvement: defined as complete regression in skin /nerve lesions. (Table 3) shows the distribution of groups A and B with respect to clinical improvement.

No patient in either group showed 'very good' improvement in the clinical assessment. The proportion of patients showing good improvement after two doses of BCG vaccine was marginally higher than those administered placebo though not significant ($P = 0·08$).

CHANGE IN DISABILITY GRADES

Disability grade was recorded, both at zero and 6 months. The change in grade overall as well as per nerve was compared and recorded under three categories: no change, deterioration in disability grade and improvement in disability grade. The proportion of patients showing improvement in disability grade was comparable while deterioration was higher in group A. However, the difference was not statistically significant ($P = 1·0$) (Table 4).

NERVE FUNCTION IMPAIRMENT

The nerve functions of the patients were measured using two of the clinical tests i.e. MF and VMT and nerve conduction studies (NCV) carried out using standard protocols. Taking zero month as the referral point, changes in nerve function following intervention was compared for both the groups (Tables 5 and 6).

In both the groups, MF, VMT and in the NCV tests the proportions (percentile) of both sensory and motor nerves showing deterioration are higher than improvement. On statistical analysis it was found that while sensory nerve deterioration as assessed through NCV is significantly higher in group A [$P = 0·005$, RR: 1·42(95%Cl 1·15-1·75)] the motor nerve deterioration as assessed through VMT (MRC scale) and NCV were significantly higher in group B [$P = 0·041$, RR: 0·73 (95% CI 0·54-0·99)].

Table 4. Change in Disability grades

	Group A ($n = 36$)	Group B ($n = 42$)
No change in DG type	29 (80%)	36 (85%)
Deterioration	5 (14%)	4 (9%)
Improved	2 (6%)	3 (7%)

P value: 1·0

Table 5. Number of sensory and motor nerve showing improvement and deterioration assessed using monofilament testing (MF) and voluntary muscle testing (VMT)

Sensory (MF) Group A	Ulnar	Median	Radial cutaneous	Sural	Total
Improved	8 (11%)	7 (9%)	6 (8%)	4 (5%)	25 (8%)
Deterioration	8 (11%)	8 (11%)	8 (11%)	13 (18%)	37 (12%)
No change	56	57	58	55	226
Group B					
Improved	7 (8%)	4 (4%)	4 (5%)	10 (12%)	25 (7%)
Deterioration	13 (15%)	12 (14%)	9 (11%)	7 (8%)	41 (12%)
No change	64	68	65	65	262
Motor(VMT) Group A	Ulnar	Median	Common peroneal	Post Tibial	Total
Improved	5 (6%)	2 (2%)	1 (1%)	2 (2%)	10 (3%)
Deterioration	1 (1%)	0	0	1 (1%)	2 (0·6%)
No change	66	70	71	69	276
Group B					
Improved	8 (9%)	1 (1%)	2 (2%)	2 (2%)	13 (3%)
Deterioration	11 (13%)	1 (1%)	1 (1%)	0	13 (3%)
No change	65	80	81	82	308

REACTION TYPE, ITS OCCURRENCE AND SEVERITY AFTER THE INTERVENTION

As depicted in Tables 7, 8 and 9 the overall proportion of patients developing reaction (T1R, T2R and neuritis combined) and its profile were comparable in the two groups. T2R was more common in each group.

Table 10 gives the distribution of patients in each group with respect to history of reactions and the occurrence of a lepra reaction following the intervention. In both groups, a majority of patients who developed reaction following intervention, reported a history of reaction (48% and 40%), The addition of BCG did not increase the risk in terms of incidence/ occurrence of reaction/neuritis (p value not significant) but the proportion of patients showing severe reaction was higher in group A (25%) as compared to group B (5%).

In relation to BCG scar (Table 9) reactions occurring in BCG scar absent group was higher, but not statistically significant.

Table 6. Sensory and Motor nerve functions using NCV parameters (8 nerves/patient)

	Sensory		Motor	
Variables	Group A ($n = 26$)	Group B ($n = 28$)	Group A ($n = 26$)	Group B ($n = 28$)
Improved	12 (6%)	11 (5%)	10 (5%)	11 (5%)
Deterioration	48 (23%)	31 (14%)	31 (15%)	56 (25%)
Same (S) Normal	50 (24%)	30 (13%)	54 (26%)	39 (17%)
(S) abnormal	17 (8%)	20 (9%)	92(44%)	96 (43%)
(S) no conduction	81 (39%)	132 (59%)	21(10%)	22 (10%)
Total	208	244	208	244

P value: 0·005 - between the groups A and B, sensory nerve deterioration values
P value: 0·04 - between the groups A and B, motor nerve deterioration values

Table 7. Type of reaction and its occurrence

	Group A ($n = 36$)				Group B ($n = 42$)			
Time interval	T1R	T2R	Neuritis	Total	T1R	T2R	Neuritis	Total
0-3m	3	5	1	9	1	5	1	7
3-6m	2	4	1	7	3	4	3	10
Total	5 (14%)	9 (25%)	2	16 (44%)	4 (10%)	9 (21%)	4	17 (40%)

P value: 0·9 between the groups A and B, with and without reaction values

TREATMENT FOR REACTION

A total of 12 patients who developed reaction including seven from group A and five from group B received corticosteroids during the course of the study for the control of moderate to severe reaction (Table 8).

BACTERIOLOGICAL FINDINGS

Table 11 depicts the decline in BI as assessed by SSS in 60 patients (group A = 32 and group B = 35) at 6 months following intervention.

The proportion of patients showing good decline in the bacteriological index is higher in the group A as compared to group B but the difference is not significant ($P = 0{\cdot}645$). On comparing the decline in BI with presence of old BCG scar (prior immunomodulation by BCG vaccination in childhood), it was found the number of patients showing good decline in BI were more likely to have no exposure to BCG vaccine in childhood. Notably, 73% of the study group of patients did not have BCG scar suggesting that they may not have been vaccinated with BCG at childhood.

VIABILITY ASSESSED USING FOOT PAD OF NON-IMMUNO-SUPPRESSED S/W MICE

As depicted in Table 12 six months (post intervention) no sample tested in group B (0/35) showed any growth in footpad, while 3/32(9%) tested positive (indicating viability) in group

Table 8. Severity of reaction following interventions

	Group A ($n = 36$)				Group B ($n = 42$)			
Time interval	Mild	Moderate	Severe	Total	Mild	Moderate	Severe	Total
0-3m								
T1R	2	0	1	3	1	0	0	1
T2R	3	0	2	5	4	0	1	5
Neuritis	0	0	1	1	1	0	0	1
3-6m								
T1R	1	1	0	2	2	1	0	3
T2R	2	2	0	4	2	2	0	4
Neuritis	1	0	0	1	2	1	0	3
Total	9	3	4	16	12	4	1	17

Table 9. Occurrence of reaction in relation to presence/absence of BCG scar

	Group A ($n = 35$)		Group B ($n = 40$)	
Type of reaction	Scar present ($n = 9$)	Scar absent ($n = 26$)	Scar present ($n = 12$)	Scar absent ($n = 29$)
T1R	3 (33%)	2 (8%)	1	3 (10%)
T2R	2 (22%)	6 (23%)	3	7 (24%)
Neuritis	0	2	2	2
No reaction	4 (44%)	16 (61%)	6 (50%)	17 (59%)

A (Table 12). Additionally, a comparison of the proportion patients in group A, who tested positive in MFP at zero and six months; there was no significant difference (Fischer's exact test two tailed *P value*: 0·4287).

Unequivocal growth in the footpads of non-immuno-suppressed mice, proving the presence of viable bacteria, was seen in nine samples from eight patients. Among the cases scoring positive in the MFP one was positive both at zero and 6 months, five cases only at zero month and in two cases only the sixth month biopsy. On comparing the relationship between the average BI and growth in the mouse foot pad, of the nine patients testing positive in MFP, the average BI was high (3 + to 4 +) in four patients and was low i.e. $<2+$ in five cases. Likewise decline in BI was graded as good in five cases and poor in three cases. On stratification, it was noted that occurrence of viable bacteria, as assessed by MFP, failed to show any direct correlation to the average bacterial index (BI) or its clearance.

LESION REGRESSION ASSESSED THROUGH HISTOPATHOLOGY

Granuloma Fraction (GF)

Granuloma fraction (GF) was estimated using Ridley's scale.[23] It was found that at 6 months, group A and group B had comparable lesion regression patterns assessed by GF (Chi square *P value*: 0·356). One patient (1/71) showed evidence of upgrading that is BT granuloma at 6 months as against BL at zero months. This patient also showed lepromin conversion and belonged to group B.

ANTIGEN CLEARANCE

It was found that the mean antigen load at zero months in group A was 3·6 ± 2·7 and in group B was 4·3 ± 3·4. The mean antigen load at 6 months in group A and group B was 3·2 ± 2·7

Table 10. Number and (%) of patients with reaction before and after intervention

Variables	Group A ($n = 36$)	Group B ($n = 42$)	Total
Reaction before and after intervention	12 (33%)	14 (33%)	26
No reaction before intervention but developed later	4 (11%)	3 (7%)	7
History of reaction but no reaction after intervention	13 (36%)	21 (50%)	44
No reaction before and after	7 (19%)	4 (9%)	12

Table 11. Decline in bacteriological index assessed in SSS

Improvement in BI	Group A ($n = 32$)	Group B ($n = 35$)
Very good	0	0
Good	21 (65%)	20 (57%)
Poor	11 (34%)	15 (42%)
Grading:		
Very good – decline in BI of $>$ 1 log at 6m		
Good – decline in BI of $>$ 0·5 and $<=$ 1 at 6m		
Poor – no decline in BI		

P value: 0·64

and 4·4 $\pm$ 3 respectively. On comparing the mean antigen load in the two groups at 6 months using independent sample 't' test it was found that there was no statistically significant difference.

LEPROMIN CONVERSION

Lepromin positive reaction is defined as a local induration of >5 mm recorded at 3 weeks following lepromin testing. The proportion of patients showing lepromin conversion is depicted in Table 13. There was no difference in the lepromin conversion rates in the two groups.

HISTOPATHOLOGY OF NERVE BIOPSIES

Seven sural nerve biopsies from six patients including two at zero months and five at 6 months were studied. All the seven nerves studied showed peri-vascular aggregates of foamy macrophages consistent with treated BL-LL leprosy. Presence of bacilli within the endoneurial region, total loss of fibres, presence of denervated Schwann bands along with increased collagen deposition were seen in five cases. Fibre loss to the extent of $\sim$90% and presence of uniformly distributed regenerating/remyelinating fibres were seen in two cases. Both were 6 months post intervention biopsies from group A and one among them showed presence of viable bacteria as assessed in MFP. The numbers are too small to come to any logical conclusion. However, no nerve showed any signs of (upgrading) reaction either in the endoneurium or in the epineurial region.

Table 12. Viability test findings assessed at 0 and 6 m using MFP (among BI positive patients)

Growth in MFP	Group A ($n = 32$)	Group B ($n = 35$)
At 0 m	5/32 (16%)	1/35 (3%)
At 6 m	3/32 (9%)	0/35
% decline	40%	–

P value: 0·42

Table 13. Lepromin conversion in groups A and B

Lepromin conversion	Group A ($n = 26$)	Group B ($n = 28$)
− ve to + ve	6 (24%)	7 (25%)
No change		
− ve to − ve	20 (76%)	21 (75%)
+ ve to + ve	1	–
ND	9	14

Discussion

Immunomodulatory strength of BCG vaccine given post MDT in BL-LL leprosy patients was investigated in a placebo controlled study. Our working hypothesis here is that BCG used as an adjunct to MDT boosts cellular immunity, hastens antigen clearance, reduces the occurrence of 'persister' bacilli, brings about lepromin conversion and helps in the recovery of nerve functions.

Immunotherapy during the period of initial chemotherapy may be interfered with possibly by immunosuppressive actions of Dapsone and Clofazimine.[26,27] Post treatment immunotherapy with BCG, as Katoch *et al.*, have observed in their study of cases poses problems of monitoring because of a small number of viable bacilli and the difficulty in obtaining suitable controls.[12] Use of multiple parameters including occurrence of 'persisters', antigenic clearance along with clinical, histopathological regression of lesions and lepromin conversion in the present study helped in overcoming some of the constraints faced by Katoch *et al.*

Evidence for a correlation between optimal BCG dose (in terms of viable bacilli) and protection has been reported in guinea pigs though there are only hints of such an association in human studies.[13] The Chingleput trial in Southern India compared two doses (0·1 and 0·01 mg) and found a consistently greater protection with the higher dose against leprosy.[3] The findings of the Burma trial also suggest that the higher dose (representing a larger dose of viable bacilli) imparted greater protection against leprosy.[28] These results indicate that BCG vaccines may generally be given at the top of their dose response curves viz. 0·1 mg. Secondly, emerging evidence in MDR TB using *M. vaccae* immunotherapy as well as in leprosy using other vaccine candidates viz *M. welchii* as well as BCG suggest that use of multiple doses may be required for a better/optimal outcome.[29,30] Therefore, in this study we used optimum dose regimen of BCG given twice at 3 month intervals.

Both the primary and the secondary outcomes assessed were closely comparable in patients receiving BCG and placebo in our study. Narang *et al.* compared the clinical and bacteriological performance of 60 patients divided on placebo, BCG and *Mw* vaccine and found that there was marked improvement in Ramu's score and bacteriological index in BCG group compared to control group.[31] Contrary to this, our study findings show no statistically significant difference between the BCG and placebo group of patients with respect to clinical regression, bacterial/antigen clearance as well as lepromin conversion rate. Upgrading towards the tuberculoid end of the spectrum along with lepromin conversion was seen in only one patient who belonged to the placebo group. Most importantly, the level of persisters remained unaltered following BCG intervention. A study by Douglas *et al.* in 270 leprosy patients who had completed treatment and receiving multiple (five times) treatment with

BCG vaccine and heat killed *M. leprae,* also showed no significant difference in the lepromin conversion rate between the two groups.[32]

The majority of our study group of patients (73%) did not have a BCG scar suggesting that they may not have been vaccinated with BCG at childhood, is in line with the probable protective efficacy of BCG against leprosy. However, immunization with BCG at childhood appeared to be counter productive in terms of bacterial clearance in group A.

In both groups, the proportion of nerves showing deterioration of function in the NCV test was significantly higher than the proportion improving. While the percentage of patients developing reaction/neuritis post intervention was closely comparable in the two groups, patients showing severe reaction was higher in the group A (25%). The deterioration in the sensory nerve conduction parameters was also higher in the group A (14 vs 9% and 23 vs 14% respectively). However, motor nerve deterioration was higher in the group B (25%) as compared to group A (14%), despite reaction severity being higher in the latter (25% vs 5%). The number is too small to carry out a subgroup analysis for an association. It should be noted that all patients developing severe and moderate reaction were treated with (40 mg tapering dose x 12 weeks) corticosteroids. Therefore relatively less deterioration among the motor nerves noted in the group A could well be due to the prompt usage of steroids. The question is why was this difference seen with motor and not sensory nerves? One explanation could be that motor nerves are affected later than sensory nerves. CMAP amplitudes are expressed in millivolts (mV); changes in conduction parameters (CMAP amplitude, distal latency, MNCV and wave form morphology) are easier to discern. On the other hand, sensory action potential being in the μV range are frequently un-recordable even in mild sensory neuropathy, making them a sensitive tool for *detecting* sensory nerve pathology but a poor one for assessing changes in follow-up studies. A similar observation was made in an earlier study by us where effect of corticosteroids on nerve function impairments was studied.[18]

Admittedly a singular limitation of this study is the small sample size. Intake and follow up were significantly restricted by reaction episodes. The high number of drop-outs (27%) after the initiation of the study made it difficult to achieve the targeted numbers (i.e. was a total of 200 cases), despite extending the study period by a year. In both groups, over 60% of the patients included were with past history of reaction introducing some selection bias. A better powered study indeed would be desirable to give a clearer picture. Nevertheless findings of various parameters assessed as outcome measures in this study were complementary to each other.

An important primary outcome assessed in this study was the impact of immunomodulation on the occurrence of viable bacteria termed as 'persisters.' Using foot pads of non-immunosuppressed S/W mice, we were able to demonstrate the presence of viable bacteria in a comparable proportion of patients both before and after the intervention indicating that BCG used as an immuno-potentiating agent did not affect viable *M. leprae or* 'persisters.' This was unlike the findings in an earlier study by Katoch *et al.* where better killing of *M. leprae* as assessed by ATP assay among the patients receiving Mw vaccine, was reported. Their study however was on a very small number of patients.[12]

Conclusion

Our study findings show that two injections of BCG given at optimal dose, post-MDT did not have any significant impact on the rate of bacterial clearance, granuloma clearance, and

lepromin conversion. More importantly, BCG vaccine did not alter the level of persisters in this study group of patients disproving our working hypothesis. On the positive side, despite severity of reaction being higher in group A there was no adverse effect on the motor nerve function impairments, in our opinion this was attributable to prompt usage of steroids in these patients.

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