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Histopathological Upgrading using *Mycobacterium indicus pranii* (MIP) Vaccine as an Immunotherapeutic with Standard Chemotherapy in Borderline Leprosy: A Double Blind Randomized Placebo Control Trial

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Abstract

Background: Immunotherapy with BCG, BCG+ *M. leprae,* ICRC, *MIP* has been observed to be effective in improving the treatment in leprosy. Histopathological upgrading using *MIP* vaccine has been reported in cases with the high bacillary load. However, very little information is available in borderline leprosy which is characterized by a state of shifting immunity and would, therefore, be ideally suited to such observations. This study is originated from the involvement of our Institute in trials aimed at improving the therapy of leprosy by using combined immunotherapy and chemotherapy.

Aims: The study aimed to assess the histopathological improvement by adding immunotherapy (MIP vaccine) with chemotherapy (WHO-MDT) in borderline leprosy.

Methods: A total of 105 new borderline leprosy cases were included, after formal written consent, a detailed clinical examination. Skin biopsies were taken from the active lesions (diagnosis blinded for histopathologist) Blinded therapeutic regimens containing *MIP* vaccine/placebo was injected intradermally at the start of therapy and every six months in addition to chemotherapy and effect was observed on histopathological parameters (Infiltration fraction by percentage of infiltration. Granuloma dimensions by diameters in maximum and minimum area. Cellular fraction by the predominance of cells present in the granuloma or infiltration and bacillary clearance of AFB in tissue section).

Results: Addition of immunotherapy resulted in faster granuloma clearance, faster reduction in infiltration fraction, faster bacillary clearance and a significant finding was an increase in the epithelioid cells population in the immunotherapy group in borderline leprosy cases.

Limitations: 105 borderline cases recruited, 90 completed follow up during therapy.

Conclusion: This study shows the usefulness of adding immunotherapy (MIP vaccine) to Chemotherapy (WHO-MDT) in borderline leprosy for faster histopathological improvement.

Keywords: Borderline leprosy; Histopathological upgrading; Chemotherapy; Immunotherapy

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Introduction

Treatment of leprosy has been improved by the introduction of immunotherapy along with chemotherapy. The immunomodulatory action of Bacillus Calmette-Guerin (BCG) vaccine alone or in combination with killed *Mycobacterium leprae*

vaccine, Indian Cancer Research Center (ICRC) *Mycobacterium indicus pranaii* vaccine has been observed to be effective in improving the treatment in leprosy [1-4]. Histopathological upgrading and faster bacterial clearance using *Mycobacterium indicus pranaii* vaccine have been reported in cases with high bacillary load [5,6]. After the declaration of elimination of leprosy in India, most of the prevailing cases are of borderline leprosy type. *Mycobacterium indicus pranaii* shows a good therapeutic response in leprosy. This study originated from a trial aimed at improving the therapy of leprosy by using combined immunotherapy and chemotherapy. Accelerated clearance of bacilli and granulomas has been documented in studies based on cases with high bacillary load [7] as well as in a pilot study on borderline leprosy [8] but these studies were not done in a double-blind manner. Hence the present study was undertaken to assess the additive effect of immunotherapy (*Mycobacterium indicus pranaii* vaccine) with standard WHO-MDT on histopathological parameters like infiltration fraction, granuloma diameter, the predominance of cells present in the granuloma on untreated clinically active borderline leprosy patients.

Methods

This was a double-blind, randomized, placebo-controlled, parallel group trial conducted during 22/3/2011 to 14/8/2013 in the Department of Clinical Medicine, National JALMA Institute for Leprosy and Other Mycobacterial Diseases, Tajganj Agra, India.

Inclusion criteria

Only borderline untreated cases of more than 18 years of age were included in this study. Patients were diagnosed according to clinical criteria and smear positivity and classified into three groups according to Ridley and Jopling Classification [9]; Borderline Tuberculoid (BT), Borderline Borderline (BB) and Borderline Lepromatous (BL).

Exclusion criteria

Patients who did not have conclusive evidence for the diagnosis of borderline leprosy, patients with HIV infection, other additional immunosuppressive illness such as diabetes mellitus, hematological and reticuloendothelial malignancies were excluded.

Ethical Permission, Informed Consent

The ethical approval for this study was taken from the Human Ethics Committee of the Institute for smear examination, intradermal injection of *Mycobacterium indicus pranaii* vaccine, for treatment by Multi-Drug Therapy (MDT) and for skin biopsies. At the time of recruitment, all the patients were informed about the diseases, the implications of treatment, possible side effects, and benefits. Once the written consent was obtained they were recruited in the study and started on treatment.

Recruitment, randomization, intervention, implementation and blinding

In this study, 105 new borderline leprosy patients attending the outpatient department were recruited; (BT-37, BB-32, and BL-36). After the formal written consent detailed clinical examination, slit skins smear examination of all patients was done and skin biopsies were taken.

From active lesions of all the patients at the start of therapy, at six months and after 6 months of post-treatment follow up (total of three times) for BT. In BB/BL also biopsies were taken at the start of therapy, at 6 months and at 12 months of completion of therapy (total of three times). Similar size (5 mm) and depth punch skin biopsy were obtained from all the patients. Clinical diagnoses of all the skin biopsies were kept blinded from histopathologist. Slit skin smears were taken from both ear lobules and from lesion sites and results were recorded on the Ridley scale. Standard WHO-MDT (PB and MB) given to all the patients according to the type of disease. Blinded therapeutic regimens (0.6 ml identical vials of **c**olourless opaque suspension with non visible cells) made by Cadila Pharma containing MIP vaccine or placebo was injected 0.1 ml (each 0.1 ml containing sodium chloride 0.9 w/v, Thiomersol IP 0.01% w/v with non visible cells (0.5×10^9 bacilli) intra-dermally at the start of therapy and every six months in addition to chemotherapy to all the patients serially using pre-coded random numbers on identical vials from no. 1 to vial no. 105. BT cases were followed up after 6 doses of MDT and 2 doses of blinded regimens (MIP vaccine or placebo) and BB/BL cases were followed up during the 12 monthly doses of MDT (at 0, 6 and 12 months) corresponding to the time they received the 3 doses of blinded regimens (MIP vaccine or placebo). The participant and health care provider were both blinded.

Assessment

Each patient was assessed at intake, at 6 months, and at 12 month during period of post-therapy follow up (clinical post MDT follow up was for 5 years but histopathological follow up was done only up to completion of therapy (12 months) for BB/BL and up to 6 months after therapy for BT cases in this study for the following histopathological parameters according to definition [10,11] **(Figures 1 and 2).**

(i) Infiltration fraction means the fraction of dermis occupied by infiltrate includes both granulomatous and non granulomatous as assessed under low power objective by using the ocular grid

(ii) The maximum diameter of the granuloma was measured by counting the divisions of an ocular micrometer which was earlier calibrated against the stage micrometer. The actual diameters were derived from the equivalence value obtained during calibration

(iii) Predominant cells in the granuloma (lymphocytes, epithelioid and others type of cells)

Methods of evaluation, concealment and statistical analysis

Data collected was deposited to Director of the Institute on 19/5/2014. Decoding of serially numbered identical vials took place on 20/05/2014 from sealed packed envelops provided to the director by Cadila Pharma. After decoding, data was made available to authors for statistical analysis. A t-test was used to compare means and X^2 for proportion.

Results

As described in **Table 1**, 105 borderline cases (BT-37, BB-32, BL-36) were recruited in the study; out of which 90 (BT-36, BB-24, BL-30) completed their follow up during therapy and it was found that 15 patients defaulted from therapy. After decoding, study and control groups were made. Forty six patients (BT-13, BB-11, and BL-22) were present in study group (MDT+MIP) and 44 patients (BT-23, BB-13, BL-8) were present in control group (MDT+Placebo). After decoding, it was known that patients who defaulted during therapy 6 belonged to the BT group (MDT+MIP) and 9 to BL group (MDT+placebo)**.** Histopathological diagnosis of 93% of patients showed concordance with the clinical classification after decoding and the remaining 7% clinically diagnosed cases showed a nonspecific pathology that is known to occur in leprosy in a proportion of cases.

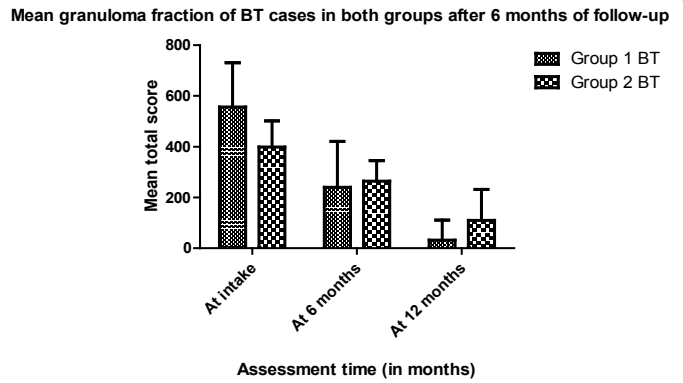

Figure 1. Effect of Immunotherapy and standard MDT on dimension of granuloma of BT cases in both groups.

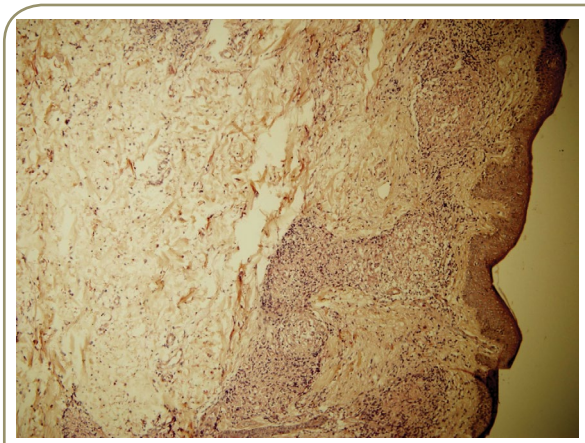

Figure 2. Photomicrograph of tissue specimen of BT case (Gp.1 MDT+MIP) showing extensive branching granuloma in upper dermis before initiation of therapy, Haematoxyline and Eosin staining (Magnification 50X).

Table 1. Distribution of the borderline patients according to type of disease.

	Study Group (MDT+MIP)		Control Group (MDT +placebo)	
Type of Disease	n	AFB Positive	n	AFB Positive
BT	13	0	23	3
BB	11	9	13	11
BL	22	22	8	8
Total	46	31	44	22

Effect of immunotherapy (*Mycobacterium indicus pranaii* vaccine) and standard MDT on the dimension of granuloma (Maximum diameter in microns)

At intake, both BT groups were statistically comparable. The initial mean granuloma diameter in the study group (MDT+MIP) decreased by 56.8% at 6 months and further reduced by 86.8% by 12 months in comparison to 46.9% at 6 months and further reduced by 58.6% at 12 months in control group (MDT+placebo). The difference of decline in granuloma diameter size (vaccine Vs placebo) at 6 months was not statistically significant, but statistically significant (86.8% vs 58.6%, $p<0.01$) during 6 to 12 months. The difference in reduction in mean diameter of granuloma in the study group was 10% more at 6 months and 28.2% more at 12 months. In BB/BL study group the initial mean granuloma diameter was reduced, from 525 to 205.15 microns (60.9%) at 6 months and further reduced to 34.09 microns (83.4%) by 12 months in comparison to control group where initial mean granuloma diameters was reduced from 466.7 to 297.5 microns (36.3%) and further reduced to 138.1 microns (53.6%) at 12 months. The difference of decline in granuloma diameter size (Vaccine Vs Placebo) at 6 months (60.9% vs 36.3%, $p<0.01$) and at 12 months (83.4% vs 53.6%, $p<0.01$) were earlier and statistically significant. The difference in reduction in mean diameter of granuloma in the study group was 24.6% more at 6 months and 29.8% more at 12 months **(Table 2 and Figures 3-5).**

Effect of immunotherapy (*Mycobacterium indicus pranaii vaccine*) and standard MDT on infiltration fraction

In BT cases, at intake, the IF of both the groups were statistically comparable. The mean infiltration fraction in study group reduced from 68.85% to 25.15% (60.6%) at 6 months and further reduced to 2.31% (90.8%) by 12 months. In the control group, the reduction was 35.9% at 6 months and 83.9% at 12 months respectively. The difference of decline in Infiltration fraction score (vaccine vs placebo) is significant at 6 months (60.6% vs 35.9%, $p<0.001$). At 12 months (90.8% vs 83.9%, $p>0.05$) the reduction was apparently rapid but not statistically significant. The difference in reduction in mean infiltration fraction in the study group was 24.7% more at 6 months and 6.9% more at 12 months. The mean of infiltration fraction in BB/BL cases at intake in both the groups also was statistically comparable. The mean infiltration fraction in the study group was reduced from 62.91 to 22.12 (64.8%) at 6 months and further reduced to 7.58 (87.9%) by 12 months in comparison to 36% at 6 months and 68.9% at 12 months decline in control group respectively. The difference of decline in infiltration fraction score (vaccine to placebo) is significant at 6 months (64.8% vs 36%, $p<0.001$). At 12 months (87.9% vs. 68.9%) the reduction was apparently rapid but not statistically significant. The difference in reduction in mean infiltration fraction in the study group was 28.8% more at 6 months and 19% more at 12 months **(Table 3 and Figures 3-5).**

Table 2. Effect of immunotherapy and standard MDT on mean score of maximum diameters of granulomas of BT and BB/BL cases at 6 and 12 months of therapy.

RJ type							
Assessment Time	Statistical Parameters	BT			BB/BL		
		Study Group (MIP+MDT)	Control Group- (MDT+Placebo)	Groups comparison	Study Group (MIP+MDT)	Control Group- (MDT+placebo)	Groups comparison
At Intake	N	13	23		33	21	
	Mean score of maximum granuloma diameter	556.82	498.08	t-test=1.08	525	466.7	t-test=1.33
	S.D.	174.3	127.1	p value>0.05	161.3	154.5	p value>0.05
At 6 Months	Mean score (% decline)	240.5 (56.8%)	267.58 (46.9%)	t test=1.90,	205.2 (60.9%)	297.5 (36.3%)	t test-2.62,
	S.D.	180.8	80.1	p value>0.05	151.9	103.5	p value<0.05
Intake vs. 6 months, t-test, p-value		4.54, <0.01	7.45, <0.001		8.3, <0.0001	4.2, <0.001	
At 12 Months	Mean score (% decline)	31.82 (86.8%)	109.62 (58.6%)	t test=2.3,	34.1(83.4%)	138.1(53.6%)	t test -3.17,
	S.D.	78.3	122.3	p value<0.05	68.98	139.8	p value<0.05
6 months vs. 12 months, t-test, p-value		3.81, <0.01	5.08, <0.001		5.9, <0.001	4.2, <0.001	

Table 3. Effect of immunotherapy and standard MDT infiltration fraction of BT and BB/BL cases at 6 and 12 months.

RJ type							
		BT			BB/BL		
Assessment Time	Statistical Parameters	Study Group (MIP+MDT)	Control Group (MDT+placebo)	Groups comparison	Study Group (MIP+MDT)	Control Group (MDT+ placebo)	Groups comparison
At Intake	N	13	23		33	21	
	Mean of IF	63.85	56.96	t-test=1.57	62.91	58.19	t-test=1.22
	S.D.	11.2	14.7	p value>0.05	13.5	14.1	p value>0.05
At 6 Months	Mean of IF(% decline)	25.15 (60.6%)	36.52 (35.9%)	t test-2.14,	22.12 (64.8%)	37.25 (36.0%)	t test-3.56,
	S.D.	14.15	17.2	p value<0.05	15.2	15.2	p value<0.05
Intake vs. 6 months(t-test, p-value)		38.7, <0.0001	4.3, <0.001		11.5, <0.0001	4.6, <.001	
At 12 Months	Mean of IF (% decline)	2.31 (90.8%)	5.87 (83.9%)	t test -1.03,	7.58 (87.9%)	18.10 (68.9%)	t test-2.24,
	S.D.	6.9	13.9	p value>0.05	14.3	18.2	p value<0.05
6 months vs. 12 months(t-test, p-value)		2.7,<0.02	6.6, <0.0001		4.0,<0.001	3.7,<0.001	

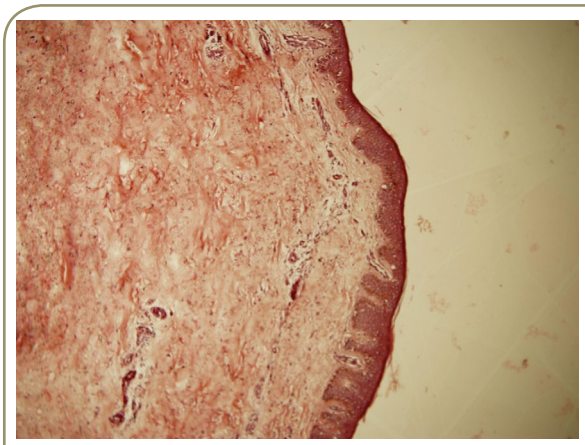

Figure 3 Photomicrograph of tissue specimen of BT case (Gp.1 MDT+MIP) after 6 month of therapy showing Dermis cleared of granuloma, Haematoxyline and Eosin staining (Magnification 50X).

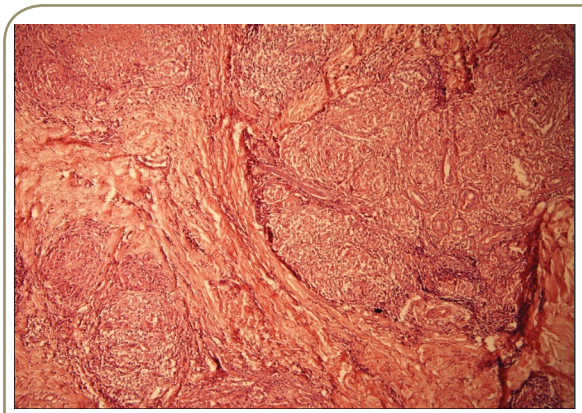

Figure 4 Photomicrograph of tissue specimen of BB/BL case (Gp.1 MDT+MIP) before initiation of therapy showing extensive granuloma , Haematoxyline and Eosin staining (Magnification 50X).

Effect of immunotherapy (*Mycobacterium indicus pranaii vaccine*) and standard mdt on mean bacillary index of bb/bl cases on the tissue section

In BB/BL patients the mean bacillary index at intake in both the groups was statistically comparable. The mean bacillary index in the study group was reduced from 3.22 to 2.15 (49.8%) at 6 months and further reduced to 0.88 (72.7%) by 12 months in comparison to 2.18 (25.6%) at 6 months and 1.47 (49.8%) at 12 months decline in control group respectively. The difference of decline in the mean bacillary index (vaccine to placebo) at 6 months was apparent but not statistically significant but at 12 months it is statistically significant (72.7% vs. 49.8%, p<0.01).

Table 4. Effect of Immunotherapy and Standard MDT on Mean bacillary index of BB, BL cases on tissue section.

Assessment Time	Statistical Parameters	Mean bacillary index of BB/BL		Statistical
		Study Group (MIP+MDT)	Control Group (MDT+placebo)	Groups comparison
At Intake	N	33	21	
	Mean of BI	3.22	2.93	
	S.D	0.18	0.668	t-test=0.092 p value>0.05
At 6 Months	Mean of BI (% decline)	2.15 (49.77%)	2.18 (25.59%)	
	S.D.	0.266	0.572	t test=0.227 p value>0.05
At 12 Months	Mean of IF (% decline)	0.88 (72.67%)	1.47 (49.83%)	
	S.D.	0.26	0.948	t test=2.79 p value<0.05

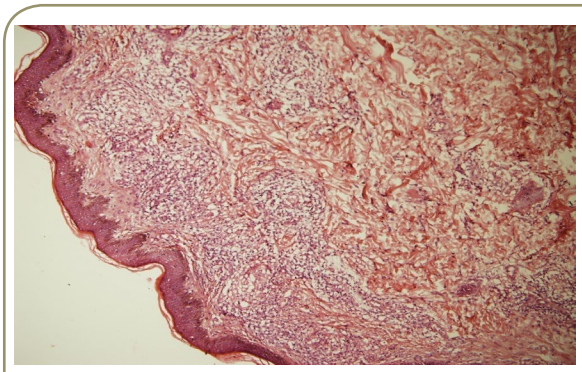

Figure 5 Photomicrograph of tissue specimen of BB/BL case (Gp.1 MDT+MIP) after 6 month of therapy showing clearance of granulomas, Haematoxyline and Eosin staining (Magnification 50X).

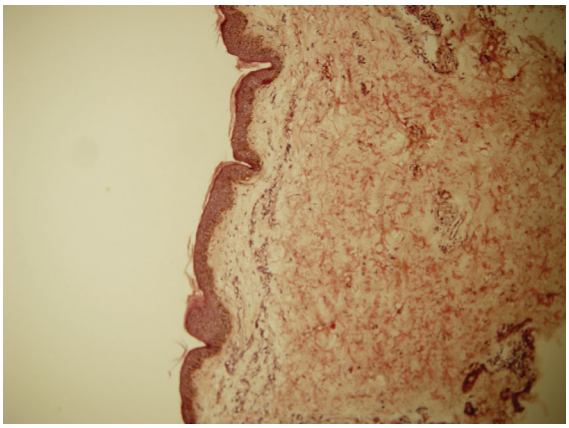

Figure 6 Photomicrograph of tissue specimen of BB/BL case (Gp.1 MDT+MIP) after 12 month of therapy showing cleared granuloma leaving diffuse band of infiltration, Haematoxyline and Eosin staining (Magnification 50X).

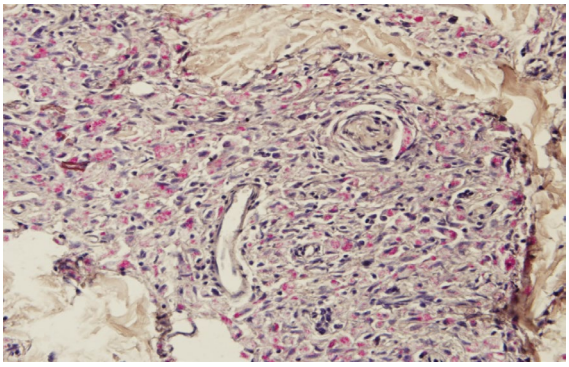

Figure 7 Photomicrograph of tissue specimen of BL case (Gp.1 MDT+MIP) at initiation of therapy showing Highly bacillated fields, Fite-faraco staining (Magnification 200X).

The difference in reduction in the mean bacillary index in the study group was 24.2% more at 6 months and 22.9% more at 12 months **(Table 4 and Figures 6 and 7).**

Local reaction to *Mycobacterium indicus pranaii* vaccine

Mycobacterium indicus pranii (MIP) was well tolerated by the patients and did not lead to any systemic side effects. There was a local reaction developed at the immunization site after 1 month, in the form of a circular area with scaling, crusting and occasional bruising in all the patients. 11 Borderline patients showed a shallow ulcer at the site of injection of MIP vaccine after 2 months of intradermal injection. On subsequent vaccination, the reaction did not produce any ulceration. MDT was well tolerated by all participants, no side effects were noted during the follow-up period of the therapy.

Discussion

As the nation is passing through the eradication phase of leprosy, reports are suggesting a change in epidemiology and symptomatology of the disease [8]. The incidence of patients of borderline leprosy as compared to a highly bacillated form of the disease is higher. The present double-blind study aimed to assess the additive effect of immunotherapy (*Mycobacterium indicus pranaii vaccine)* with standard MDT (WHO) on the histopathological profile of borderline patients of leprosy till completion of therapy.

Mycobacterium indicus pranaii used in this study is a known immunomodulator. The immunomodulatory action of MIP has been shown to induce histological upgrading and also faster bacterial clearance (5.7).

In the present study in BT cases, the overall decline in granuloma diameter was significant in both groups (94.3% vs 78%, p<0.01). Reduction in mean diameter of granuloma in the study group was 10% more at 6 months and 16.3% more at 12 months. In BB/BL study group also, the overall decline in granuloma diameter was significant in both groups (93.5% vs 70.4%). The reduction was rapid and statistically significant (p<0.0001) in the study group as compared to the control group**.** The reduction of infiltration fraction in BT cases was significant in both groups (96.5% vs

89.7%) and also in BB/BL cases (88% vs 68.9%). This study is first to report the impact of immunotherapy on Infiltration fraction in borderline leprosy cases.

The present study also observed the effect of Immunotherapy (*Mycobacterium indicus pranaii vaccine)* and standard MDT on Mean bacillary index of BB/ BL cases on the tissue section. The difference of decline in the mean bacillary index in both groups at 6 months was apparent but not statistically significant but at 12 months it is statistically significant in both groups (72.7% vs 49.8%, $p<0.01$). The difference in reduction in the mean bacillary index in the study group was 24.2% more at 6 months and 22.9% more at 12 months.

Addition of immunotherapy resulted in earlier granuloma clearance, earlier reduction in infiltration fraction, earlier bacillary clearance on tissue section and a significant finding was an increase in the epithelioid cells population in the immunotherapy group. This suggests a possible immunoactivation of the macrophages, especially in the BB/BL immunotherapy sgroup.

Histopathological upgrading and accelerated clearance have also been reported using MIP [12-17] and more recently the similar changes have been reported. Several studies have reported similar changes using BCG [18-20], using BCG+ killed *M leprae* and with ICRC.

In the present study, histological upgrading (unpublished observation), clearance of bacilli and healing without granuloma formation is achieved earlier in the interventional group, without an increase in the incidence of reactions (unpublished observation). This was not seen with the use of MDT alone. The vaccine was more effective in BB/BL cases as compared to BT cases because of immune status in BB/BL cases is lower and immune-enhancing effect of the vaccine is more evident in this group. The effects were better observed in the initial phases of treatment. Hence immunotherapy in the form of MIP can be recommended as an adjuvant with chemotherapy in borderline leprosy for better response.

One of the limitations of the study was that although 105 borderline cases were recruited, only 90 completed follow up during therapy.

Conclusion

This study shows the usefulness of adding immunotherapy (*Mycobacterium indicus pranaii vaccine)* to standard MDT in borderline leprosy. Addition of immunotherapy resulted in earlier granuloma clearance, earlier reduction in infiltration fraction, earlier bacillary clearance on tissue section Such information is expected to be useful in improving the immunotherapeutic approaches for treating granulomatous conditions in general and in leprosy in particular.

Acknowledgment

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