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**Original Article**

# Clinical and histopathological evaluation of the effect of addition of immunotherapy with *Mw* vaccine to standard chemotherapy in borderline leprosy

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This study reports detailed analysis of clinical parameters and clearance of granuloma in borderline leprosy patients treated with immunotherapy and chemotherapy. It aims to assess the additive effect of immunotherapy (*Mw* vaccine) with standard MDT on clinical status of untreated borderline leprosy cases and on granuloma fraction of untreated borderline leprosy cases. Patients attending the OPD were serially recruited in two groups. A total of 150 cases in one treatment (trial) group (*Mw* vaccine plus MDT) and 120 cases in another treatment (control) group (MDT only) of border line leprosy have been included. After the formal written consent, detailed clinical examination, charting, smear examination of all untreated borderline patients of both groups was done, biopsies were taken from the active lesions of all patients of both groups at start of therapy and every six month thereafter till the completion of therapy. The same procedure was repeated every six months during the follow-up period. Standard MDT was given to all the patients of both groups according to type of disease. *Mw* vaccine 0.1 ml (0.5 x $10^9$ bacilli) was injected intra-dermally at the start of therapy and every six months in addition to chemotherapy to the treatment group. The BT cases were followed up after 6 doses of MDT and 2 doses of *Mw* vaccine, and, the BB, BL cases were followed up after 24 doses of MDT plus 5 doses of *Mw* vaccine. Clinically, greater and faster improvement was observed in all the clinical parameters, faster attainment of smear negativity and two episodes of lepra reaction occurred in cases treated with combined chemotherapy and immunotherapy, as compared to controls (chemotherapy alone) wherein clinical improvement was slower in all parameters, slower attainment of smear negativity in bacillary index and seven showed the occurrence of reactions. histipathologically in addition to more rapid clearance of granuloma in immunotherapy treated group, a significant finding was an increase in the epithelioid cells population in this group. This suggests a possible immunoactivation of the macrophages especially in BB/BL immunotherapy group. Overall comparison of regression induced by chemotherapy alone with that induced by combined chemotherapy and immunotherapy shows a greater reduction in clinical parameters as well as granuloma fraction in BT cases as well as in BB/BL cases. This trial shows the potential usefulness of this approach of addition of immunotherapy to standard chemotherapy in borderline leprosy cases which leads to in faster recovery from disease reduced chances of reactions and faster granuloma clearance. Such information is expected to be useful in improving the immunotherapeutic approaches for treating granulomatous conditions in general and in leprosy in particular.

**Keywords:** Skin biopsies, Immunotherapy, Chemotherapy, Borderline leprosy, Granuloma

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## Introduction

Immunotherapy with BCG, BCG+ *M. leprae, Mw,* ICRC has been observed to be effective in improving the treatment in leprosy (Convit et al 1982, Bhatki and Chulawala 1992, Kar et al 1993, Talwar 1999. Clinical improvement with accelerated bacterial clearance and histological up grading using *Mw* vaccine has been reported in highly bacillated cases (Natrajan et al 1992, Sharma et al 2000, Katoch et al 2004). 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. *Mw* in general shows a good response in leprosy and in TB (Patel and Trapathi 2003).

This study has originated from involvement of our Institute in trials aimed at improving the therapy of leprosy and other mycobacterial diseases like tuberculosis by using combined immunotherapy and chemotherapy. Accelerated killing as well as clearance of bacilli and accelerated granuloma clearance has been documented in our earlier studies in highly bacillated leprosy cases (Katoch et al 2004). A trial has been carried out to assess the clinical and histopathological changes of the addition of *Mycobacterium w* (Mw) vaccine as a immunotherapeutic agent to standard chemotherapy in the immunological labile borderline leprosy which is characterized by a state of shifting immunity and there is presence of reactive episodes and persistence of granuloma. The objective of the present article is to present the findings of the aforesaid study which assess the additive effect of immunotherapy (*Mw* vaccine) with standard MDT on clinical status of untreated borderline leprosy cases and granuloma fraction of untreated borderline leprosy cases.

## Patients and Method

A non randomized trial was conducted. In this study, patients attending the OPD were serially recruited in two treatment groups. Group-1 (*Mw* vaccine plus MDT) A total of 150 cases BT-61, BB-54, BL-35 and in Group-2 (MDT only) 120 cases BT-51, BB-43, BL-26 of border line leprosy have been included. After the formal written consent, detailed history, clinical examination, charting of all clinical parameters of all untreated border line patients of both groups was done.

Skin biopsies were taken from active lesions of all the patients at the start of therapy followed by every six monthly till completion of therapy. Similar size and depth punch skin biopsy were obtained at all time from all the patients. The same procedure was repeated every six months during the follow-up period. Slit skin smears were taken from four sites and results were recorded on the Redley scale.

Standard MDT was given to all the patients of both groups according to type of disease. *Mw* vaccine 0.1ml ($0.5 \times 10^9$ bacilli) was injected intradermally at the start of therapy and every six months in addition to chemotherapy to the treatment group. The BT cases were followed up after 6 doses of MDT and 2 doses of *Mw* vaccine, and, the BB, BL cases were followed up after 24 doses of MDT plus 5 doses of *Mw* vaccine.

Therapeutic regimens –

1. Standard MDT plus Mw vaccine
2. Standard MDT

Follow up was done with respect to the following :

1. Local reaction to *Mw* vaccine.
2. Clinical progress of lesions with respect to following parameters.
   (i) Number and size of lesions.
   (ii) Erythema
   (iii) Infiltration
   (iv) Sensation
3. Reduction in bacillary index (B.I)
4. Incidence of reactions
5. Histopathology for bacillary and granuloma clearance.

It may reiterated that this was not a double blind trial and the patients were allotted serially in the two treatment groups. These patients were comparable clinically (clinical score), bacteriologically (BI), and histologically. It is, therefore, expected that the results using these parameters will be unbiased and comparable.

**Diagnosis of Borderline leprosy**

Borderline leprosy cases will be classified into three groups according to Ridley and Jopling Classification based on immunohistological scale

**1. Borderline Tuberculoid (BT) –** Skin lesions are single or few in number, variable in size and dry, impaired touch, pain and temperature sensation. Lepromin response is positive and usually skin smears are negative. Histopathology shows narrow clear sub epidermal zone above the granulomas, Lymphocyte are plentiful but less well focalized. Nerves swollen but recognizable. B.I. = 0-2+(AFB).

**2. Borderline Borderline (BB) –** Skin lesions are numerous, variable in size and shiny. Sensation is impaired. Smear is moderately positive and Lepromin test is negative. Histopathology shows sheets of epitheliod cells with no giant cells. Lymphocytes are rather sparse and diffusely infiltrating nerves show structural disorganization but no granulomas. B.I.=3–4+ have few lymphocytes.

**3. Borderline Lepromatous (BL) –** Large no. of lesions, variable in size, shiny surface, and sensation slightly diminished. Skin smears are strongly positive, but Lepromin response is negative. Histopathologically seen histiocytic granulomas with cells of slightly epitheliod appearance, heavily laden with bacilli, few lymphocytes. There is infiltration with foamy cells but no golbi. Numerous diffuse lymphocytes are seen, more than in BB or BT. B.I= 4-5 +.

**Exclusion criteria :**

Patients who were failed to provide conclusive evidence for the diagnosis of borderline leprosy.

Patients of Tuberculosis, HIV infection , additional immunosuppressive illness such as Diabetes mellitus, Hematological reticuloendothelial malignencies and mental illness were excluded.

**Clinical Scoring**

- Each patient was assessed at admission at $6^{th}$ month, at $12^{th}$ month, at $18^{th}$ month and at $24^{th}$ month.
- For each lesion maximum possible score at intake was 12 and the minimum score at follow up was zero.

Each patient was clinically assessed and scores were given as shown in the following table.

| Clinical parameters | Marked | Moderate | Mild |
|---|---|---|---|
| *Size of lesion | 3 (clearly visible) | 2 (faintly visible) | 1 (Doubtful) |
| Erythema | 3 | 2 | 1 |
| Infiltration | 3 | 2 | 1 |
| Anasthesia | 3 (complete loss) | 2 (Definite impairment) | 1 (Doubtful impairment) |

Size (Area of lesion = Length x Breadth), Maximum score: 3 (for every up to 20% increase or decrease, subtract 0.5, lesion disappeared - 0)

Maximum clinical sore for each parameter was 3 at the time of intake and subsequent reduction in this score was noted at every six monthly interval.

*The above scoring system is same as used in the 2-3 lesion multi-centric trial group. *Indian J Lepr.* 2005 Jan-Mar; **77(1)**: 19-25.

### Histopathology

Biopsies of the patients were fixed in buffer formalin and subsequently embedded in paraffin. Every sample were stained with hematoxylin-eosin for morphology, and fite-faraco for detection of bacilli Histopathological characteristics in granuloma morphology and their cellular populations and bacterial load was studied.

The ethical approval for this study was taken from the Ethical Committee of the Institute. Data were analyzed using tabular and graphic presentation and statistical procedures viz. summary statistics and proportion test for comparison.

## Results

As described, 150 borderline cases in trial group and 120 borderline cases in control group were recruited in the study. They were further distributed according to sex, age and type of diseases. Out of 150 subjects in first treatment (trial) group, there were 85 (56.7%) males and 65 (43.3%) females. In the second treatment (control) group, out of 120 subjects, there were 64 (53.3%) were males and 56 (46.7%) were females (Table 1).

The age distribution of subjects in both the treatment groups is comparable. About one-third subjects in both the treatment groups are of age 15 or less; 43.3% subjects belong to adult age (16-50) years in both the treatment groups. About a quarter (23.3% in first treatment group and 25% in second treatment group) subjects are aged (above 50 years (Table 2).

Table 3 provides distribution of subjects by the type of disease, Borderline Tuberculoid (BT), Borderline Borderline (BB) and Borderline Lepromatous (BL) in both the treatment groups. In Group 1, out of 150 subjects, 61 were BT with 12 cases as AFB+, 54 were BB with 42 AFB+ and rest 35 with 30 AFB+. In Group 2, 51 cases were BT with 9 as AFB+, 43 were BB with 32 as AFB+ and 26 were BL with 534 as AFB+.

This is first report which has observed improvement in the clinical parameters of BT leprosy (Table 4). The clinical parameters included to observed the clinical progress of disease like size of lesion, erythema, infiltration, sensation all showed faster improvement with Mw vaccine as compared to controls (only chemotherapy)

**Table 1 : Distribution of the borderline patients Group-1 (MDT + Mw) and Group-2 (MDT only) according to Sex**

| SEX | Group-1 ( MDT+Mw ) | Group-2(MDT only) |
|---|---|---|
| MALE | 85(56.66%) | 64(53.33%) |
| FEMALE | 65(43.33%) | 56(46.66%) |
| TOTAL | 150(100%) | 120(100%) |

**Table 2 : Distribution of the borderline patients Group-1 (MDT+Mw) and Group-2 (MDT only) according to Age**

| AGE | Group-1 ( MDT+Mw ) | Group-2(MDT only) |
|---|---|---|
| UP TO 15 YEARS | 50 (33.33%) | 38 (31.66%) |
| 16 TO 50 YEARS | 65 (43.33%) | 52 (43.33%) |
| > 50 YEARS | 35 (23.33%) | 30 (25.00%) |
| TOTAL | 150 (100%) | 120 (100%) |

**Table 3 : Distribution of the borderline patients Group-1 (MDT+Mw) and Group-2 (MDT only) according to type of disease**

| TYPE OF DISEASE | Group-1 ( MDT+Mw ) | Group-2 (MDT only) |
|---|---|---|
| BT | 61(12)* | 51(9)* |
| BB | 54(42)* | 43(32)* |
| BL | 35 (30)* | 26(21)* |
| TOTAL | 150(72)* | 120(53)* |

*AFB positive

**Table 4 : The clinical progress of BT cases in both Groups after 24 months of follow up**

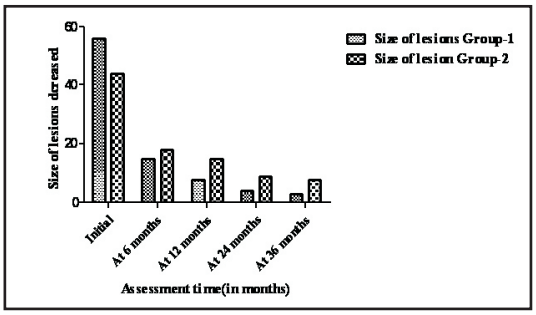
| Clinical Parameters | Study Groups | BT During Treatment At 6mths | BT During follow-up At 12 mths | BT During follow-up At 24 | BT During follow-up At 36 |
|---|---|---|---|---|---|
| **Size and No. of** | **Group-1( MDT+Mw ) 56** | 73%(41) | 86%(48) | 93%(52) | 95%(53) |
| **lesion decreased** | **Group-2(MDTonly)44** | 55%(24) | 66%(29) | 80%(35) | 82%(36) |
| P Value | | .026 | .010 | .025 | .021 |
| **Erythema Decreased** | **Group-1( MDT+Mw ) (56)** | 91%(51) | 100%(56) | 100%(56) | 100%(56) |
| | **Group-2(MDTonly) (44)** | 66%(29) | 75%(33) | 89%(39) | 95%(42) |
| P Value | | .001 | .0001 | .026 | .045 |
| **Infiltration Decreased** | **Group-1( MDT+Mw ) (56)** | 93%(52) | 100%(56) | 100%(56) | 100%(56) |
| | **Group-2(MDTonly) (44)** | 68%(30) | 75%(33) | 86%(38) | 91%(40) |
| P Value | | .0007 | .0001 | .002 | .011 |
| **Sensory Improvement** | **Group-1( MDT+Mw ) (56)** | 61%(34) | 80%(45) | 88%(49) | 89%(50) |
| | **Group-2(MDTonly) (44)** | 52%(23) | 61%(27) | 68%(30) | 70%(31) |
| P Value | | .183 | .018 | .007 | .009 |

where the clinical improvement was slower at 6, 12, 24 and 36 months of assessment period. The difference between the values of the two groups was statistically significant. The improvement in each clinical parameter is also depicted through the bar graphs 1,2,3,4.

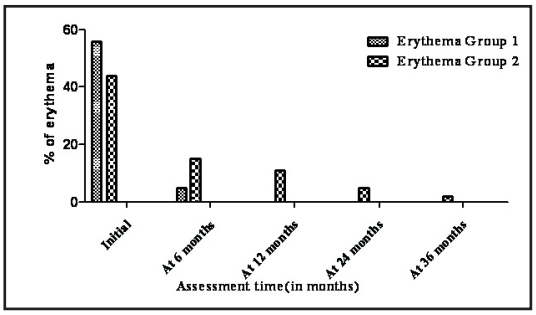
The clinical progress of all parameters in BB, BL cases was also observed after 5 doses of Mw vaccine plus MDT at 6, 12, 24 and 36 months of assessment period. There was good clinical improvement in these cases (Table 5).

The mean clinical score of cases treated with Mw+ chemotherapy showed significant and rapid reduction of clinical score at 6, 12, 24 and 36 months of assessment period as compared to only chemotherapy group where we observed slower and lesser reduction.

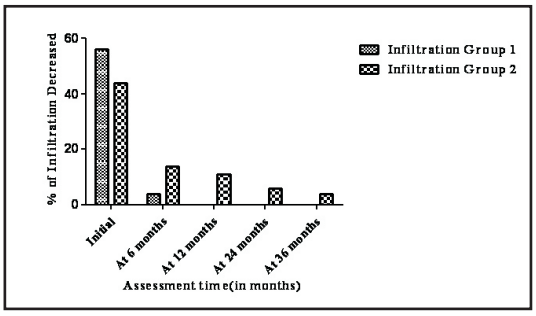
(Table-6). In BT patients in both the regimens mean clinical score decreased substantially over to 36months: from 7.80 $\pm$1.69 to 0.53 $\pm$ 1.11 and from 7.77$\pm$1.62 to 2.20$\pm$ 1.30 in the both group respectively. The differences between the mean values of the two groups were continuous & statistically significant ($p<0.01$) at 6, 12, 24 & 36 months. These observations are also shown in bar graph 5.



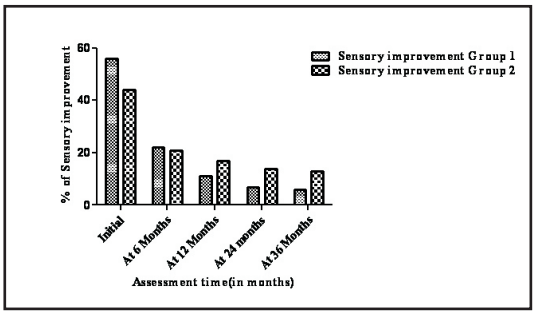

**Graph-1 : Effect of immunotherapy on Size of lesions of BT patients of group-1 & group-2 at different intervals**



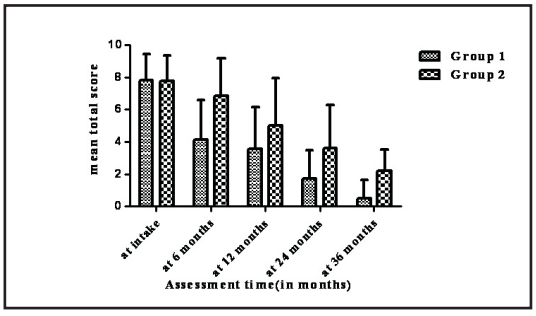

**Graph-2 : Effect of immunotherapy on erythema of BT patients of group 1 & group 2 at different intervals**



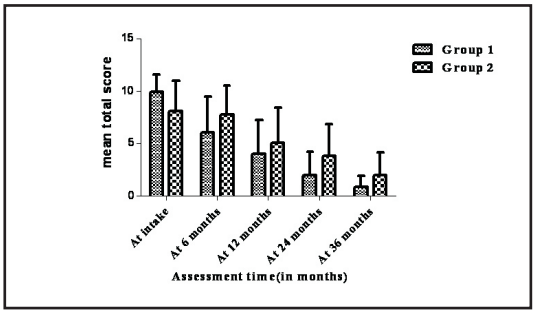

**Graph-3 : Effect of immunotherapy on infiltration of BT patients of group 1 & group 2 at different intervals**




**Graph-4 : Effect of immunotherapy on sensory improvement of BT patients of group 1 & group 2 at different intervals**




**Graph-5 : Effect of immunotherapy on mean total score of BT patients of group 1 & group 2 at each assessment**




**Graph-6 : Effect of immunotherapy on mean total score of BTpatients of group 1 & group 2 at each assessment**

The BB, BL cases were followed up after 5 doses of Mw vaccine plus 24 doses of MDT. In BB/BL group also in both the regimens mean clinical score decreased substantially over to 36 months: from 9.92 $\pm$1.62 to 0.85 $\pm$1.03 and from 8.136$\pm$2.86 to 1.97 $\pm$ 2.17 in the both group respectively

**Table 5 : The clinical progress of BB/BL cases in both Groups after 12 months of follow up**

| Clinical Parameters | Study Groups | During Treatment | | | During follow-up |
|---|---|---|---|---|---|
| | | At 6 Months | At 12 Months | At 24 Months | At 36 Months |
| **Size and No. of** | **Group-1 (MDT+Mw) (80)** | 56%(45) | 68%(54) | 78%(62) | 91%(73) |
| **lesion decreased** | **Group-2(MDTonly) (66)** | 35%(23) | 45%(30) | 61%(40) | 67%(44) |
| P Value | | .005 | .004 | .013 | .0001 |
| **Erythema Decreased** | **Group-1 (MDT+Mw) (80)** | 53%(42) | 63%(50) | 75%(60) | 88%(70) |
| | **Group-2(MDTonly) (66)** | 35%(23) | 44%(29) | 58%(38) | 71%(47) |
| P Value | | .016 | .013 | .013 | .007 |
| **Infiltration Decreased** | **Group-1 (MDT+Mw) (80)** | 53%(42) | 68%(54) | 78%(62) | 89%(71) |
| | **Group-2(MDTonly) (66)** | 30%(20) | 48%(32) | 61%(40) | 76%(50) |
| P Value | | .004 | .010 | .013 | .019 |
| **Sensory Improvement** | **Group-1 (MDT+Mw) (80)** | 55%(44) | 63%(50) | 69%(55) | 73%(58) |
| | **Group-2(MDTonly) (66)** | 33%(22) | 47%(31) | 50%(33) | 58%(38) |
| P value | | .004 | .030 | .011 | .029 |

**Table 6 : Effect of immunotherapy on mean total score at each assessment BT patients**

| Assessment time | Statistical parameters | Group-1(BT) (56) | Group-2(BT )(44) | P values |
|---|---|---|---|---|
| At Intake | Mean score | 7.8 | 7.77 | 0.9287 |
| | S.D | 1.69 | 1.62 | |
| At 6 months | Mean score | 4.12 | 6.88 | 0.000 |
| | S.D. | 2.47 | 2.32 | |
| At 12 months | Mean score | 3.57 | 5.04 | 0.0088 |
| | S.D. | 2.58 | 2.91 | |
| At 24 months | Mean score | 1.73 | 3.59 | 0.0001 |
| | S.D. | 1.75 | 2.71 | |
| At 36 months | Mean score | 0.53 | 2.2 | 0.000 |
| | S.D. | 1.11 | 1.3 | |

(Table 7). The differences between the mean values of the two groups were continuous & statistically significant ($p<0.01$) at 6, 12, 24 & 36 months. These observations are also shown in bar graph 6.

All the patients in the present series were grouped in to BT with high GF ranging 70-75% and BT with moderate ranging 43-45% like this BB/BL were also grouped in to BB/BL with high GF ranging 70-75% and BB/BL with moderate ranging 42-45% showing a macrophage granuloma with the granuloma fraction ranging from 60 to 70% (Table 8).

In BT cases (Table 8), the initial high granuloma fraction (mean value 72.69%) decreased to mean of 45.3% after first dose of Mw with MDT, further

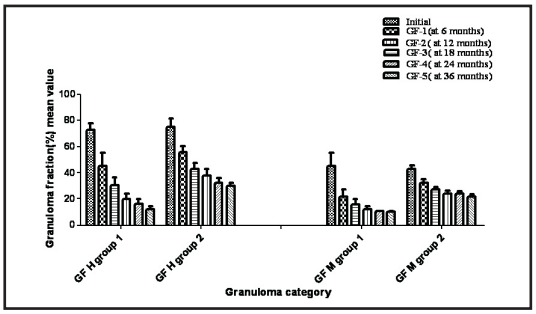
**Table 7 : Effect of immunotherapy on mean total score at each assessment BB/BL patients**

| Assessment time | Statistical parameters | Group-1BB/BL (80) | Group-2BB/BL (66) | P values |
|---|---|---|---|---|
| At Intake | Mean score | 9.92 | 8.13 | 0.9135 |
| | S.D | 1.62 | 2.86 | |
| At 6 months | Mean score | 6.07 | 7.8 | 0.000 |
| | S.D. | 3.41 | 2.71 | |
| At 12 months | Mean score | 3.95 | 5.09 | 0.0015 |
| | S.D. | 3.24 | 3.28 | |
| At 24 months | Mean score | 1.98 | 3.83 | 0.000 |
| | S.D. | 2.21 | 2.96 | |
| At 36 months | Mean score | 0.85 | 1.97 | 0.000 |
| | S.D. | 1.03 | 2.17 | |

**Table 8 : Histological findings of BT cases in both groups after 24 mths of follow-up**

| Type of diseases | Statistical parameters | Initial | At 6 months | At 12 months | At 18 months | At 24 months | At 36 months |
|---|---|---|---|---|---|---|---|
| Group-1BT (23) | Mean | 72.69 | 45.3 | 30.43 | 19.91 | 16.26 | 12.26 |
| High GF | S.D. | 5.18 | 10.02 | 5.74 | 3.99 | 3.37 | 2.00 |
| Group-2BT (21) | Mean | 75.00 | 55.85 | 43.33 | 37.8 | 32.19 | 30.14 |
| High GF | S.D. | 6.81 | 4.69 | 4.01 | 5.18 | 3.65 | 2.41 |
| P value | | 0.2100 | 0.0001 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| Group-1BT (22) | Mean | 45.36 | 22.04 | 15.36 | 12.18 | 10.31 | 10.13 |
| Moderate GF | S.D. | 10.17 | 5.57 | 4.61 | 1.91 | 0.56 | 0.35 |
| Group-2BT (18) | Mean | 43.11 | 32.05 | 27.33 | 24.22 | 24.00 | 22.05 |
| Moderate GF | S.D. | 2.65 | 3.11 | 1.84 | 1.7 | 1.53 | 1.43 |
| P value | | 0.3677 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |

reduced to 30.43% after second dose and was last 12.26% after 2 years of follow up period. In the controls the initial mean value of granuloma fraction decreased from 75% to 56% at 6 months, then further reduced to 43% (at 12 months) and was last 30% at 2 years follow up. Same reduction was observed in moderate GF BT cases with immunotherapy. The reduction was rapid and statistically significant at different time intervals as compare to Group-1 BT, also shown in bar graph 7.



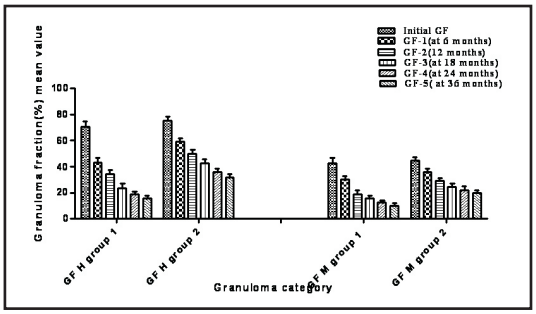

**Graph-7 : Histological findings with group 1 & group 2 (BT patients) after 24 months of follow-up**

Table-9 showing the reduction of granuloma fraction and in both sub groups of high and moderate GF. In BB/BL cases with high GF, initial 75% mean value of granuloma fraction reduced to 43% after first dose and last 15% after 2 years as compared to controls where initial 75% of granuloma fraction remained 32% after 2 years. And in BB/BL cases with moderate GF initial 42.72% last 10.03% after 2 years as compare to BB/BL controls where the decrease is from 45.04% to 19.63% depicting promising effect of Immunotherapy on granuloma clearance. Which is also shown in bar graph 8 below.

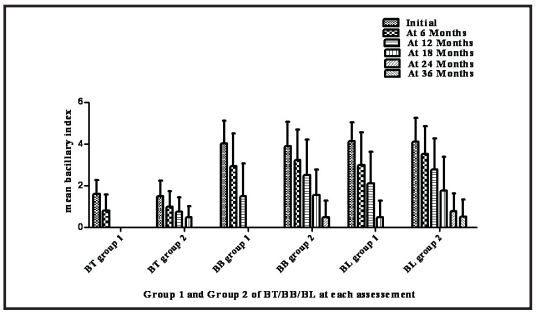
In addition to more rapid clearance of granuloma in immunotherapy treated group, a significant finding was an increase in the epithelioid cells population in this group. This suggests a possible immunoactivation of the macrophages especially in BB/BL immunotherapy group. Overall comparison of regression induced by chemotherapy alone with that induced by combined chemotherapy and immunotherapy, shows a greater reduction in clinical parameters as well as granuloma fraction in BT cases as well as in BB/BL cases.

**Table 9 : Histological findings with BB/BL cases in both groups after 12 months of follow-up**

| Type of diseases | Statistical parameters | Initial | At 6 months | At 12 months | At 18 months | At 24 months | At 36 months |
|---|---|---|---|---|---|---|---|
| BB/BL Cases (38) | Mean | 70.97 | 42.84 | 34.05 | 23.23 | 18.26 | 15.34 |
| High GF | S.D. | 3.97 | 3.77 | 3.14 | 3.56 | 2.64 | 2.45 |
| BB/BL Controls(30) | Mean | 75.00 | 59.2 | 49.9 | 42.5 | 35.93 | 31.96 |
| High GF | S.D. | 3.26 | 2.51 | 2.85 | 3.25 | 2.67 | 2.28 |
| P value | | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| BB/BL Cases(33) | Mean | 42.72 | 30.00 | 18.42 | 15.30 | 12.06 | 10.03 |
| Moderate GF | S.D. | 3.77 | 2.88 | 3.00 | 2.32 | 1.81 | 1.62 |
| BB/BL Controls(22) | Mean | 45.04 | 36.13 | 29.13 | 23.18 | 21.95 | 19.63 |
| Moderate GF | S.D. | 2.31 | 2.39 | 1.83 | 3.45 | 2.64 | 1.91 |
| P value | | 0.0130 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |




**Graph-8 : Effect of immunotherapy on mean ganuloma fraction of BB/BL patients of group 1 & group 2**




**Graph-9 : Effect of immunotherapy on mean bacillary index of BT/BB/BL patients of group 1 & group 2 at each assessment**

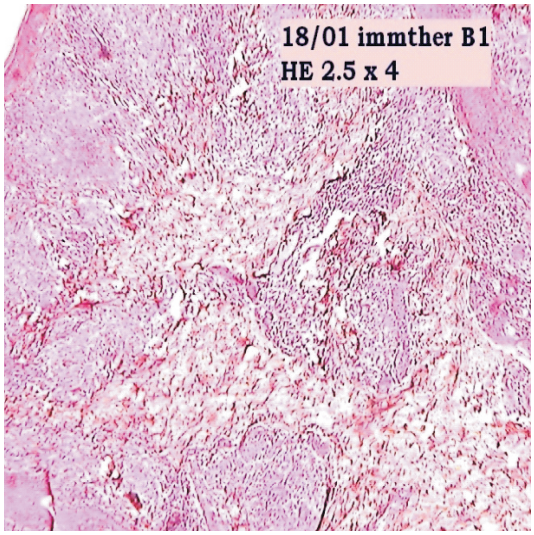



**Fig 10 : Tissue specimen of BT case showing extensive branching granuloma before initiation of therapy**

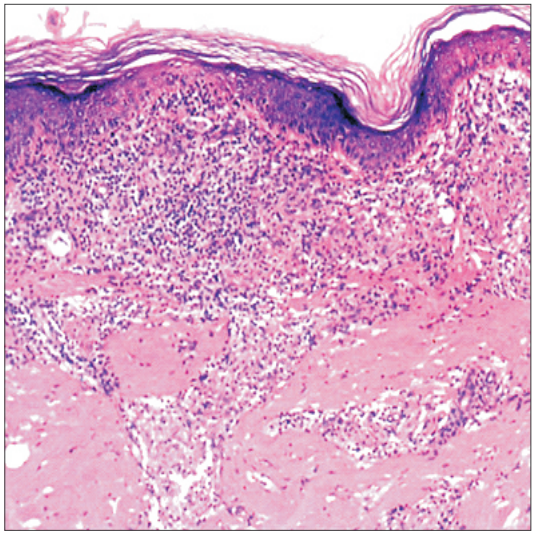


**Fig. 11 : BT, after Ist dose of *Mw* showing few giant cells and sub- epidermal zone clearing of infiltrate**

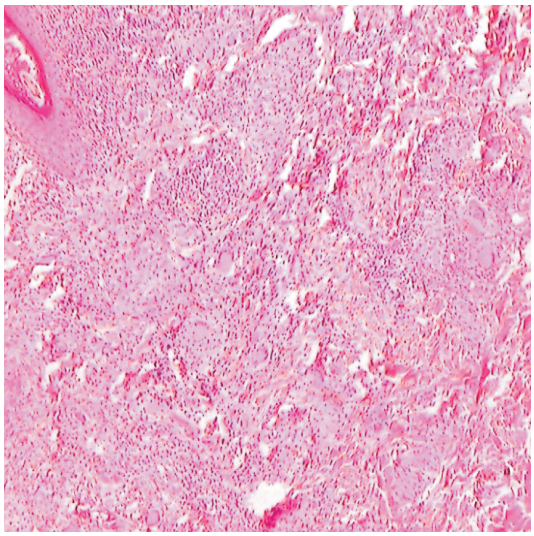


**Fig. 10 A : Tissue specimen of BT control before initiation of therapy**

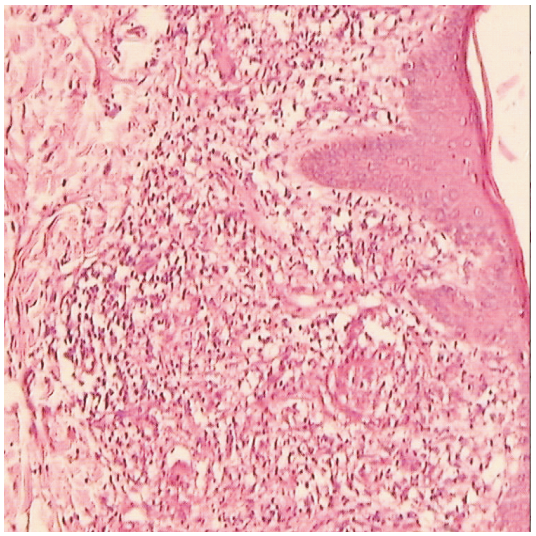


**Fig. 11 A : BT control, after 6 months of MDT showing separation of cells within granuloma**

The fall in BI (Table 10) was much faster in cases group-1 (MDT + immunotherapy groups) as compared to group-2 (MDT alone). As a result of a faster fall in the BI, the patients in (MDT + immunotherapy group) became negative much earlier than the patients in (MDT alone) in BT, BB

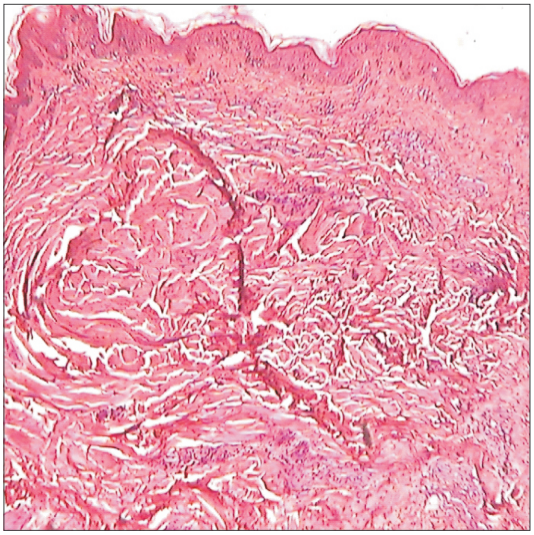


**Fig. 12 : BT, after 2 doses of mw plus 6 months of MDT and12 months follow up showing replacement of granuloma with fibrous tissues**

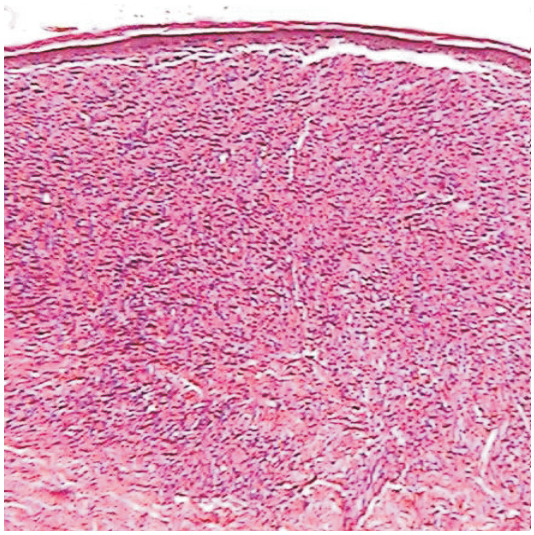


**Fig. 13 : Tissue specimen of BB case showing granuloma before initiation of therapy**

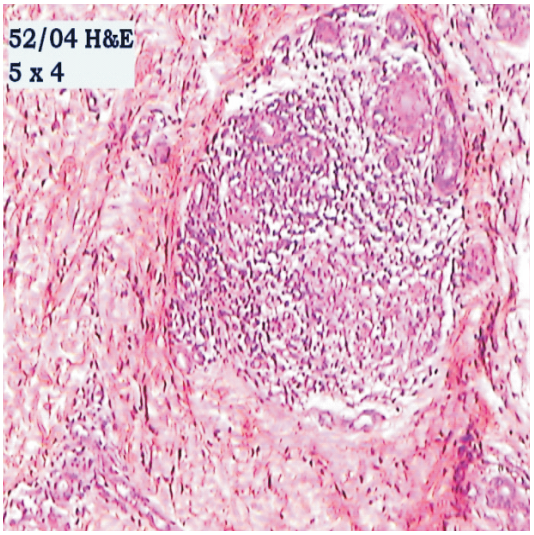



**Fig. 12 A : BT control, after 6 mths of MDT and 12 months of follow-up MDT showing slow clearance of cells within granuloma**

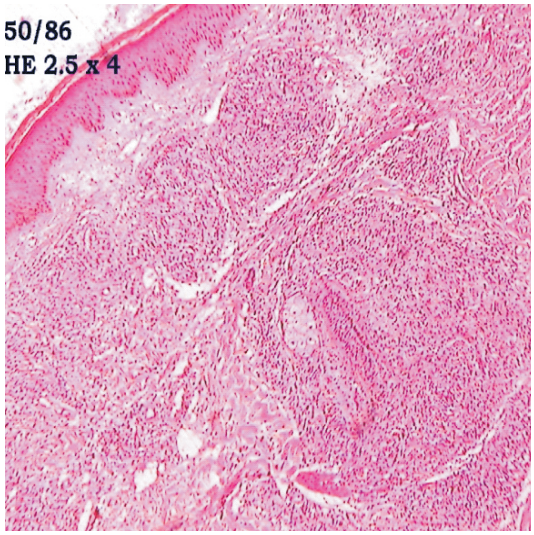



**Fig. 13 A : Tissue specimen of BB control, Before initiation of therapy**

and BL sub groups of all the borderline patients. Table showing greater decrease in mean bacillary index in BT, BB and BL cases from 1.6, 4.02 and 4.14 to 0.00, 0.00 and 0.00 (on 12, 18 and 24 months respectively) at different time intervals which is earlier and statistically significant as

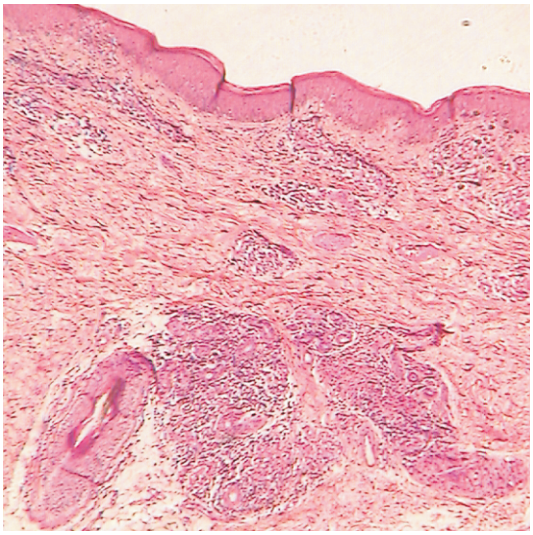


**Fig 14 : BB case after 12 mth of MDT+ 3 doses of Mw**

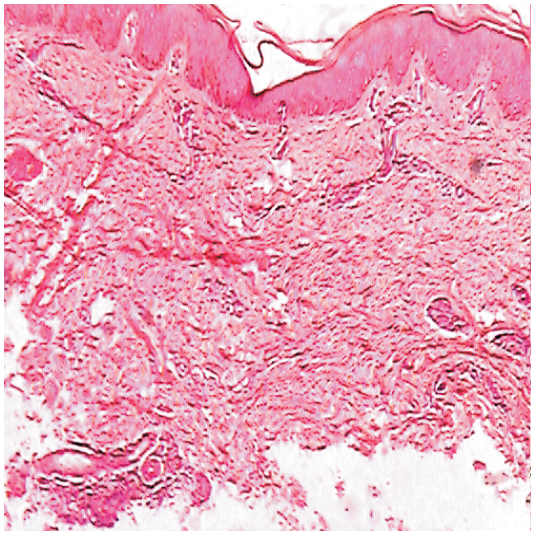


**Fig. 15 : BB case after 18 mth of MDT+4 doses of Mw**

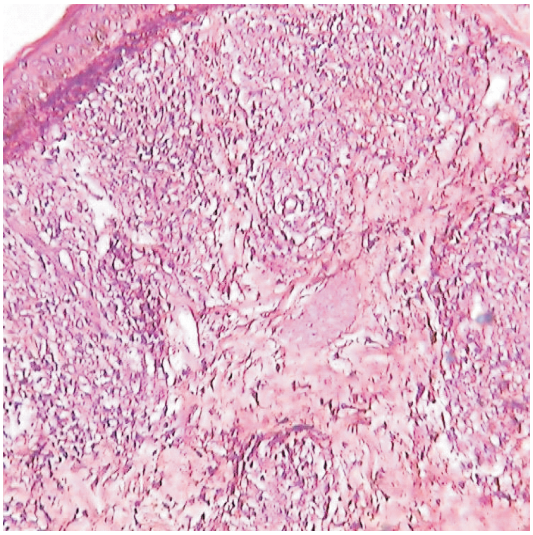


**Fig. 14 A : BB control, after 12 mths of MDT**

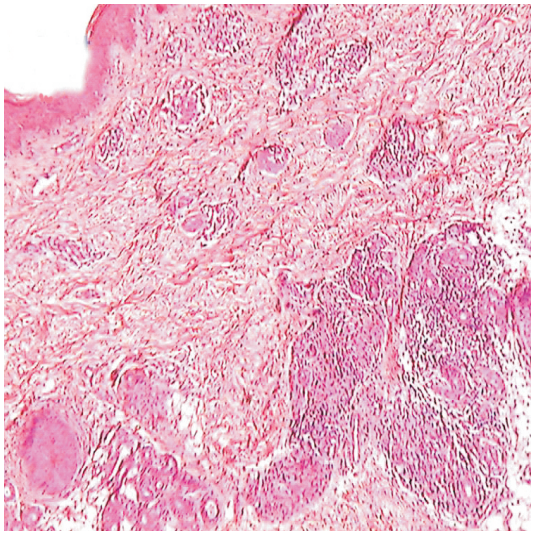


**Fig. 15 A : BB control, after 18 mths of MDT**

compare to BT, BB and BL controls where the decrease is from 1.5, 3.9 and 4.10 to 0.00, 0.00 and 0.52 (on 24, 36 and 36 months respectively) also shown in the bar graph 9.

Table 11 showing greater fall of mean bacillary index in tissue section, the mean bacillary index in group 1 (BT, BB and BL) from 1.7, 4.2 and 4.1 to 0.0, 0.0 and 1.6 (on 18 months and 0.0 at 24

**Table 10 : Effect of immunotherapy on mean bacillary index of BT, BB, BL cases in both groups**

| Type of diseases | Mean bacillary index | At Intake | At 6 months | At 12 months | At 18 months | At 24 months | At 36 months |
|---|---|---|---|---|---|---|---|
| Group-1BT (10) | Mean bacillary index | 1.6 | 0.8 | 0.00 | 0.00 | 0.00 | 0.00 |
| | S.D. | 0.69 | 0.78 | 0.00 | 0.00 | 0.00 | 0.00 |
| | Successive p-values | | 0.0258 | 0.0045 | | | |
| Group-2BT Cases (08) | Mean bacillary index | 1.5 | 1.00 | 0.75 | 0.5 | 0.00 | 0.00 |
| | S.D. | 0.75 | 0.75 | 0.70 | 0.53 | 0.00 | 0.00 |
| | Successive p-values | | 0.2037 | 0.5019 | 0.4341 | 0.0184 | |
| Group-1BB (39) | Mean bacillary index | 4.02 | 2.92 | 1.51 | 0.00 | 0.00 | 0.00 |
| | S.D. | 1.11 | 1.59 | 1.57 | 0.00 | 0.00 | 0.00 |
| | Successive p-values | | 0.0007 | 0.0002 | 0.0000 | | |
| Group-2BB (30) | Mean bacillary index | 3.9 | 3.23 | 2.5 | 1.56 | 0.5 | 0.00 |
| | S.D. | 1.15 | 1.45 | 1.69 | 1.22 | 0.77 | 0.00 |
| | Successive p-values | | 0.0521 | 0.0778 | 0.0165 | 0.0002 | 0.0008 |
| Group-1BL (28) | Mean bacillary index | 4.14 | 3.00 | 2.10 | 0.5 | 0.00 | 0.00 |
| | S.D. | 0.89 | 1.56 | 1.54 | 0.79 | 0.00 | 0.00 |
| | Successive p-values | | 0.0014 | 0.0342 | 0.0000 | | |
| Group-2BL (19) | Mean bacillary index | 4.10 | 3.52 | 2.78 | 1.78 | 0.78 | 0.52 |
| | S.D. | 1.15 | 1.34 | 1.51 | 1.6 | 0.85 | 0.84 |
| | Successive p-values | | 0.1608 | 0.1188 | 0.0552 | 0.0214 | 0.3493 |

months) at different time intervals which is earlier and statistically significant as compare to group-2 (BT, BB and BL) where the decrease is from 1.6, 4.0 and 4.1 to 0.00, 0.4 (on 36 months).

*Mycobacterium* w was well tolerated by the patients and did not lead to any systemic side effects. There was a local reaction to the vaccination in the form of erythema and induration at 24–48 h and nodule formation at 4 weeks. In some cases there was ulceration of the nodule which healed on its own in few days. On subsequent vaccination the reaction did not produce any ulceration. In the present trial one patients of BB (MDT + immunotherapy group)

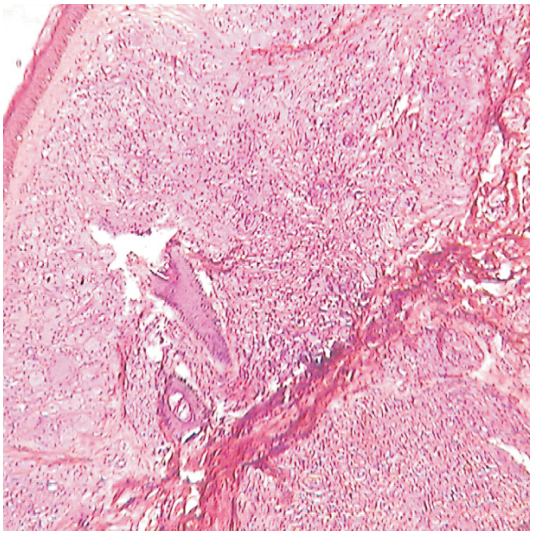


**Fig. 16 : Tissue section of BL patient, before therapy showing extensive granuloma formation with atrophy of epidermis and clear sub epidermal zone**

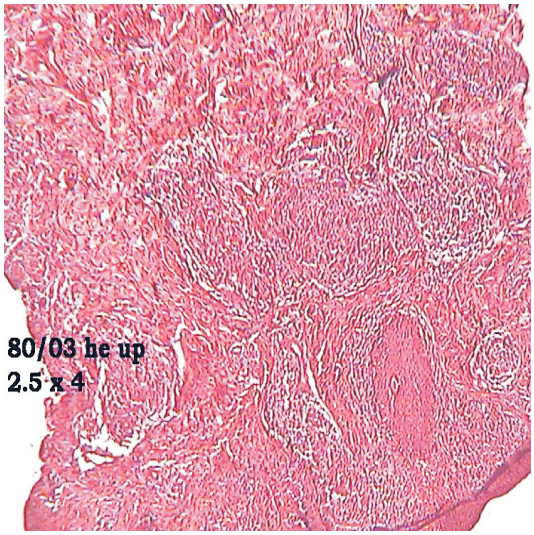



**Fig. 17 : BL after12 doses of MDT plus 3 doses of Mw showing faster clearance of granuloma**

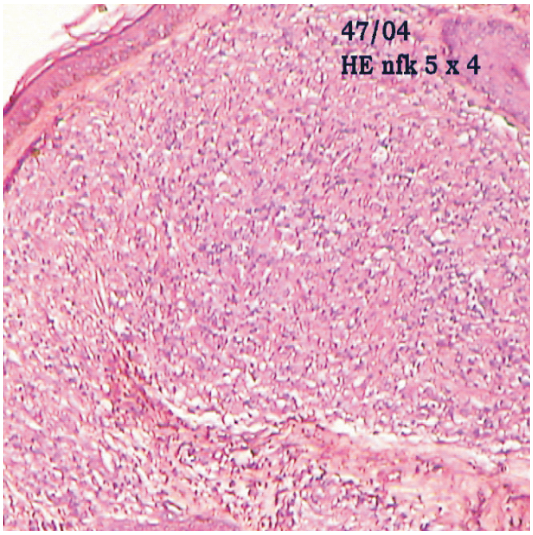



**Fig. 16A : BL control before of initiation of therapy**

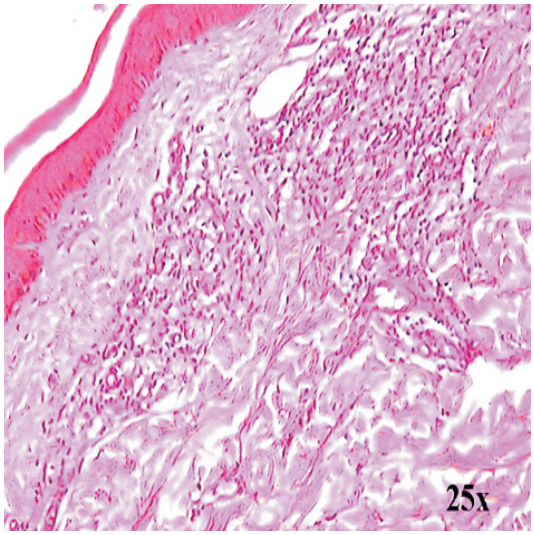



**Fig. 17A : BL control after 12 doses of MDT showing slow Clearance of granuloma**

suffered from type-1 reaction during the therapy and one patients of BL (MDT+immunotherapy group) suffered from type-1 reaction in the post-treatment follow-up at 10 months of 2 and half year. None of the patients from BT sub group suffered from any kind of reaction during therapy

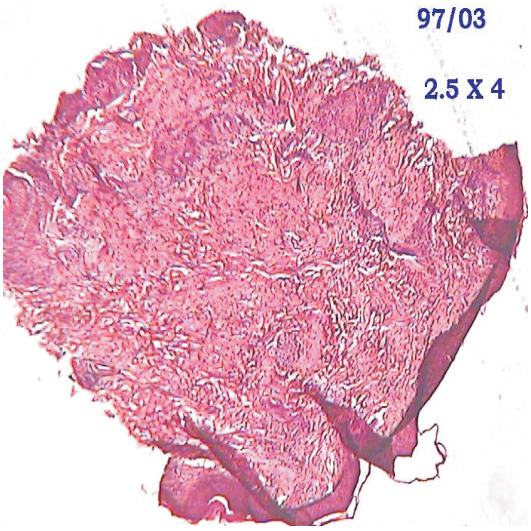



**Fig. 18 : BL, after 4 doses of Mw plus 18 months of MDT therapy showing clearance of granuloma**

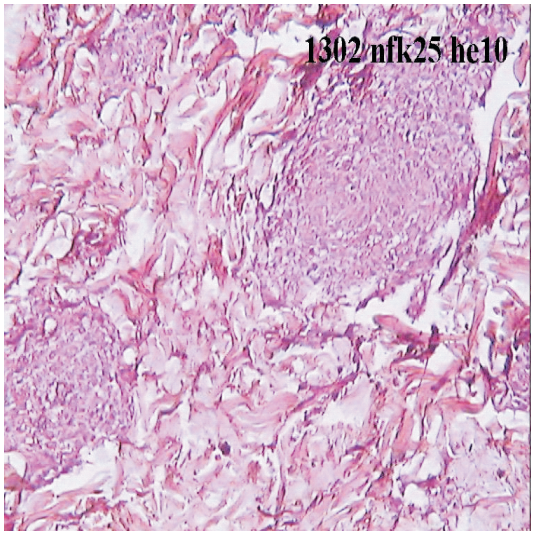



**Fig. 18 A : BL control, after 18 months of MDT only showing slower clearance of granuloma**

**Table 11 : Fall in bacillary index in tissue sections in both groups**

| Type of diseases | Mean bacillary index | Initial | At 6 months | At 12 months | At 18 months | At 24 months | At 36 months |
|---|---|---|---|---|---|---|---|
| Group-1 BT (10) | Mean bacillary index | 1.7 | 0.8 | 0.0 | 0.0 | 0.0 | 0.0 |
| Group-2 BT (08) | Mean bacillary index | 1.6 | 1.0 | 0.8 | 0.6 | 0.0 | 0.0 |
| Group-1 BB (39) | Mean bacillary index | 4.2 | 3.0 | 1.6 | 0.0 | 0.0 | 0.0 |
| Group-2 BB (30) | Mean bacillary index | 4.0 | 3.4 | 2.6 | 1.6 | 0.6 | 0.0 |
| Group-1 BL (28) | Mean bacillary index | 4.1 | 2.9 | 2.1 | 0.6 | 0.0 | 0.0 |
| Group-2 BL (19) | Mean bacillary index | 4.1 | 3.4 | 2.8 | 1.7 | 0.78 | 0.4 |

and in follow up period as compared to (MDT only) group where 7 patients showed the occurrence of reactions (3 ENL and 4 reversal reaction). (1BT, 2BB, 3BL) suffered from Reversal reaction and ENL during the treatment and one patients of BL suffered from ENL reaction during follow up period. Overall two incidence of reaction occurred in the cases (Mw + MDT) as compared to controls (MDT only) where 7 patients showed the occurrence of reactions (Table 12).

**Table 12 : Effect of immunotherapy on the incidences of reactions**

| Type of Disease | Type of Category | During Therapy | During Follow-up |
|---|---|---|---|
| **BT** | **Group-1 (MDT+Mw)** | **0** | **0** |
| | **Group-2 (MDT only)** | 1 (Type-1Reaction) at 6th Month of therapy | **0** |
| **BB** | **Group-1 (MDT+Mw)** | 1(Type-1Reaction) 10th month of therapy | **0** |
| | **Group-2 (MDT only)** | 2(Type-1Reaction) at 6th & 9th Month of therapy | **0** |
| **BL** | **Group-1 (MDT+Mw)** | 1(Type-1Reaction) at 12th month of therapy | **0** |
| | **Group-2 (MDT only)** | 3(1 Type-2Reaction) at 3rd Month after therapy, 2 (Type-1 reactions) at 9th & 12th month of therapy | 1(1 Type-2Reaction) at 10th Month of follow up |

## Discussion

As the nation is passing through the eradication phase of leprosy, reports are suggesting a change in epidemiology and symptomatology of the disease. More patients of borderline leprosy as compared to highly bacillated forms of the diseases are prevailing, and in our earlier studies it was recommended to consider the addition of immunotherapy (both BCG and Mw) to chemotherapy to achieve faster bacteriological and histological responses. In the present study by the addition of Mw as immunotherapy to MDT an attempt has been made to effectively reduce the duration of treatment in borderline leprosy. This will help in substantially reducing the morbidity of the disease in terms of both reactions and relapses.

The clinical parameters were included to observe the clinical progress of disease like size of lesion, erythema, infiltration, sensation (Table 4). All showed faster improvement with combination of MDT and Mw vaccine as compared to controls (only MDT) where the clinical improvement was slower at 6/12/24 and 36 months of assessment period. The clinical progress of all parameters in BB, BL cases was also observed after 5 doses of Mw vaccine plus MDT at 6/12/24 and 36 months of assessment period (Table 5). There was also good clinical improvement in these cases. In BT group in both the regimens mean clinical score decreased substantially over 36 months: from 7.80$\pm$1.69 to 0.53$\pm$1.11 and from 7.77$\pm$1.62 to 2.2$\pm$1.3 in the study and control group respectively. The differences between the mean values of the two groups were continuous and statistically significant ($p<0.01$) at 6, 12, 24 and 36 months. In BB/BL groups also, in both the regimens, mean clinical score decreased substantially over 36 months: from 9.92$\pm$1.62 to 0.85$\pm$1.03 and from 8.136$\pm$2.86 to 1.97$\pm$2.17 in the study and control groups respectively. The difference between the mean values of the two groups were continuous and statistically significant ($p<0.01$) at 6/ 12/ 24 and 36 months. The mean clinical score of cases treated with Mw+MDT showed significant and rapid reduction of clinical score at 6, 12, 24 and 36 months of assessment period as compared to only chemotherapy group where we observed slower

and lesser reduction in BT/BB/BL leprosy (Table 6, 7). This rapid clinical improvement by the addition of immunomodulators to MDT in BB/BL leprosy has also been reported in our earlier study (Katoch et al 2004). Narang et al (2005) also reported that using Mw and BCG vaccines, the mean reduction in clinical scores in BCG and Mw groups was significantly more when compared to controls. At 12 and 24 months, the patients in BCG group had significantly greater reduction in Ramu 's score as compared to those in the Mw group. BCG exhibited slightly better and faster effect on bacteriological clearance and clinical improvement as compared to Mw vaccine in borderline lepromatous (BL)/polar lepromatous (LL) patients with a high initial B.I.

Although the initial BI was comparable in both groups, the fall in BI during the course of treatment was different. BI is a semi-quantitative measure of the total load and includes both the live as well as the dead bacilli. The fall in BI was much faster in MDT + immunotherapy groups as compared to controls MDT alone (Table 10). As a result of a faster fall in the BI, the patients in MDT + immunotherapy group became negative much earlier than the patients in (MDT alone) in BT, BB and BL sub groups of all the borderline patients. This rapid attainment of smear negativity by the addition of Mw as a immunomodulators to MDT has also been reported in our earlier study (Katoch et al 2004). The average fall in BI with the standard MDT is reported about 1 log per year (Kaplan et al 1991). Zaheer et al (1993) reported a statistically significant fall of 1.84± 0.18 in LL per year patients who received MDT + Mw as compared to a fall of 0.98± 0.11 in patients on MDT alone. Sharma et al (2000) reported that 63% of BL and LL cases on MDT + Mw became skin smear negative compared to 25% in the MDT group in the same period. Sarkar et al (2001) reported a fall in BI of 2.05 per year in the group of patients who received MDT + Mw as compared to a BI fall of 1.05 per year in patients who received MDT alone. Bhatki and Chulawala (1992) used killed ICRC as an immunomodulator with MDT and reported a more rapid fall in the BI, more so during the 2nd year. These and the present study do show that the addition of immunotherapy to MDT does help in a greater and a more rapid fall in BI and achievement of a early smear negativity status. None of the patients in the present study have relapsed in the 2 year & 6 months post-treatment follow-up of BT patients and 1year post-treatment follow-up of BB/BL patients. This is therefore more effective in borderline leprosy.

Two incidence of reaction occurred in the trial group cases (Mw+MDT) as compared to controls (MDT only) where 7 patients showed the occurrence of reactions. The results, therefore, indicate that in patients treated with MDT and immunotherapy the suffering and morbidity of reaction was reduced, which is a significant advantage. These reactions were easily controlled by concurrent administration of steroids and none of the patients suffered from permanent nerve damage. Katoch et al (2004), Zaheer et al (1993), Stanford et al (1990) using Mw, Convit et al (1992) using BCG + killed *M. leprae,* Bhatki and Chawla (1992) using immunopotentiaters like ICRC and Kaplan et al (1991) using interleukin-2 reported milder and less frequent ENL reactions when leprosy patients were treated with the addition of these immunomodulators, respectively in highly bacillated leprosy patients. Sharma et al (2000) using *Mycobacterium* w in multibacillary patients observed no change in the incidence of type-2 reactions. Similarly Sarkar et al also observed no change in the severity and incidence of type-2 reactions in multibacillary patients treated with MDT + Mw. In their series, the incidence of type-2 reactions was more in the control group (MDT group) as compared to the Mw vaccinated group.

*Mycobacterium w* used in this study is a known immunomodulator. The immunomodulatory action of Mw has been shown to induce lepromin positivity, histological upgrading and also faster bacterial clearance (Natrajan et al 1992, Sharma et al 2000, Zaheer et al 1993, Sarkar et al 2001, Katoch et al 1995, Mukharjee et al 1992). All the patients in the present series were grouped into BT with high GF ranging 70-75% and BT with moderate ranging 43-45% like this BB/BL were also grouped in to BB/BL with high GF ranging 70-75% and BB/BL with moderate ranging 42-45% showing a macrophage granuloma with the granuloma fraction ranging from 60 to 70% (Table-8, 9). In BT cases the initial granuloma fraction (mean value 72%) decreased to mean of 45% after first dose of Mw with MDT, further reduced to 30% after second dose and was finally reduced to 12% after 2 years of follow up period. In the control the initial mean value of granuloma fraction decreased from 75% to 56% at 6 months, then further reduced to 43% (at 12 months) and was finally reduced to 30% at 2 years follow up. In BB/BL cases initial 75%mean value of granuloma fraction reduced to 43% after first dose and last 15% after 2 years as compared to controls where initial 75% of granuloma fraction remained at 32% after 2 years. In addition to more rapid clearance of granuloma in immunotherapy treated group, a significant finding was an increase in the epithelioid cells population in this group. This suggests a possible immunoactivation of the macrophages especially in BB/BL immunotherapy group. Overall comparison of regression induced by chemotherapy alone with that induced by combined chemotherapy and immunotherapy shows a greater reduction in clinical parameters as well as granuloma fraction in BT cases as well as in BB/BL cases. In immunotherapy treated patients, there was much faster clearance of granuloma. In trial group (MDT+ Chemotherapy), 6 months after the first vaccination there was a fall in the granuloma fraction and lymphocytic infiltration in nearly half of the cases at the local site. After the second vaccination there was a further fall in the granuloma fraction at the local site with lymphocytic infiltration and these changes were seen in some patients distally. There was also a reduction in the number of bacilli more at the local site. After the third vaccination there was appearance of epithelioid cells at the local site, with lymphocytic infiltration and reduction in the number of AFB. These changes were also observed at the distal sites (Table 11). By 2 years of therapy there was marked reduction in the number of AFB both at the local and distal sites with lymphocytic infiltration and marked fall in the granuloma fraction. By 2 years of treatment hardly any bacilli were observed and there was non-specific healing with lymphocytic infiltration at both the sites as compared to the patients in control group (MDT alone) responded more gradually. There was a fall in the granuloma fraction but more gradually. The granuloma consisted mainly of macrophages with very few lymphocytes but there was not much change distally. After 2 years the granuloma fraction was reduced to about half in nearly all the patients. AFB were observed in all the cases and there was sparse lymphocytic infiltration with no epitheloid cells. By 2 years of therapy the bacilli were reduced but still visible, macrophages were predominant. And there was slight reduction in the granuloma fraction. Histological upgrading and accelerated bacterial clearance has also been reported using *Mw* (Zaheer et al 1993, Kar et al 1993, Mukherjee et al 1992, Sharma et al 2000, Sarkar et al 2001). And more recently the similer changes have been reported by (Katoch et al 2004). Using BCG Fernandez (1993), Katoch et al (1989), using BCG + killed *M. leprae* Convit et al (1982) and with ICRC Bhatki and Chawla (1992).

Hastings and Job (1978) using transfer factor observed reversal reaction clinically and increased influx of lymphocytes locally but the effect was transient and at the local site only. Kaplan et al (1991) using interleukin-2 also reported upgrading of lesions with increased bacterial killing at the local site and also a higher incidence of reactions. Similar results have also been reported by Mathur et al (1992) using intra-lesional recombinant interferon. However, this was associated with the occurrence of reversal reactions.

In the present study, histological upgrading, influx of lymphocytes locally, clearance of bacilli and healing without granuloma formation is achieved at the distal site also at a much earlier date, without increase in the incidence of reactions. This was not seen with the use of MDT alone.

## Conclusion

This study shows the usefulness of adding immunotherapy (Mw vaccine) to standard MDT in borderline leprosy. Addition of immunotherapy resulted in faster clinical recovery from diseases, faster granuloma & bacillary clearance and lesser incidence of reactions. This trial shows the potential usefulness of this approach of addition of immunotherapy to standard chemotherapy in borderline leprosy cases, Such information is expected to be useful in improving the immunotherapeutic approaches for treating granulomatous conditions in general and in leprosy in particular.

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