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Impact of Combined *Mycobacterium w* Vaccine and 1 Year of MDT on Multibacillary Leprosy Patients[1]

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Treatment with multidrug therapy (MDT) results in effective control of leprosy, but there are still some problems seen predominantly in patients with a high bacterial index (BI) indicating a high bacterial load. With MDT, *Mycobacterium leprae* are killed but they are not completely cleared from the body and some viable bacilli (persisters) lie dormant in certain specific sites, such as peripheral nerves, smooth muscles, lymph nodes, etc. This leads to increased risk of a) transmission and relapse of the disease, b) recurrent reactions and c) drug resistance ([4]).

To overcome these problems, the addition of an immunomodulator as an adjunct to MDT was proposed, and many vaccines such as BCG, BCG plus killed *M. leprae, Mycobacterium w, M. phlei, M. habana,* etc., have been tried as immunotherapeutic agents. Based on laboratory studies, *Mycobacterium w* was found to be antigenically nearest to *M. leprae* ([2, 9]).

Mycobacterium w (*M. w*) is a nonpathogenic, rapidly growing, cultivable strain of an atypical mycobacterium classifiable in Runyon's group IV, but differing in some respects from bacteria currently listed in that group. It shares a number of B- and T-cell epitopes with *M. leprae,* which may partly be the basis for its immunomodulatory effects ([8, 11]).

All of the previous studies of combined chemotherapy and immunotherapy with *M. w* vaccine in multibacillary (MB) leprosy patients consisted of WHO/MDT of 2 years' duration. In our study, we have given MDT for 1 year according to current WHO guidelines ([15]) and compared our results with previous similar studies. Ours is the first study to evaluate the efficacy of a shortened MDT regimen and *M. w* vaccine for MB leprosy patients.

MATERIALS AND METHODS

A total of 20 untreated, bacteriologically positive MB (BI ≥2) patients classified as BL/LL of an age more than 18 years who were attending the leprosy clinic of the Postgraduate Institute of Medical Education & Research, Chandigarh, India, comprised the study group. They were given standard WHO/MDT for MB patients for 12 consecutive months ([15]) and, in addition, they received four doses each of 0.1 ml *M. w* vaccine. The vaccine was injected intradermally into the deltoid region of the right arm at 3 monthly intervals (Study group). Each dose of vaccine contained 1×10^9 autoclaved bacilli in 0.1 ml normal saline (0.85% NaCl). Twenty age-matched, untreated MB patients (BI ≥2) formed the Control group. They were given WHO/MDT only for 12 months. Due to strict inclusion criteria we could include only 20 patients in each of the groups so as to complete the study in a reasonable time period.

Informed consent was taken from all the patients before inducting them into the study. Patients who were pregnant, in reaction, and those who had any immunodeficiency disorder or were taking immunosuppressive drugs were excluded from the study.

Criteria used for evaluation of vaccine efficacy

Clinical. Patients were evaluated clinically by Ramu's clinic score ([9]) initially and at 3, 6, and 12 months. In this scoring system, the body is divided into seven regions

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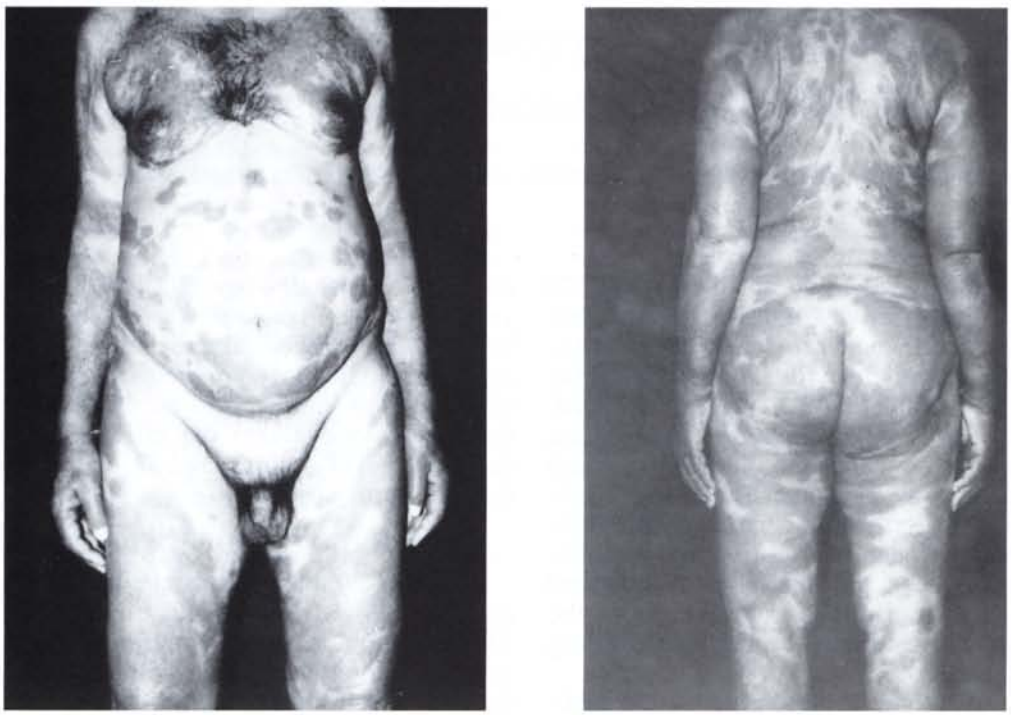

FIG. 1. Clinical lesions of a vaccinated patient before treatment showing widespread symmetrical erythematous plaques.

(face, head and neck, right and left upper limbs, chest and abdomen, back and buttocks, right and left lower limbs). Each region is independently scored. A score of 1 is given to predominantly macular lesions, 2 to diffuse infiltration, 3 to few papules or plaques and 4 to predominantly papulonodular lesions. Each of the seven regions received a score of 1–4.

Bacteriological. The bacterial index (BI) was estimated by taking slit-skin smears (SSS) using Ridley's log scale ([10]) taken initially and at 6 and 12 months from the same sites. SSS were taken from 5 sites—2 earlobes and 3 of the most active skin lesions.

Histological. Skin biopsies were taken from the most representative skin lesions at the start of treatment and at the end of 1 year of treatment. Repeat skin biopsies were taken from the same sites as far as possible. Biopsy results were compared with respect to clearance of dermal granulomas measured by estimating the granuloma fraction (GF) (i.e., fraction of dermis occupied by the granulomas) and clearance of acid-fast bacilli (AFB). The GF was then converted into a six-point score which was calculated as follows:

Score	Granuloma fraction
0	0 (no granuloma)
1	>0–02
2	0.21–0.4
3	0.41–0.6
4	0.61–0.8
5	≥0.81

The magnitude of the fall in the BI for each patient was noted and the percentage fall from baseline was calculated for each patient.

Immunological. The lepromin test was performed on all patients at the start of the treatment and after 1 year of treatment, and the results between the two groups were compared. A reading of ≥3 mm in diameter of induration was considered as positive ([5]).

The laboratory technicians reporting the SSS, the histopathologist and the statistician were blinded to the Study and the Control groups. The clinician assessing the response was not blinded since he was required to check for any local or systemic adverse effects of the vaccine.

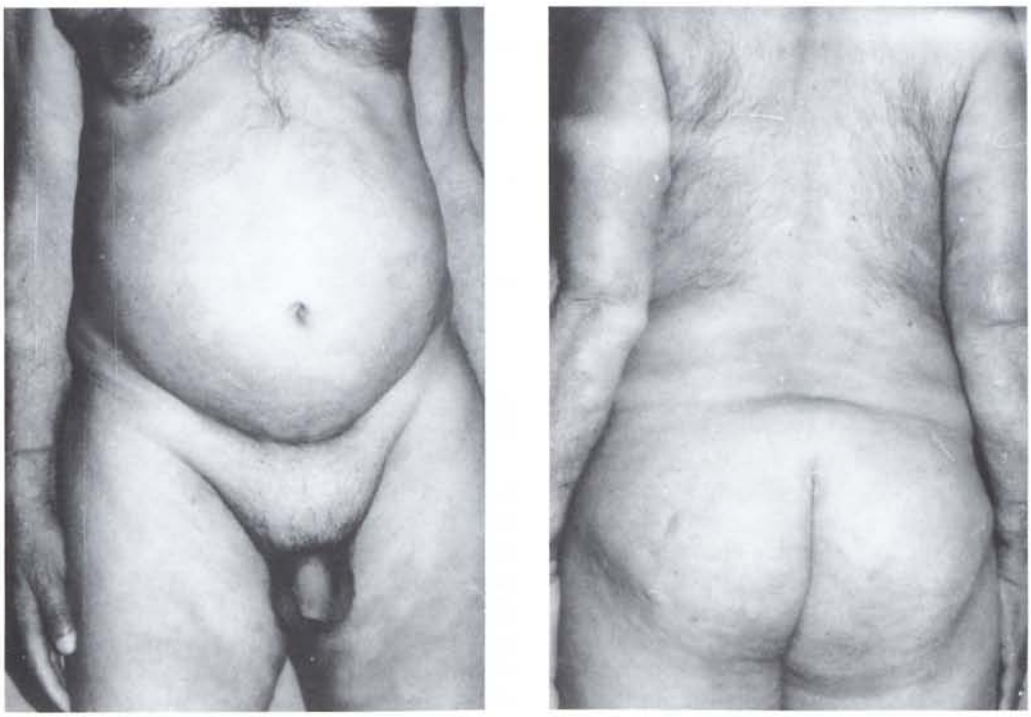

FIG. 2. Clinical lesions of the same patient after treatment showing complete subsidence of lesions after 1 year.

Statistical analysis. The data were analyzed using the Mann-Whitney U test and a modified *t* test.

RESULTS

The mean age of the patients in each group was similar (30.1 ± 12.5 in the Study group and 33 ± 16.9 in the Control group). The male : female ratio was 2.3:1 in the study group and 9:1 in the Control group. Clinically, 13 patients in the Study group and 10 patients in the Control group were LL, 7 Study group patients and 10 Control group patients were BL (Fig. 1).

A reduction in the values of Ramu's clinical scores was noted in both groups during therapy (Fig. 2). There was no appreciable change of scores at 3 months, but change was noticeable from month 6 onward and was more marked in the Study group than in the Control group both at 6 and 12 months (Table 1). The differences in the two groups were statistically significant only at 12 months ($p < 0.05$) but not at 6 months (Table 1).

There was no significant difference in the baseline BI scores between the two groups. The fall in the BI in the Study group was more rapid than in the Control group, and was apparent even at 6 months. However, the difference in the BI between the two groups was significant ($p < 0.01$) only at 12 months and not at 6 months (Table 2).

Almost all patients in both groups showed a rapid decline of the morphological index (MI) following treatment. Only three (15%) patients in the Study group had shown any viable bacilli after 6 months compared to six (30%) patients in the Control group carrying solid-staining bacilli. The MI became zero in all patients of both groups after 12 months.

TABLE 1. *Clinical scores before and after treatment.*

Group	No.	Clinical scores (mean ± S.E.M.)		
		Initial	After 6 mos.	After 12 mos.[a]
Study	20	15.8 ± 6.48	10.85 ± 4.48	6.05 ± 2.43
Control	20	14.8 ± 5.01	11.20 ± 4.33	8.05 ± 2.83

[a]The decrease in clinical scores after 12 months was significantly higher ($p < 0.05$) in the Study group as compared to the Control group (*t* test).

TABLE 2. *Bacterial index (BI) before and after treatment.*

Group	No.	Mean BI (mean ± S.D.)		
		Initial	After 6 mos.	After 12 mos.[a]
Study	20	3.6 ± 0.22	2.8 ± 0.24	1.55 ± 0.22
Control	20	3.2 ± 0.22	2.95 ± 0.28	2.15 ± 0.38

[a]BI value of the Study group was significantly lower than the Control group ($p < 0.01$) at 12 months (Mann-Whitney U test).

TABLE 3. *Granuloma fraction score before and after treatment.*

Group	No.	Granuloma fraction score (mean ± S.D.)	
		Initial	After 12 mos.
Study	20	3.3 ± 1.08	1.1 ± 0.31
Control	20	3.5 ± 0.76	1.7 ± 0.92

[a]GF score of the Study group was significantly lower ($p < 0.05$) than the Control group (modified *t* test).

There was no significant difference in the granuloma fraction (GF) of both groups at the beginning of the study. All patients on treatment showed a reduction of the GF. It was more marked in patients in the Study group, and the inter-group difference in the GF score was significant only at 12 months ($p < 0.05$) (Table 3). As a measure of the magnitude of the fall in the GF, the mean percentage fall in the GF score at 12 months was also significantly more in the Study group than in the Control group (79.4% ± 18.9 vs 49.4% ± 24.7; $p < 0.001$).

At the beginning of the study, all patients in both groups were negative for the late lepromin (Mitsuda) reaction read at 4 weeks. After 1 year of treatment eight (40%) patients in the Study group became lepromin positive (induration >3 mm); whereas no patient in the Control group showed conversion.

In the Study group, at 12 months 10 (50%) patients (five each with BL and LL), who were initially graded as having granulomatous pathology, showed nonspecific infiltration (NSI), which indicates only a mild-to-moderate degree of lymphohistiocytic infiltration in the dermis, either randomly scattered or found around blood vessels and appendages. No definite granulomas or AFB were identifiable. In another three (15%) BL patients, the infiltrate became well circumscribed, and was composed of epithelioid cells, occasional Langhans' giant cells and lymphocytes suggestive of the BT type of histology. In two (10%) LL patients, conversion occurred to the BL type of histopathology showing change from diffuse to relatively more compact granulomas consisting of a reduced number of foam cells and more lymphocytes and plasma cells and a reduced degree of positivity for AFB.

In the Control group by comparison, only 3 (15%) patients (2 BL, 1 LL) who initially had shown granulomas were graded as having NSI after 1 year. The histopathological picture in two (10%) more BL patients also changed into a more definite epithelioid cell granulomatous response, resembling BT. In others, the histopathological picture did not alter significantly.

Type 1 reactions were seen more frequently in the Study group. Six (30%) patients of the Study group showed type 1 reaction after 3–6 months of treatment compared to two (10%) patients of the control group, who showed type 1 reaction after 6 months of treatment. In two of these Study group patients the reactions were severe, associated with neuritis and required corticosteroids for control. Both of the Control group patients with type 1 reaction had neuritis and were managed similarly. In the remaining patients of the Study group, reactions were mild and were managed with nonsteroidal antiinflammatory drugs (NSAIDs).

However, type 2 reactions occurred more frequently in the Control group. Five (25%) patients from the Control group had type 2 reaction compared to three (15%) patients in the Study group. Type 2 reactions in both groups occurred after an average period of 4 to 6 months of treatment. In two patients from the Control group the reactions were associated with neuritis and required corticosteroids. None of the patients with type 2 reaction in the Study group had neuritis or required steroids.

Ulceration at the site of the vaccine injection occurred in 12 (60%) Study group patients. The ulcers were superficial, well-de-

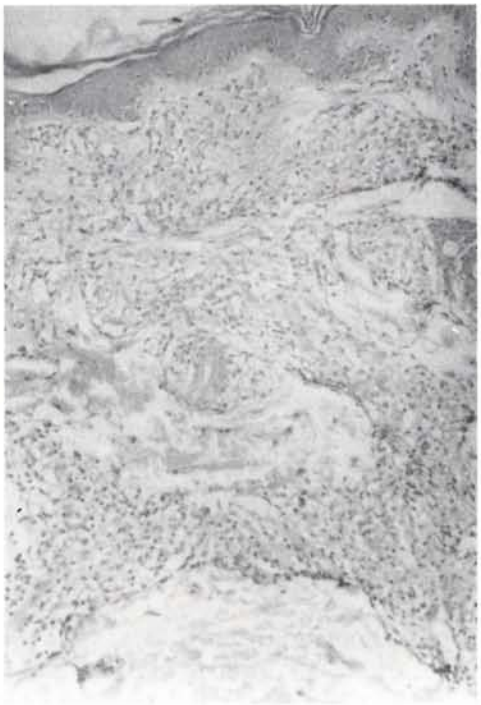

FIG. 3. Pretreatment skin biopsy of a patient treated with MDT plus vaccine showing extensive dermal granulomatous infiltrate (H&E ×140).

FIG. 4. Skin biopsy of the same patient after treatment for 1 year showing reduction in granuloma fraction (H&E ×140).

fined and associated with mild pain and induration. Ranging from 3–9 mm in size, they healed spontaneously in 3–4 weeks with scar formation. There was no regional lymphadenopathy.

The vaccine was well tolerated by all of the patients and no systemic side effects were noted.

DISCUSSION

In the present study, the immunotherapeutic effects of the *Mycobacterium w* (*M. w*) vaccine were analyzed by four parameters: clinical improvement, bacteriologic clearance, histopathologic changes and lepromin conversion.

Clinical improvement in the Study group was marked by a significant fall in the clinical scores associated with reduction of infiltration and flattening of papulonodular lesions (Figs. 1 and 2), the values reaching significance ($p < 0.05$) at 12 months compared to the Control group. All previous studies have reported similar results of significant clinical improvement at 12–24 months in the clinical scores of patients who were given the vaccine. the results were attributed to faster clearance of bacilli due to the addition of vaccine ([6, 12–14, 16–18]).

With MDT alone, the average decline in the BI has been reported to be 0.6–1.0 log/year. In the Study group, the mean BI of the patients receiving the vaccine fell by 2.05 log in 1 year, while in patients receiving MDT alone, the BI fell by 1.05 log in the same period. The difference between the BIs of the two groups was statistically significant at 12 months. The results are comparable with prior studies which also reported a significant fall in the BI at 12 months in the vaccine-treated group ([6, 12–14, 16–18]) (Table 2).

Shifts in immunologic status after vaccination were reflected by lepromin conversion from negative to positive. Lepromin conversion occurred in 40% of the patients given the vaccine. None of our patients in the Control group showed lepromin conversion. The lepromin conversion rate in our study is much lower than the reported rates of conversion of more than 70% in the earlier studies ([1, 6, 12–14, 16–18]). However, all pre-

vious studies except one reported the results of repeat testing done after 2 years of MDT and eight doses of vaccine; whereas our results are after 1 year of MDT and four doses of vaccine. Individual variations of upgradation of cell-mediated immunity (CMI) may also be responsible for this difference in the lepromin conversion rates.

The overall clinical, bacteriological and immunological improvement was also evident on histopathology. After vaccination, patients showed rapid bacillary clearance and more marked reduction in the size of their granulomas (Figs. 3 and 4). In all patients receiving treatment, there was a reduction in the number of foam cells and macrophages, reappearance of few epithelioid cells and even giant cells, with an increase in the number of lymphocytes. The changes were more marked in patients in the Study group. The bacillary load was markedly reduced and the remaining bacilli were in the granular or fragmented form. There was significantly more reduction in the granuloma fraction at 12 months and this was seen in more patients in the Study group than in the Control group. This is in agreement with previous studies ([6, 7, 12–14, 16–18]) which showed that the histopathological picture in 40%–60% of the vaccinated patients converted from the LL/BL type of infiltration into mild nonspecific infiltration.

The vaccine seems to upgrade the CMI response, which was reflected in the more frequent occurrence of type 1 reactions in the vaccinated patients. In our study, six (30%) patients of the Study group experienced type 1 reactions compared to two (10%) patients in the Control group. In a previous study by Zaheer, *et al.* ([17]) the incidence of type 1 reaction was 26% in the vaccine group compared to 13% in the control group.

Type 2 reactions are immune complex mediated, and they occur in the presence of *M. leprae* antigens. Fewer episodes of type 2 reactions were noted by us in the Study group compared to the Control group (15% vs 25%), possibly due to more rapid clearance of the bacilli and a subsequent reduction of interleukin-1 (IL-1) and tumor necrosis factor-alpha (TNF-α) synthesis by the macrophages. However, this difference in the incidence of type 2 reactions between the two groups in previous studies ([6, 12, 14, 16–18]) was not as marked as in our study. In the study by Zaheer, *et al.* ([17]), the incidence of type 2 reactions was 29% in the vaccine group and 28% in the control group.

It was reassuring to note that there was a lower incidence of neuritis (associated with both type 1 and type 2 reactions) in the Study group (10% vs 20%) and that the *M. w* vaccine did not increase the incidence of neuritis associated with reactions. This is in concurrence with the reports of an earlier study by Talwar, *et al.* ([12]) in which the control group experienced significantly more episodes of neuritis compared to the vaccine group. In another study ([17]) the incidence of neuritis was equal (9 patients in the vaccine group vs 8 patients in the control group) in both groups. The more efficient and rapid clearance of bacilli from the nerves may be responsible for this observation.

Local ulceration, which healed in 3–4 weeks with scar formation, was seen in 60% of the patients given the vaccine. Frequent ulceration at the site of injection also has been reported in other studies ([13, 16, 17]).

There were no systemic side effects in any of the patients given the vaccine, and the vaccine was well tolerated. Systemic side effects of the *M. w* vaccine have not been reported in any prior study ([6, 12–14, 16–18]).

Ours is the first study done in which WHO/MDT was given for 1 year, according to current WHO recommendations, along with the *M. w* vaccine. In all previous similar studies MDT was given for 2 years. In one study by Talwar, *et al.* ([13]), good results were shown after 1 year but MDT was continued for 2 years. The results of this preliminary study suggest that the addition of the *M. w* vaccine to the currently recommended shortened WHO/MDT of 1 year is more beneficial than giving MDT alone since it leads to more rapid clearance of bacilli, and this therapy leads to a faster improvement of the patients, as assessed clinically, bacteriologically, immunologically and histopathologically. The improvement observed by us in various parameters is comparable to the results shown in previous studies when MDT was combined with *M. w* ([6, 12–14, 16–18]).

On the basis of our observations, although based on a small number of patients, we recommend that MB patients with a

high BI should be given the *M. w* vaccine along with WHO/MDT for rapid clinical and bacteriological improvement. Immunological improvement, as evidenced by the appearance of lepromin positivity, seems to be responsible for the rapid killing and faster clearance of the bacilli. Larger studies with longer follow-up times are required to confirm the preliminary promising results seen in our patients.

SUMMARY

A total of 20 bacteriologically positive multibacillary (MB) leprosy patients older than 18 years of age with a bacterial index (BI) of 2+ or greater were given standard World Health Organization multiple drug therapy (MDT-MB) for 12 consecutive months plus four intradermal doses of *Mycobacterium w* vaccine at 3 monthly intervals (Study group). Twenty age-matched MB patients were given WHO/MDT alone (Control group). The patients of both groups were followed up for 1 year.

Improvements in the patients were periodically monitored by clinical (Ramu's score), bacteriological (SSS), histopathological (skin biopsy) and immunological (lepromin conversion) parameters. Study group patients showed more significant improvements in all parameters except for lepromin conversion compared to patients in the Control group. The incidence of type 1 reaction was more in the Study group (30% vs 10%), while the incidence of type 2 reaction was more in the Control group (25% vs 15%). Neuritis associated with reactions was seen more often in the Control group compared to the Study group (20% vs 10%).

The addition of *Mycobacterium w* vaccine as an adjunct to the 1-year WHO/MDT regimen appears to be significantly more beneficial in MB leprosy patients with a high initial BI compared to WHO/MDT given alone. Studies on larger numbers of patients with extended follow up will be in order.

RESUMEN

Veinte pacientes con lepra multibacilar, MB (índice bacteriológico de 2 o más), mayores de 18 años de edad, se trataron con la poliquimioterapia recomendada por la Organización mundial de la Salud (PQT/OMS) durante 4 meses consecutivos, y con 4 inyecciones de una vacuna preparada con *Mycobacterium w* administrada a intervalos de 3 meses (grupo de estudio). Otros veinte pacientes multibacilares, similares a los anteriores, se trataron sólo con la PQT/OMS (grupo control). Los pacientes de ambos grupos se evaluaron durante un año.

La mejoría en los pacientes fue periódicamente analizada por criterios clínicos (escala de Ramu), bacteriológicos (SSS), histopatológicos (biopsia de piel) e inmunológicos (reactividad a la lepromina). Excepto en la prueba de la lepromina, en todos los otros parámetros los pacientes del grupo de estudio mostraron mejor evolución que los pacientes del grupo control. La incidencia de reacciones del tipo 1 fue mayor en el grupo de estudio que en el grupo control (30% vs 10%), mientras que la incidencia de reacciones tipo 2 fue más común en el grupo control (25% vs 15%). La neuritis asociada con reacciones fue más común en el grupo control que en el grupo de estudio (20% vs 10%).

En los pacientes con lepra multibacilar y altos índices bacteriológicos, la adición de la vacuna *Mycobacterium w* al tratamiento de un año con la PQT/OMS, resulta más satisfactoria que la PQT/OMS sola. Son imperativos los estudios con mayor número de pacientes y con tiempos más prolongados de seguimiento.

RÉSUMÉ

Un total de 20 patients hanséniens multibacillaires bactériologiquement positifs, âgés de 18 ans et plus avec un index bactérioscopique (IB) supérieur ou égal à 2+, ont reçu une polychimiothérapie stadard (PCT) recommendée par l'Organisation Mondiale de la Santé (OMS) pendant 12 mois consécutifs, ainsi que quatre injections intra-dermiques de vaccin à Mycobactéries w à trois mois d'intervalle (groupe traité). Vingt autres patients MB controllés pour l'âge reçurent la PCT/OMS seule (groupe témoin). Les patients des deux groupes furent suivis pendant un an.

L'amélioration des patients fut suivie périodiquement par des paramètres cliniques (score de Ramu), bactériologiques (SSS), histopathologiques (biopsies cutanées) et immunologiques (conversion du test à la lépromine). Comparé au groupe témoin, les patients du groupe traité ont montré une amélioration plus importante de tous les paramètres examinés, à l'exception de la conversion du test à la lépromine. L'incidence des réactions de type I était plus importante dans le groupe traité (30% contre 10%), tandis que l'incidence des réactions de type II était plus importante dans le groupe témoin (25% contre 15%). Les névrites associées à ces réactions étaient plus souvent observées chez le groupe témoin comparé au groupe traité (20% contre 10%).

L'addition du vaccin à Mycobactérie w comme complément du traitement PCT/OMS de un an apparait être significativement plus bénéfique chez les patients hanséniens avec un IB initial haut comparé au traitement PCT/OMS donné seul. Des études sur un

échantillon de patients plus large et un suivi plus étendu sont envisagées.

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