Award Article

A double-blind, randomized controlled trial to compare the effectiveness and safety of purified protein derivative of tuberculin antigen with *Mycobacterium w* vaccine in the treatment of multiple viral warts

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Abstract

Background: Present day therapeutic modalities for viral warts are mostly ablative in nature, limited by high recurrence rates and are unsuitable for numerous lesions. Immunotherapy has the potential to overcome these limitations.

Aims: This study aimed at comparing efficacy and safety of and quality of life changes with intradermal purified protein derivative (PPD) of tuberculin antigen and *Mycobacterium w* (*Mw*) vaccine in immunotherapy of warts.

Methods: Patients with multiple (≥5) warts were randomized (1:1) into two groups (PPDand, *Mw* vaccine groups). Fortnightly, 0.1 ml of either medicine was injected intradermally over the deltoidregion till complete resolution or a maximum of six doses. Patients were followed-up for another 3 months for recurrence.

Results: Sixty-four participants received either PPD or *Mw* vaccine. The number of warts were comparable at baseline ($P = 0.089$, Mann–Whitney test), and reduced significantly with treatment in both groups ($P < 0.001$, Friedman's ANOVA), as seen from the fourth follow-up onwards with *Mw* and fifth follow-up onwards with PPD ($P < 0.05$, *Post hoc* Dunn's test). Intergroup comparison showed significantly more ($P < 0.05$, Mann–Whitney test) reduction with *Mw* than PPD at the sixth and seventh follow-up. The size of warts also reduced significantly ($P < 0.001$) in both groups from the third follow-up onwards. Complete remission was more ($P = 0.539$, Fischer's exact test) in the *Mw* group (68.8%) than the PPD group (50%); and was significantly higher ($P = 0.049$, Mann–Whitney test) in patients having shorter duration of warts. Adverse events were significantly more ($P < 0.001$) with *Mw* including ulceration (50%), discharge (15.6%), pain-swelling-induration and scar at the injection site (97% each), whereas some of those receiving PPD noted erythema and scaling at the injection site (18.8%), and post-inflammatory hyperpigmentation (12.5%). No recurrence was seen till the end of the study.

Limitation: Unicentric trial.

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Conclusion: Intradermal injection of *Mw* vaccine was more effective but had a higher incidence of adverse effects compared to PPD of tuberculin antigen in patients with warts.

Key words: Immunotherapy, *Mycobacterium w* vaccine, purified protein derivative, randomized controlled trial, viral warts

Introduction

Management of verrucae is often frustrating to the patient and the physician alike.[1] Unfortunately, even with years of medical literature on this subject, high-quality evidence for efficacy is lacking for almost all treatments. Primary treatment modalities for verrucae include ablative therapies, e.g. topical chemical cautery, cryotherapy, electrocautery, excision, bleomycin sulfate injection and laser vaporization, but none gives a guarantee of cure, and recurrence and scarring are common.[2,3] These destructive modalities are designed to remove visible lesions; nonvisible infected tissues are not targeted. Moreover, in patients with numerous lesions, there is no effect on lesions other than the treated ones, resulting in repeated and long-drawn treatment sessions.

Immunotherapy in warts utilizes the ability of the immune system to mount a delayed-type hypersensitivity response to various antigens in wart tissue. It has been found to be associated with the production of Th1 cytokines which activate cytotoxic and natural killer cells to eradicate the HPV infection. This clears not only treated warts but also distant warts, unlike conventional therapies.[4]

Antigens such as BCG, PPD, candida, mumps, MMR and *Mycobacterium w* (*Mw*) vaccine as well as interferons have shown promise as immunotherapeutic agents.[3] Purified protein derivative (PPD) is an extract of *Mycobacterium tuberculosis* used for testing exposure to tuberculin protein. It is an especially promising immunotherapeutic agent in countries where vaccination against tuberculosis is performed routinely.[5] *Mw* vaccine contains killed nonpathogenic, saprophytic, cultivable, atypical mycobacterium belonging to Runyon Group IV, which has now been renamed *Mycobacterium indicus pranii*.[6] Due to its strong immunogenic and immunomodulatory actions, it has found place in treatment of both genital[7] and extragenital warts.[6]

India is endemic for tuberculosis, with a high prevalence rate (249 per 100,000 population). It is estimated that 40% of the Indian population is infected with *M. tuberculosis*.[8] Considering this along with the practice of routine immunization against tuberculosis, it can be argued that the Indian population is already sensitized to mycobacterial antigens; this was our rationale in choosing two mycobacteria-derived antigens for immunotherapy.

This study was undertaken to evaluate efficacy, safety and tolerability of, and quality of life changes with intradermal tuberculin PPD versus those with *Mw* vaccine in the treatment of multiple warts in an Indian setting. The study was planned to provide evidence to clinicians about which modality to adopt or which immunotherapeutic antigen to use when confronted with a patient having numerous cutaneous warts.

Methods

The study was carried out as a double-blind, randomized, controlled parallel-group trial of tuberculin PPD versus *Mw* vaccine in the Dermatology OPD of Medical College, Kolkata. Institutional Ethics Committee permission was taken and trial was registered with CTRI (registration number CTRI/2015/03/005433). Adult consenting patients (18–65 years) of either sex suffering from multiple viral warts (≥5 in number) were included. Pregnant, lactating women, patients immunosuppressed due to drug or disease, those suffering from liver or kidney diseases, and those with mucosal, ulcerated, inflamed or genital warts were excluded.

Randomization and blinding

The participants were randomized equally in a 1:1 ratio (unstratified) into two treatment groups by a computer-generated random number table using WINPEPI software. Randomization was concealed by the sequentially numbered opaque sealed envelope (SNOSE) technique. Insulin syringes were each prepared with 0.1 ml of trial medicines and packed in envelopes by a departmental nurse not associated with the trial, making both the treating physician and the participants blind to the treatment received.

Study medications

- Tuberculin PPD (10 TU/0.1 ml) – the formulation marketed by Span Diagnostics Private Limited, Gujarat, India, (Tuberculin Diluted; Batch no. 4000013401, Mfg. Dt: 07-2014, Exp. Dt: 10-2015) was utilized.
- Mycobacterium w vaccine formulation marketed by Cadila Pharmaceuticals, Licenced by National Institute of Immunology, New Delhi, India, (Immuvac; Batch no. 14001; Mfg. Dt: 12-2014, Exp. Dt: 11-2016) was utilized.

Both medications were purchased from the respective company for the trial by the investigator.

Sample size

The sample size was 29 patients of viral warts in each treatment group considering a superiority trial. Sample size was calculated considering complete clearance of warts of 83% with *Mw* vaccine[6] and 50% with tuberculin PPD,[7] with

80% power and 0.05 probability of Type I error. Considering a possible 10% dropout rate, this translated to a recruitment target of 32 subjects in each group or 64 subjects overall.

Visits and follow-ups

The study was carried out for 18 months. Each participant received intradermal injections (PPD or *Mw*) every fortnight for a total of 6 doses; then the patient was advised to come for follow-up every month for 3 months to assess recurrence. The first participant was included on May 2014 and recruitment was continued till January 2015. Follow-up of the last recruited participant was done till April 2015.

At the screening visit, history was recorded and a clinical examination was done. Tuberculin (Mantoux) test was done on all patients with 0.1 ml of 10 TU PPD and the reading was taken after 72 h. Baseline laboratory values of hemoglobin, total leucocyte count, differential counts, platelet count, erythrocyte sedimentation rate, fasting blood sugar, serum urea, serum creatinine and liver function tests were recorded.

The baseline visit was scheduled 3 days after the screening visit. Erythema and induration of the tuberculin test was recorded by measuring the maximum horizontal diameter. Number and size of warts at presentation were recorded in a pre-tested case-record-form, and the vernacular version of the Dermatology Life Quality Index (DLQI) form was filled by the patient. The investigational product was injected into the left arm (first dose), and patients were asked to telephone the investigator and report immediately if any untoward reactions occurred.

Treatment visits were scheduled at week 2, 4, 6, 8, and 10 with subsequent follow-up visits at week 14, 18, and 22 weeks. At each fortnightly visit, intradermal injections were administered into alternate arms as per the randomization for a total of six doses or less in case of complete resolution. Efficacy and safety parameters were tested at every visit. The size of warts was estimated by measuring the largest diameter of the largest wart with a ruler. Laboratory investigations were repeated at week 10. The DLQI was reassessed at week 22.

Study parameters

Efficacy parameters were reduction in the total number of lesions, decrease in thesize of existing lesions, and physicians' and patients' global assessments of disease activity improvement on a five-point Likert scale (from 0 to 4).[9] Safety parameters recorded were the adverse events reported by participants or elicited by clinicians and the changes in laboratory parameters after active treatment. Quality of life was assessed by vernacular version of DLQI questionnaire administered at baseline and at the study end.[10]

Statistical analysis

Continuous variables were compared between groups by independent samples *t*-test and within group by paired *t*-test. Mann–Whitney U-test and Wilcoxon's test was carried out for unpaired and paired nonparametric data. Categorical data were compared between groups by Chi-square test or Fisher's exact test, as appropriate. Freidman's analysis of variance (ANOVA) followed by post-hoc Dunn's test was carried out with nonparametric data for within group repeated measures comparisons. Efficacy analysis was done on a modified intention-to-treat basis for the 64 subjects reporting for at least two post-baseline follow-up visits. Missing values were dealt with by the last observation carried forward strategy. Subgroup analysis was planned taking the presence of a BCG immunization scar as the grouping variable. Pre and posttreatment laboratory data were obtained from patients who had come for at least five follow-ups. This included 29 patients of PPD group and 30 patients of *Mw* group. For analysis of adverse effects, all patients who had received at least one dose of the injection were considered, i.e. all 32 patients each from the PPD group and *M*w group.

Results

Of the 105 participants screened, 64 were randomized equally into the treatment groups. The flow of study participants is depicted in Figure 1.

Clinicodemographic data of study participants are highlighted in Table 1. Both groups were found to be comparable on those parameters.

The number of warts in the PPD group was 58.37 ± 53.44 while in the *Mw* group it was 41.25 ± 43.83 at baseline, and they were comparable ($P = 0.089$). Reduction in wart numbers started from the first follow-up itself in both groups; however, the reduction was significant ($P < 0.001$) from the fourth follow-up onwards in the PPD group and third follow-up onwards in the *Mw* group, and this decline was maintained till the end of the study. Intergroup comparison revealed significantly more reduction in the *Mw* group than the PPD group at 6th and 7th follow-up visits, but this difference was not noted in the next visit. Changes in the number of warts during 3 months of active treatment (baseline to fifth

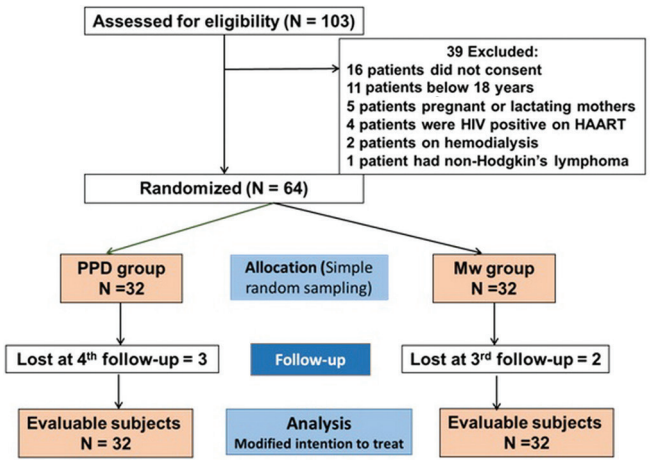

Figure 1: Flowchart of study participants

Table 1: Clinical profile of study participants

Parameters	PPD (*n*=32), *n* (%)	*Mw* (*n*=32), *n* (%)	*P* (between groups)
Duration of illness (years)			
Mean±SD	2.78±3.48	2.97±3.33	0.994
Median (IQR)	2 (1,3.5)	2 (1,3.25)	
Type of wart			
Verruca vulgaris	15 (46.9)	15 (46.9)	0.796
Verruca plana	12 (37.5)	6 (18.8)	
Periungual	5 (15.6)	7 (21.9)	
Palmoplantar	7 (21.9)	7 (21.9)	
History of previous treatment			
None	5 (15.6)	11 (34.4)	0.221
Chemical cautery	12 (37.5)	9 (28.1)	
Electrical ablation (RF/ED)	3 (9.4)	5 (15.6)	
Alternative medicine (homeopathy/Ayurveda)	21 (65.6)	15 (46.9)	
Aggravating factors			
None identified	22 (68.8)	24 (75)	0.899
Threading	5 (15.6)	3 (9.4)	
Shaving	3 (9.4)	3 (9.4)	
Walking	2 (6.2)	2 (6.2)	
Koebnerization			
Absent	16 (50)	15 (46.9)	1.000
Present	16 (50)	17 (53.1)	
BCG immunization scar			
Present	24 (75)	26 (81.3)	0.763
Absent	8 (25)	6 (18.7)	
Tuberculin positivity			
Present	11 (34.4)	11 (34.4)	1.000
Absent	21 (65.6)	21 (65.6)	
Tuberculin induration (mm)			
Mean±SD	8.25±6.73	8.69±7.28	0.898
Median (IQR)	6 (3.5, 13)	8 (3, 12.5)	

P value is from Student's unpaired *t*-test for duration of illness, number and size of warts and tuberculin induration; Fisher's exact test for type of wart, previous treatment, aggravating factor, koebnerisation, BCG immunization scar and tuberculin positivity. SD: Standard deviation, BCG: Bacille CalmetteGuerin, IQR: Interquartile range, RF: Radiofrequency, PPD: Purified protein derivative, RF: Radiofrequency ablation

Table 2: Comparison of the number of warts in two treatment groups

Visit	PPD (*n*=32)	*Mw* (*n*=32)	*P* (between groups)
Baseline			
Mean±SD	58.37±53.44	41.25±43.83	0.089
Median (IQR)	28.5 (19, 94)	20 (12, 62)	
1st follow - up			
Mean±SD	55.63±53.18	42.98±20.0	0.126
Median (IQR)	32.5 (17.5, 82)	20 (11.5, 61)	
2nd follow - up			
Mean±SD	45.44±48.79	34.88±38.11	0.424
Median (IQR)	22 (11.5, 53)	21 (11, 47)	
3rd follow - up			
Mean±SD	39.31±48.01	27.25±30.62*	0.397
Median (IQR)	18 (6.5, 51)	12.5 (6, 38)	
4th follow - up			
Mean±SD	35.5±46.77*	19.97±26.78*	0.179
Median (IQR)	13.5 (4.5, 50)	9.5 (1.5, 23)	
5th follow - up			
Mean±SD	29.94±41.29*	14.19±20.88*	0.067
Median (IQR)	12.5 (1.5, 39.5)	4.5 (0, 19.5)	
6th follow - up			
Mean±SD	28.41±40.81*	9.66±16.10*	0.045
Median (IQR)	12 (0, 37.5)	1 (0, 12)	
7th follow - up			
Mean±SD	26.34±40.58*	8.34±16.11*	0.018
Median (IQR)	10 (0, 37.5)	0 (0, 11.5)	
8th follow - up			
Mean±SD	23.94±41.03*	8.0±15.99*	0.099
Median (IQR)	3.5 (0, 27)	0 (0, 11.5)	
Percentage reduction (%)	50.42	80.6	
P (within groups)	<0.001	<0.001	

*Significant reduction from baseline. *P* value between groups determined by Mann-Whitney U-test. *P* value within groups determined by Friedman's ANOVA followed by *post hoc* Dunn's test. PPD: Purified protein derivative, SD: Standard deviation, IQR: Interquartile range

follow-up) and 3 months of posttreatment follow-up (sixth, seventh, and eighth follow-ups) are depicted in Table 2 and Figures 2a, 2b, 3a and 3b. The number of warts declined significantly ($P < 0.001$) in the active treatment phase in both treatment arms in the initial 3 months, and there was a decrease in the number of warts posttreatment during the sixth to eighth follow-up visits. With treatment, PPD achieved 50.4% and *Mw*, 80.6% reduction in the total number of warts from their respective baseline values [Table 3].

The two groups were comparable at baseline with regard to the size of the warts ($P = 0.403$), with the average size of the largest wart in the PPD group being 7.9 ± 5.1 cm and Mw group being 10.4 ± 8.2 cm. There was a significant reduction ($P < 0.001$) in size from the third follow-up onwards in both groups, which was persistent till the end of the study. Intergroup comparison showed significantly more ($P = 0.059$) reduction in wart size in *Mw* group in the seventh follow-up, however, the difference was not maintained till the end of study. With PPD, patients achieved 53.7% and with *Mw* 70.5% reduction in wart size from their respective baseline values [Table 3].

Assessment of disease severity by the physician showed that at baseline the disease was severe in both treatment arms (Physicians' global assessment of disease activity improvement scale value at baseline was 0, since the disease was most severe). Severity reduced significantly ($P < 0.001$) in in both groups from the first follow-up onwards. Comparison between the treatment groups showed that decrease in disease severity was significantly more with *Mw* vaccine than with PPD at the sixth, seventh, and eighth follow-up visits. Patients' global assessment of disease activity improvement scale also showed similar results. [Figures 4a and 4b].

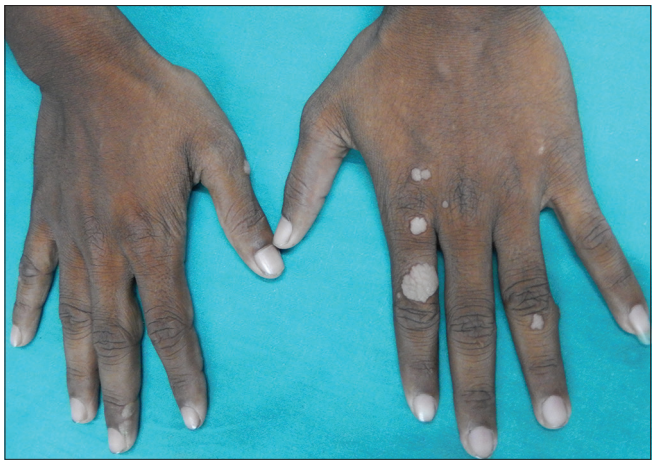

Figure 2a: Pre-treatment photograph of verruca vulgaris on dorsa of hand treated by purified protein derivative

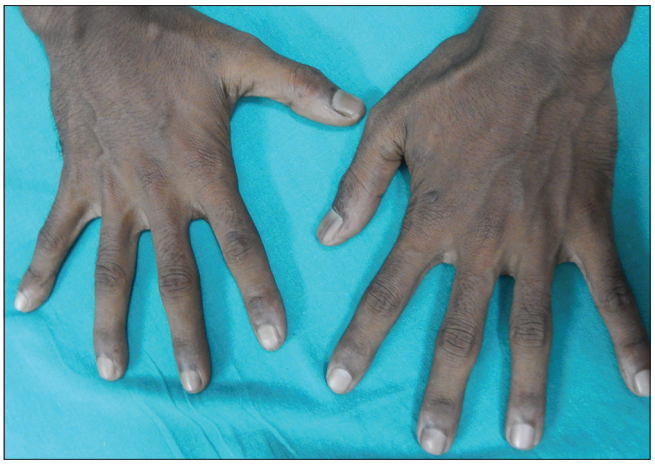

Figure 2b: Post-treatment photograph of verruca vulgaris on dorsa of hand treated by purified protein derivative (at 10 weeks)

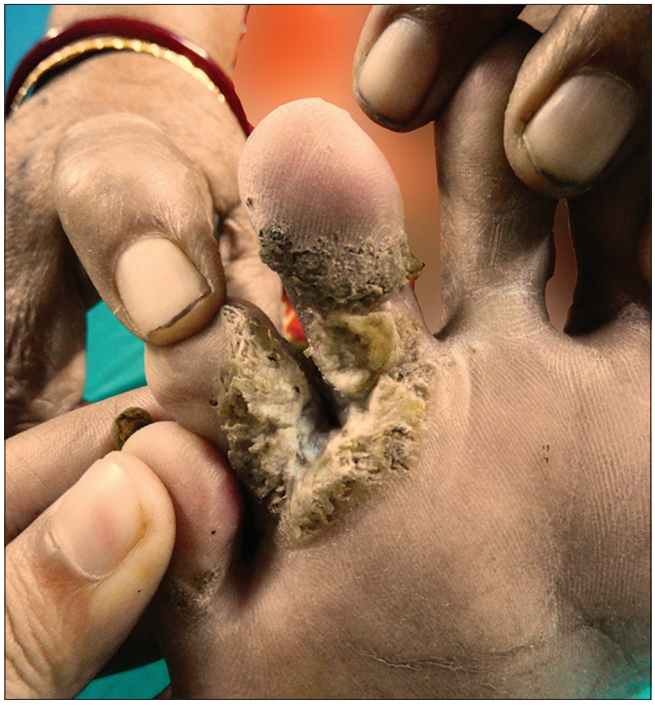

Figure 3a: Pre-treatment photograph of plantar warts treated by purified protein derivative

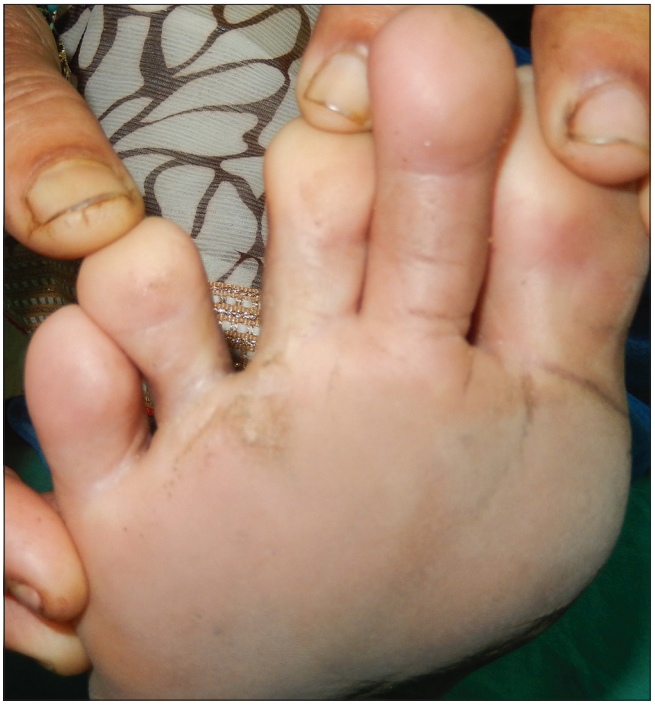

Figure 3b: Post-treatment photograph of plantar warts treated by purified protein derivative (at 12 weeks)

Out of a total of 64 patients, 38 achieved complete clearance of their warts. Complete resolution of warts was seen from the fourth follow-up onwards with PPD and third follow-up onwards with *Mw*, with 50% (16/32) of patients achieving complete remission in the former group compared to 68.8% (22/32) of patients in the latter group (P = 0.539). It was seen that age, sex, type and baseline number, size of warts and tuberculin positivity status were comparable between complete and partial responders; however, patients having shorter durations of warts achieved complete clearance significantly more (P = 0.049) than those having warts for longer durations [Table 4].

A BCG immunization scar was present in 24 (75%) patients in the PPD group whereas 26 (81.3%) had a BCG scar in the *Mw* group [Table 1]. Subgroup analysis with BCG immunization scar as a grouping variable showed no significant change in the reduction of number of warts between the two subgroups at any follow-up (P = 0.872 in PPD group; P = 0.231 in *Mw* group at final visit). Tuberculin reactivity status too did not have any effect on the reduction of the number of warts either in the PPD group (P = 0.276 at the last follow-up) or the *Mw* group (P = 0.399).

Quality of life (assessed by DLQI) was comparable (P = 0.114) in both treatment arms at baseline. The DLQI improved

Table 3: Comparison of the size of warts in two treatment groups

Visit	PPD (*n*=32)	*Mw* (*n*=32)	*P* (between groups)
Baseline			
Mean±SD	7.97±5.15	10.38±8.21	0.403
Median (IQR)	6.5 (4, 11)	7 (4, 13)	
First follow-up			
Mean±SD	7.75±4.89	9.53±7.55	0.622
Median (IQR)	6.5 (4, 10)	5 (4, 11)	
2nd follow - up			
Mean±SD	6.34±5.08*	7.63±6.17*	0.311
Median (IQR)	5 (3, 9)	5 (4, 8)	
3rd follow - up			
Mean±SD	5.69±4.82*	6.5±5.96*	0.569
Median (IQR)	4 (2.5, 6.5)	4 (3, 7.5)	
4th follow - up			
Mean±SD	4.94±4.91*	5.47±5.75*	0.745
Median (IQR)	3.5 (2, 6)	4 (3, 5)	
5th follow - up			
Mean±SD	4.47±5.04*	4.59±6.02*	0.792
Median (IQR)	3 (2, 5.5)	3 (0, 5)	
6th follow - up			
Mean±SD	4.03±5.28*	3.69±6.06*	0.392
Median (IQR)	2 (0, 5)	1 (0, 5)	
7th follow - up			
Mean±SD	4.06±5.28*	3.13±6.19*	0.059
Median (IQR)	2 (0,5.5)	0 (0,4.5)	
8th follow - up			
Mean±SD	3.69±5.47*	3.06±6.22*	0.209
Median (IQR)	1 (0, 5.5)	0 (0, 4.5)	
Percentage reduction (%)	53.7	70.5	
P (within groups)	<0.001	<0.001	

*Significant reduction from baseline. *P* value between groups determined by Mann-Whitney U-test. *P* value within groups determined by Friedman's ANOVA followed by *post hoc* Dunn's test. PPD: Purified protein derivative, SD: Standard deviation, IQR: Interquartile range

significantly from baseline at the end of the study with both PPD ($P < 0.001$) and *Mw* ($P < 0.001$), though intergroup comparison showed no difference between the two treatment groups ($P = 0.583$) at the end of the study.

Adverse events were encountered in 31.3% (10/32) of patients in the PPD group and all patients in the *Mw* group, and the difference was statistically significant ($P < 0.001$). In the PPD group, 12.5% (4/32) of patients developed hyperpigmentation at the site of the lesions after receiving five injections, which persisted till the end of study; 18.8% (6/32) of patients developed transient mild erythema and scaling at injection site after four doses, which subsided within 2–3 weeks without treatment; one patient reported a sudden onset of pruritus with erythema over the warts after receiving three doses of injection which was treated with antihistamines for 7 days, followed by rapid regression of the wart lesions. In the *Mw* group, almost all patients developed an indurated nodule at the injection site after every dose which healed with scarring [Figure 5a and 5b]. 37.5% (12/32) patients' nodules were painful requiring tablet paracetamol (650 mg) twice daily till resolution; 34.4% (11/32) patients additionally developed ulceration of the nodule, and in 12.5% patients (4/32) there was a discharge from the injection site nodule. The patients with ulceration with or without discharge were treated with a standard atypical mycobacterial drug regimen comprising tablet doxycycline (100 mg) twice daily + tablet ofloxacin (200 mg) twice daily + tablet azithromycin (500 mg) once daily till healing of their ulcer. Self-limiting erythema and scaling over the injection sites occurred in one patient after three doses and one reported pruritus and erythema over the warts after receiving two injections that was followed by rapid clearance of the lesions over 10 days.

Laboratory parameters were within normal limits and comparable between the two groups.

Discussion

The most troublesome factor in the management of warts is the high recurrence rate of at least 30% after apparently successful treatment, possibly by recrudescence of the virus from the surrounding tissue reservoir.[3] In the present study, 21 and 8 patients respectively had recurrences after chemical cautery and electrosurgery. Immunotherapy for warts addresses the limitations of conventional ablative therapy as it enhances the cell mediated immunity and helps clear the virus-infected tissues, irrespective of whether they are visible or not. In this sense, immunotherapy can target lesions situated remotely from the site of its administration, making it a better option in multiple warts, warts on inaccessible or difficult-to-treat sites (like sub- or periungual regions) and in cosmetically sensitive areas (such as the face). Various agents like PPD,[11] BCG vaccine,[12,13] MMR vaccine,[4] and Candida vaccine,[14] have been used for immunotherapy of warts in the past with favorable results.

In the present study, there were significant reductions in the number and size of warts in both groups (PPD and *Mw* arms) over 3 months of active treatment and another 3 months of follow-up, demonstrating that both the study medications were effective in wart treatment. The results of other similar studies with respect to reduction in the number of warts, size of warts and complete clearance of warts are shown in Table 5. Table 6a and 6b respectively show a comparison between our study and other studies on PPD and *Mw* vaccine in viral warts. Our study obtained complete cure in 15.6% (5/32) and 34.4% (11/32) patients in PPD and *Mw* groups, respectively, at the end-of-treatment visit; this increased to 50% (16/32) and 68.8% (22/32) in the respective groups at the final visit (3 months after end of treatment). With respect to complete clearance, though achieved early with *Mw*, the final difference in outcome was not statistically significant ($P = 0.203$). This highlights the fact that immune enhancement and clinical effects persist beyond the treatment schedule, and patients need to be

Table 4: Comparison between complete responders (both with purified protein derivative and *Mw*) and partial responders

Parameters	Complete cure (*n*=38), *n* (%)	Partial cure (*n*=26), *n* (%)	*P* (between groups)
Age			
Mean±SD	29.47±12	26.69±9.39	0.569
Median (IQR)	24 (20, 38)	24 (20, 30)	
Sex			
Male	16 (42.1)	16 (61.5)	0.203
Female	22 (57.9)	10 (38.5)	
Duration of illness (years)			
Mean±SD	2.11±1.78	4±4.68	0.049
Median (IQR)	1.75 (1, 3)	2.5 (1, 4)	
Size of lesions at baseline (cm)			
Mean±SD	8.61±7.23	10±6.46	0.219
Median (IQR)	5 (4, 10)	9 (5, 15)	
Number of lesions at baseline			
Mean±SD	51.53±53.49	47.31±43.19	0.994
Median (IQR)	23 (16, 80)	25 (16, 75)	
Tuberculin positivity			
Present	15 (39.5)	7 (26.9)	0.422
Absent	23 (60.5)	19 (73.1)	
Type of warts			
Verruca vulgaris	15 (39.5)	15 (57.7)	0.502
Verruca plana	12 (31.6)	6 (23.1)	
Periungual	6 (15.8)	6 (23.1)	
Palmo-plantar	9 (23.7)	5 (19.2)	

P-value is from Mann-Whitney U-test for age and duration of illness and size of warts, Fisher's exact test for sex distribution, Chi-square test for type of warts

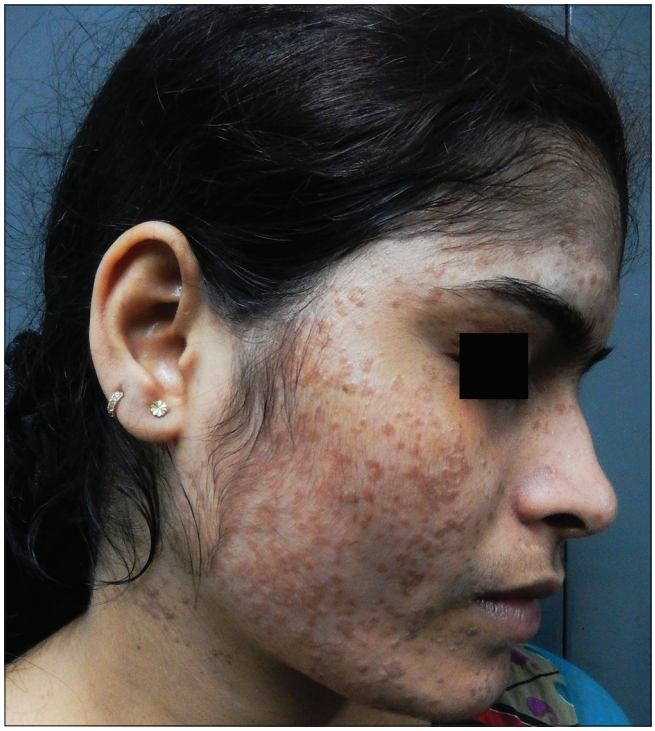

Figure 4a: Pre-treatment photograph of verruca plana treated by *Mycobacteium w* vaccine

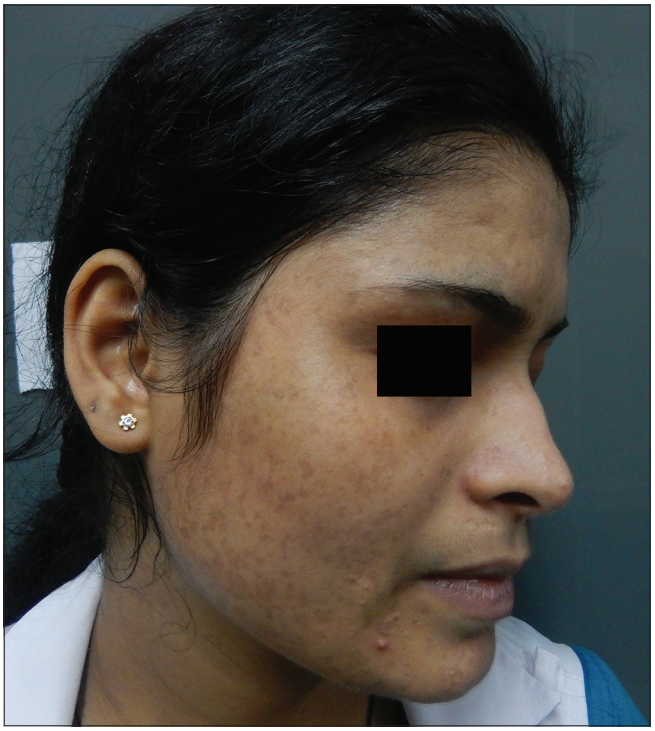

Figure 4b: Post-treatment photograph of verruca plana treated by *Mycobacteium w* vaccine (at 8 weeks)

Table 5: Comparing the present study with similar studies regarding efficacy end points

Parameter	Present study	Other studies		Comment
Number of warts Reduction (%) Significant reduction (after which dose)	PPD - 50.4% *Mw* - 70.6% PPD - after 4 doses *Mw* - after 3 doses	Podder *et al*.[13] PPD - 49.3% BCG - 63.62% PPD - after 1st dose BCG - after 1st dose		The intradermal injections were given at 4-week intervals and as the assessment was done at longer intervals, it might partly explain the early onset of a statistically significant decline[13]
Size of lesions Reduction (%) Significant reduction (after which dose)	PPD - 53.7% *Mw* - 70.5% PPD - after 2 doses *Mw* - after 2 doses	Eassa *et al*.[11] PPD - 75% PPD - after 4 doses	Kumar *et al*.[15] *Mw* - 83%	Eassa *et al*., using intradermal PPD every week for 12 weeks for ano-genital warts[11] Kumar *et al*., used intralesional *Mw* for ano-genital warts[15]
Complete cure	PPD - 50%	Studies with response <50% Eassa *et al*.[11] 18.5% Kus *et al*.[16] 29.4%	Studies with response >50% Nimbalkar *et al*.[17] 62.2% Saoji *et al*.[18] 76% Amirnia *et al*.[19] Intralesional PPD - 77.1% versus Placebo - 0% versus Cryotherapy - 18.2% Wananukul *et al*.[20] 93%	Complete response rate achieved was highly variable in various studies. The relatively lower response rate reported in the study by Kus *et al*.[16] may be attributed to the lesser number PPD doses employed (3 doses in total in contrast to 6 doses by other authors) No significant difference has been found between intradermal and intralesional PPD, though the latter had a slightly better result[5]
Complete cure	*Mw* - 68.8%	Studies with comparable results Singh *et al*.[21] 54.5% Dhakar *et al*.[22] 66.7% Kumar *et al*.[15] 67.6%	Studies with a higher cure rate Meena *et al*.[6] 83% Gupta *et al*.[7] 88.9% Garg and Baveja[23] 93%	This difference can be explained by the fact that the later studies[6,7,23] were carried out on anogenital warts had higher cure rate than those on extragenital warts; reflecting the point that response may vary with site of lesions and HPV type

PPD: Purified protein derivative, BCG: Bacille Calmette-Guerin

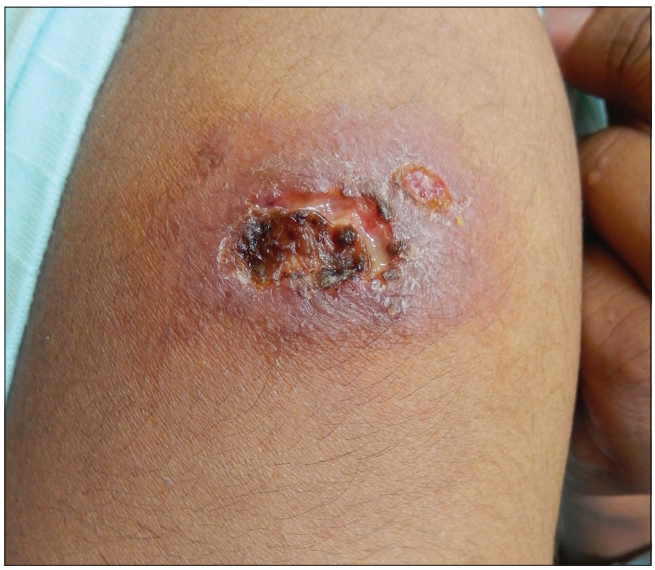

Figure 5a: Injection site reaction with *Mycobacterium w* vaccine (deltoid region)

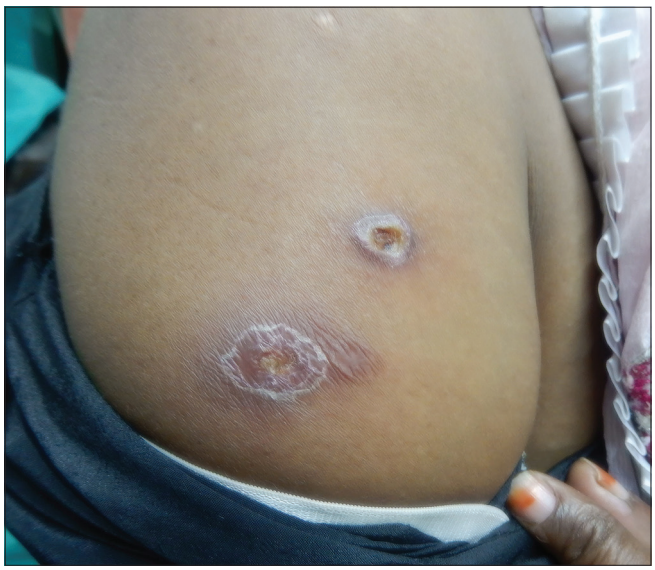

Figure 5b: Healing with atrophic scar and hyperpigmentation at *Mycobacterium w* injection site

counseled that the improvement may continue and that in case of partial response one should wait for at least 3 months before resorting to other treatment modalities.

While analyzing the prognostic factors to complete resolution, our study demonstrated a significantly better response only in lesions of shorter duration, a finding in line with other studies.[5,25] This might be due to virus-specific immune apathy in long-standing lesions. Other factors such as age, sex, baseline size and number of lesions, type of warts, presence of BCG immunization scar and tuberculin positivity had no correlation with response rates. Some previous studies have

Table 6a: Comparison between present study and other studies on purified protein derivative in viral warts

	Chandra S, *et al*. (present study)	Podder *et al*.[13]	Kus *et al*.[16]	Nimbalkar *et al*.[17]	Saoji *et al*.[18]	Elela *et al*.[5]	Kerure *et al*.[24]	Amirnia *et al*.[19]	Wananukul, *et al*.[20]
Site of study	Eastern India	Tertiary care center in Eastern India	Istanbul, Turkey	Tertiary care center in Central India	Maharashtra, India	Egypt	Tertiary care center in South India	Tabriz, Iran	Bangkok, Thailand
Type of study	Double-blind, randomized, controlled parallel -group comparative trial	Double-blind, randomized, active -controlled trial	Open labelled trial	Open labelled trial	Open uncontrolled trial	Single blinded, controlled trial	Open labelled trial	Double-blind, randomized, placebo -controlled trial	Open labelled trial
Type of wart	Multiple viral warts, (≥5) in number	Multiple warts - VV, VP, Palmoplantar wart >5 in number	Recalcitrant wart present for >2 years	Single or multiple warts in patients >12 years	Difficult-to-treat warts palmoplantar warts, periungual warts, >10 facial warts, >10 VV, >10 VP; untreated or off treatment for 1 month	Unspecified	Single or multiple warts except mucosal warts	Recalcitrant wart present >2 years, unresponsive to at least 1 treatment modality	Palmoplantar and periungual warts
Study population	PPD group: 32 Mw group: 32	BCG group: 33 PPD group: 27	18 patients	45 patients	55 patients	Group 1: 40 patients Group 2: 34 patients Group 3 (control): 20 patients	89 patients	Group 1 (PPD): 35 Group 2 (control): 34 Group 3 (cryotherapy): 33	42 patients
Drug, dosage, route	0.1 ml of PPD or *Mw* given I/D into deltoid every 2 weekly for 6 sessions or till CR	0.1 ml BCG or PPD; I/D in the right arm every 4 weekly till CR or max 3 doses	I/L PPD into target wart 3 weekly, total 3 injections. Dose was 0.1 ml, 0.2 ml or 0.3 ml (depending on tuberculin positivity)	0.1 ml of 10 TU PPD; I/L into largest wart 2 weekly till CR or max 6 doses	2.5 TU of PPD injected I/L into maximum 10 lesions with maximum dose per session being 25 TU, repeated 2 weekly for 4 sessions	Group 1: 0.1 ml of I/L PPD Group 2: 0.1 ml I/D PPD Group 3 (control): 0.1 ml I/L saline Two weekly injections given for 10 sessions or till CR	0.1 ml of 10 TU PPD injected I/L every 2 weeks till 6 sessions or CR	Group 1: 0.3 ml, 0.2 ml or 0.1 ml of PPD injected I/L (depending on tuberculin reactivity) Group 2: 0.3 ml of saline injected I/L Group 3: Cryotherapy - 3 freeze cycles followed by 10 s of thawing	0.1 ml of PPD injected I/L into largest lesion, every 2 weekly for 6 sessions or CR
Response	CR: 16 (50%) and 22 (68.8%) in PPD and *Mw* groups respectively.	BCG group: CR 16 (48.48%) PPD group: CR 5 (18.52%)	CR 5 (29.4%) Partial response 5 (29.4%) Minimal response 5 (29.4%) No response 2 (11.8%)	CR: 28 (62.2%) Partial response: 8 (17.8%) No response: 9 (20%) Response started at the end of 6 weeks	CR: 42 (76%) No response: 13 (24%)	Group 1: CR - 48 (96%), no response - 2 (4%) Group 2: CR - 32 (94.1%), no response - 2 (5.9%) Group 3: CR - 83 (69.45), no response - 21 (30.6%)	CR: 84 (94.4%) No response: 5 (5.6%)	CR: 77.1% in PPD group and 18.2% in cryotherapy group 50%-99% improvement: 22.9% in PPD, 33.35 in cryotherapy and 14.7% in control groups respectively No response: 48.5% in cryotherapy group and 85.3% in control group	CR: 39 (93%) No response: 3 (7%)

Contd...

Table 6a: Contd...

	Chandra S, *et al*. (present study)	Podder *et al*.[13]	Kus *et al*.[16]	Nimbalkar *et al*.[17]	Saoji *et al*.[18]	Elela *et al*.[5]	Kerure *et al*.[24]	Amirnia *et al*.[19]	Wananukul, *et al*.[20]
Adverse effect	PPD: Erythema and pruritus over the lesion, pain at injection site *Mw*: Painful indurated nodule, discharge, scar, erythema and pruritus over the lesion	BCG: Pain, injection site abscess, Scar	Erythema, edema, pain	Pain, injection site abscess, PIH, alopecia areata at injection site, urticaria	Redness, swelling, edema, eczematous reaction at injection site constitutional symptoms	Not mentioned	Pain	Cryotherapy: Pain, erythema, blister, hyperpigmented scar PPD group: Not mentioned	Pain, erythema, swelling, painful purpura
Follow up period and recurrence	3 months after the last dose. No recurrence	1 month No recurrence	Not mentioned	3 weeks after completion of treatment. No recurrence	At 1 month and 6 months after the last dose 1 recurrence	Not mentioned	3 months after the last dose No recurrence	6 months after the last dose. Recurrence rate: 8.6% after PPD and 22.4% after cryotherapy	6 months after the last dose. One recurrence

PPD: Purified protein derivative, BCG: Bacille Calmette-Guerin, IL: Intra lesional, TU: Tubeculin unit, CR: Complete response

found a positive correlation between younger age and a better respone[25] while one (Elela *et al.*) found a better response with older age.[5] Another study reported better response in patients with strongly positive tuberculin test.[11] Our study did not find such associations.

We found significant improvement in physicians' and patients' global assessment of disease activity and DLQI in both groups. This might reflect the frustration with the disease and other therapeutic modalities tried before, leading to physicians' and patients'satisfaction with reductions achieved both despite adverse events, which were generally mild.

Injection site reactions were probably due to local immunological responses against the injected antigen and the subsequent elaboration of cytokines leading to an inflammatory reaction. It was found in both the groups, occurring in almost all patients receiving the *Mw* vaccine compared to only 6 patients receiving PPD. These side-effects have been commonly documented by other authors as well.[11,16-18]

Our study indicates that immunotherapy with either *Mw* vaccine or PPD are potentially effective therapeutic modalities for treatment of warts. The route of injection though being intradermal were nonetheless effective in both the treatment arms. They might be especially useful in Indian settings where the population is widely sensitized to mycobacteria.[26,27] The phenomenon of immunological uplift is supported by the fact that resolution of warts continues even after the scheduled dosage of injections., and patients opting for immunotherapy need to be counseled about this favorable fact. The *Mw* vaccine may provide a slightly more rapid benefit, but otherwise the choice between the *Mw* vaccine and PPD would be guided by the availability and affordability of the agents. It is also to be highlighted that nodule with ulceration at the injection site and scarring are universal with *Mw* vaccine; thus preprocedural patient counseling is important.

Conclusion

Immunotherapeutic antigens PPD and *Mw* vaccine are effective and safe in the treatment of multiple warts in the Indian setting. *Mw* vaccine, though more effective, is associated with more adverse events. Our study provides strong evidence (level Ib) that intradermal immunotherapy has potential for consideration as first-line therapy in patients having multiple cutaneous warts and to replace conventional ablative methods as the treatment of choice in such patients.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patients have given their consent for their images and other clinical information to be reported in the journal. The patient understands that name and initials will not be published and due efforts will be made to conceal identity, but anonymity cannot be guaranteed.

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Table 6b: Comparison between present study and other studies on *Mw* vaccine in viral warts

	Chandra S, *et al* (present study)	Gupta *et al*.[7]	Meena *et al*.[6]	Garg and Baveja[23]	Singh *et al*.[21]	Dhakar *et al*.[22]
Site of study	Eastern India	New Delhi, India	Jaipur, India	New Delhi, India	New Delhi, India	Chandigarh, India
Type of study	Double-blind, randomized, controlled parallel-group comparative trial	Open labelled pilot study	Open labelled uncontrolled trial	Prospective, uncontrolled, open labelled trial	Retrospective review	Prospective, randomized, parallel group comparative trial
Type of wart	multiple viral warts, (≥5) in number	Multiple and/or giant anogenital warts	Multiple cutaneous wart ≥3	Untreated single or multiple warts with at least 1 lesion present on sole, palm, volar aspect of fingers, periungual or subungual areas	>5 extragenital warts on >1 body site or on difficult to treat sites (periungual, palms and soles)	Single or multiple refractory extragenital warts
Study population	PPD group: 32 *Mw* group: 32	10 patients	40 patients	30 patients	44 patients	*Mw* group: 33 Cryotherapy group: 33
Drug, dosage, route	0.1 ml of PPD or *Mw* given I/D into deltoid every 2 weekly for 6 sessions or till CR	I/L injection into ≤3 lesions every week for 10 sessions or till CR after an initial sensitizing dose of 0.1 ml *Mw* vaccine injected I/D into the deltoid	I/L injection into 3-5 lesions every week for 10 sessions or till CR after an initial sensitizing dose of 0.1 ml *Mw* vaccine injected I/D into the deltoid	0.1 ml of *Mw* vaccine injected I/L into single lesion, repeated 4 weekly for 10 sessions or CR	I/L injection into 2-4 lesions every 2 weekly for 10 sessions or till CR after an initial sensitizing dose of 0.1 ml *Mw* vaccine injected I/D into the deltoid	Mw group: I/L injection of 0.1 ml into 1-3 lesions every 2 weekly for 12 sessions or till CR after an initial sensitising dose of 0.1 ml *Mw* vaccine injected I/D Cryotherapy group: Liquid nitrogen spray repeated every 2 weekly till 12 sessions or CR
Response	CR: 16 (50%) and 22 (68.8%) in PPD and *Mw* groups respectively	CR: 8 (88.9%) Reduction of volume of the lesion to <5%: 1 Lost to follow-up: 1 Mean time to complete response: 5.9 weeks	CR: 33 (83%) 50% clearance: 1 25%-30% reduction: 3	CR: 28 (93.3%) No response: 2 (6.67%) Mean time to complete response: 43.71 days	CR: 24 (54.5%) >75% clearance: 37 (84.1%) Mean time to complete response: 6.75 weeks	CR: 20 (60.6%) and 19 (57.6%) in *Mw* and cryotherapy groups respectively
Adverse effect	PPD: Erythema and pruritus over the lesion, pain at injection site *Mw*: Painful indurated nodule, discharge, scar, erythema and pruritus over the lesion	Pain and edema at injection site; granulomatous balanitis and reactivation of herpes zoster and genital herpes in immunocompromised	Tender erythematous papule/pustule, BCG vaccine-like scar, erythema, swelling, superficial ulceration, low-grade fever, submandibular lymphadenitis	Redness, swelling, induration, spontaneous ulceration, fever, myalgia, headache	Pain, nodule, ulceration, atrophic scar at injection site, fever, paresthesia of limbs	Pain, swelling in both groups fever, regional lymphadenopathy, injection site scar, cellulitis in *Mw* group. Blister, scar, dyspigmentation in cryotherapy group
Follow up period and recurrence	3 months after the last dose. No recurrence	3-10 months (mean 5.1 months) after the last dose. No recurrence	Recurrence in 3 patients	Mean follow up: 11 months recurrence in 4 (14.28%)	3-9 months after the last dose. No recurrence	1 month after the last dose. Recurrence in 1 patient in cryotherapy group, none in *Mw* group

PPD: Purified protein derivative, BCG: Bacille Calmette-Guerin, I/L: Intra lesional, I/D: Intra deltoid, CR: Complete response, TU: Tuberculin unit

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Nil.

Conflicts of interest

There are no conflicts of interest.

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