Immunotherapy in viral warts with intradermal *Bacillus* Calmette–Guerin vaccine versus intradermal tuberculin purified protein derivative: A double-blind, randomized controlled trial comparing effectiveness and safety in a tertiary care center in Eastern India

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Abstract

Background: Current therapeutic modalities for viral warts are mostly ablative and are limited by high recurrence rates besides being unsuitable for numerous lesions. Immunotherapy has the potential to overcome these limitations.

Aims: The aim of this study was to compare the effectiveness and safety of *Bacillus* Calmette–Guerin vaccine versus tuberculin purified protein derivative in the immunotherapy of warts.

Methods: Patients received three doses of 0.1 ml of *Bacillus* Calmette–Guerin vaccine or tuberculin purified protein derivative intradermally over the deltoid region at 4-weekly intervals. They were followed-up for another month. Number of warts, complete cure rates and quality of life were assessed.

Results: A total of 60 patients were included. Complete clearance was noted in 16 (48.5%) out of 33 patients in the *Bacillus* Calmette–Guerin group and in 5 (18.5%) out of 27 in the tuberculin purified protein derivative group ($P = 0.121$). The number of lesions reduced statistically significantly from baseline in both the groups ($P < 0.001$) from the first follow-up visit onward ($P < 0.05$). The reduction was statistically significantly more in the *Bacillus* Calmette–Guerin group than in the tuberculin purified protein derivative group from the second follow-up onward. Dermatologic life quality index improved statistically significantly with both treatments. Adverse events (pain during injection, abscess formation and scarring at injection site) were more frequent with *Bacillus* Calmette–Guerin. No recurrence was seen after lesions cleared.

Limitations: Patients were not followed up for more than 4 weeks after treatment. We could not estimate the cytokine levels or the peripheral blood mononuclear cell proliferation in response to *Bacillus* Calmette–Guerin/ tuberculin purified protein derivative injections.

Conclusion: Both intradermal *Bacillus* Calmette–Guerin and tuberculin purified protein derivative hold promise in the treatment of viral warts. *Bacillus* Calmette–Guerin may be more effective, though it had more adverse events in our study.

Key words: *Bacillus* Calmette–Guerin, immunotherapy, tuberculin, purified protein derivative, viral warts

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Introduction

Viral warts, caused by human papillomaviruses, are among the most common dermatological diseases and are notorious for being contagious, recurrent and recalcitrant. They can affect people of both sexes, no age group being spared. Treatment of warts becomes a challenge when they are numerous or present over inaccessible areas. There are many ablative modalities of therapy such as electrocautery, chemicocautery, cryotherapy, laser surgery, curettage and topical keratolytics. Most of these take months and many of them may result in pain, scarring, and recurrences.[1] Ablative therapies are also limited by the fact that they only remove visible lesions; non-visible infected tissues are not targeted, resulting in a high chance of recurrence.[2]

Recalcitrant, multiple viral warts often cause considerable concern to the patient and management of such cases is frustrating. In an attempt to deal with this challenge, dermatologists have come up with several intuitive management strategies, many of which may act by strengthening the immune system. Diphenylcyclopropenone, squaric acid dibutyl ester, imiquimod, tuberculin jelly, *Candida* antigen, autologous vaccines, human papillomavirus vaccination and measles, mumps and rubella vaccine have all been tried as immunotherapy. Diphenylcyclopropenone and squaric acid dibutyl ester have the potential to cause allergic contact dermatitis, urticarial lesions and pigmentary disturbances, while autologous vaccines have oncogenic potential.[1,3-7]

Tuberculin antigen (purified protein derivative or tuberculin purified protein derivative, PPD) and *Bacillus* Calmette–Guerin (BCG) have garnered the attention of the dermatological fraternity for the treatment of multiple resistant warts. Purified protein derivative achieved 75% clearance of recalcitrant multiple viral warts and this was significantly better that the response in the saline control arm in a study by Abd-Elazeim *et al*. in Egypt.[8] Treatment of anogenital warts in pregnant women with intradermal purified protein derivative injection was found safe and effective with 47.5% demonstrating complete clearance and 37.5% partial response.[9] *Bacillus* Calmette–Guerin vaccination has been used for immunotherapy of viral warts in Iraq where a single-blind, placebo (distilled water) controlled study on 154 patients showed a significantly higher cure rate.[10]

Bacillus Calmette–Guerin was introduced as a prophylactic agent against tuberculosis, but is now also used in the treatment of malignant melanoma, transitional cell carcinoma of the bladder, alopecia areata and recurrent oral aphthosis.[10] It is thought to act by the stimulation of macrophages, T lymphocytes and natural killer cells. Toll-like receptor 7 may also play a role.

This study aimed to assess and compare the effectiveness of *Bacillus* Calmette–Guerin vaccination and tuberculin antigen (purified protein derivative or tuberculin purified protein derivative) for the treatment of multiple viral warts. We also aimed to look at the safety of these treatment regimens. We were unable to find any previous reports of a randomized trial of either with an active control.

Methods

The study was designed as a unicenter, double-blind, randomized, parallel group, active-controlled trial. Clearance from the Institutional Ethics Committee was obtained before the start of the study and written informed consent was obtained from all study participants or their legally authorized representatives. The study was registered in the Clinical Trial Registry-India (CTRI registration number: CTRI/2014/12/005244). The study was conducted from May 2014 for 8 consecutive months. All consecutive patients of either sex suffering from clinically diagnosed cutaneous warts and having more than five warts attending the dermatology outpatient department of Medical College and Hospital, Kolkata were included. Pregnant or lactating women, patients suffering from immunosuppression due to drug or disease, those with mucosal warts, nonconsenting patients, those with advanced diseases of vital organs, those unable to come for monthly follow-ups, and alcohol or other substance users were excluded from the study.

Visits

Screening visit

Patients were enrolled based on inclusion and exclusion criteria; written informed consent was obtained. All patients were referred to an integrated counseling and testing center and only those who were human immunodeficiency virus non-reactive were included. A thorough clinical examination was done. Routine hemogram, fasting blood glucose, serum urea, creatinine and liver function tests were also done.

Baseline visit

This visit was scheduled 7 days after the screening visit. The patients were randomized into two groups (*Bacillus* Calmette–Guerin or tuberculin purified protein derivative group) by a computer-generated random number table. The number of warts was counted and recorded in a standard case record form. Patients then received their first injection dose.

Follow-ups

Three follow-up visits were scheduled, at 4-weekly intervals. At each follow-up, the effectiveness parameters and adverse events were assessed. *Bacillus* Calmette–Guerin or tuberculin purified protein derivative injections were administered at the first and second follow-up visits. Injections were given till complete clearance of lesions, or till three sessions had been carried out.

Administration of Bacillus Calmette–Guerin/tuberculin purified protein derivative

According to the randomization, 0.1 ml of *Bacillus* Calmette–Guerin or tuberculin purified protein derivative was injected intradermally in the right arm (at the deltoid muscle insertion) with an insulin syringe.

Bacillus Calmette–Guerin

TUBERVAC® (Manufacturer: Serum Institute of India Ltd., Pune, Maharashtra, India) was used. Each ml of reconstituted vaccine contains between 1×10^6 and 33×10^6 colony forming units. The freeze-dried, powdered *Bacillus* Calmette–Guerin vaccine was diluted with 1 ml of normal saline supplied with the vaccine vial. The vials were stored between 2° and 8° after reconstitution in a refrigerator and returned immediately after drawing the vaccine dose for each patient. Each prepared vial was used within 4 h of opening, as per standard norm.

Tuberculin purified protein derivative

APLISOL® (Manufacturer: JHP Pharmaceuticals, Rochester, USA) was used. Aplisol is bioequivalent in potency to the standard purified protein derivative-S* (5 tuberculin units/0.1 ml) of the U.S. Public Health Service, National Centers for Disease Control. The vial was

stored between 2° and 8° in a refrigerator and returned immediately after drawing each patient's dose.

Effectiveness parameters

The primary effectiveness parameter was the number of visible warts on the skin. The secondary effectiveness parameters were the number of patients showing complete resolution of warts and the quality of life assessed by the vernacular version of the Dermatology Life Quality Index (with permission from the developer Prof. Andrew Finlay).

Safety parameters

Vital signs and adverse events reported by the patient or elicited by the clinician were assessed at each follow-up. Laboratory parameters were recorded at baseline and third follow-up.

Randomization and allocation concealment

Simple unstratified randomization to divide the patients into two groups was done using a computer-generated random number table. Concealment of randomization allocation was done by the sequentially numbered opaque sealed envelopes technique.

Blinding

After evaluation, the investigator referred the patient to an independent coordinator seated in another room. The coordinator was responsible for randomization, filling the insulin syringe with the appropriate trial medication and dispensing it to the investigator. The treating physician injected the medicine and noted clinical parameters. Both the investigator and the patients were blind to treatment allocation.

Sample size estimation

There were 27 patients in each treatment group. Sample size was calculated considering complete clearance of warts in 39.7% with *Bacillus* Calmette–Guerin and 75% with tuberculin purified protein derivative, with 80% power and 0.05 probability of type 1 error.[8,10] Considering a 10% possible dropout rate, this translated into a recruitment target of approximately thirty patients per group, or sixty patients overall.

Statistical analysis

Continuous variables (age, duration of illness) were compared between the groups by the independent samples *t*-test and within each group by a paired *t*-test. Mann–Whitney U-test and Wilcoxon matched-pairs signed-ranks test were employed for comparisons of unpaired and paired nonparametric data (number of warts, presence of *Bacillus* Calmette–Guerin immunization scar). Friedman's analysis of variance was carried out with non-parametric data for within-group repeated measures comparisons, followed by a *post hoc* Dunn's test. Categorical data were compared between the groups by Chi-square test or Fisher's exact test as appropriate. MedCalc version 11.6 (Mariakerke, Belgium: MedCalc Software, 2011) and GraphPad Prism version 5 (San Diego, California: GraphPad Software Inc., 20057) software were used for statistical analysis. $P < 0.05$ was considered statistically significant.

Effectiveness analysis was done on a modified intention-to-treat basis with subjects reporting for at least one post-baseline follow-up visit. Missing values were dealt with by the last observation carried forward strategy. Pre- and post-treatment laboratory values were compared in patients for whom both sets of data were available. For other safety analysis, all patients who had received at least one dose of a study drug (essentially all sixty patients) were considered.

Results

The participant flow is depicted in Figure 1. Sixty patients were randomized into two groups. Five patients were lost to follow-up. Of them, two patients in the *Bacillus* Calmette–Guerin group complained of scarring and did not come for subsequent follow-ups. In the tuberculin purified protein derivative group, one patient said that pain was the reason for his absence from follow-ups, one had migrated to a neighboring state on account of a family matter, and the third could not be contacted over the phone.

Men outnumbered women and patients were mostly in their late twenties or thirties. Both groups were comparable with respect to age, sex, residence (rural or urban) and income (above poverty line, below poverty line). The duration of illness was 11 ± 13.64 months in the *Bacillus* Calmette–Guerin group and 13.15 ± 8.85 months in the tuberculin purified protein derivative group with no significant difference between them. Both groups were also comparable in terms of the mean size of lesions at baseline ($P = 0.132$) and the type of warts seen ($P = 0.116$) [Table 1].

The number of warts was comparable initially in the two treatment arms. In the *Bacillus* Calmette–Guerin group, the number of warts significantly decreased from the first follow-up onwards ($P < 0.001$) till the end of the study [Figures 2a, b and 3a, b]. Similar results were obtained in the tuberculin purified protein derivative group. However, when the groups were compared, the reduction in the mean number of warts was found to be significantly more in the *Bacillus* Calmette–Guerin group from the second follow-up onwards [Table 2].

Complete resolution of warts could be seen from the first follow-up onward in the *Bacillus* Calmette–Guerin group; at the end of the study, 48.5% of patients achieved complete remission in this group compared to 18.5% in the tuberculin purified protein derivative group ($P = 0.028$) [Table 3]. Among the sixty patients observed, complete resolution of warts was obtained in 21 patients while the remaining 39 responded partially. Age, sex, duration and type of warts were all comparable amongst the complete cure and partial response

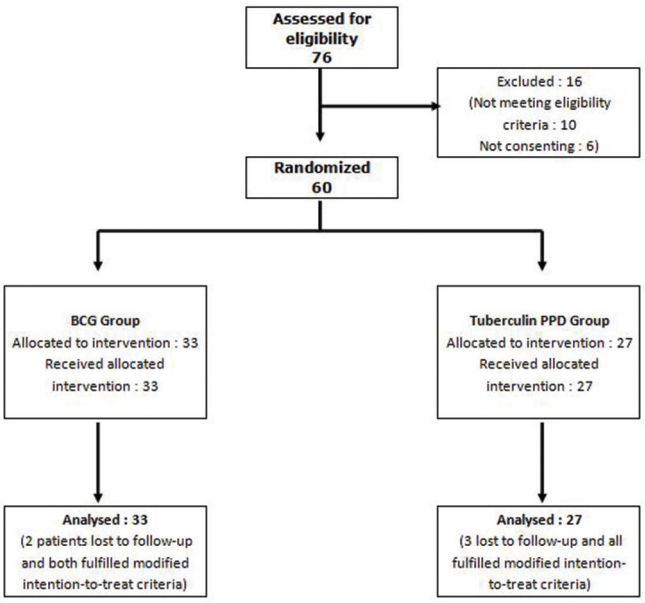

Figure 1: Flow of study participants

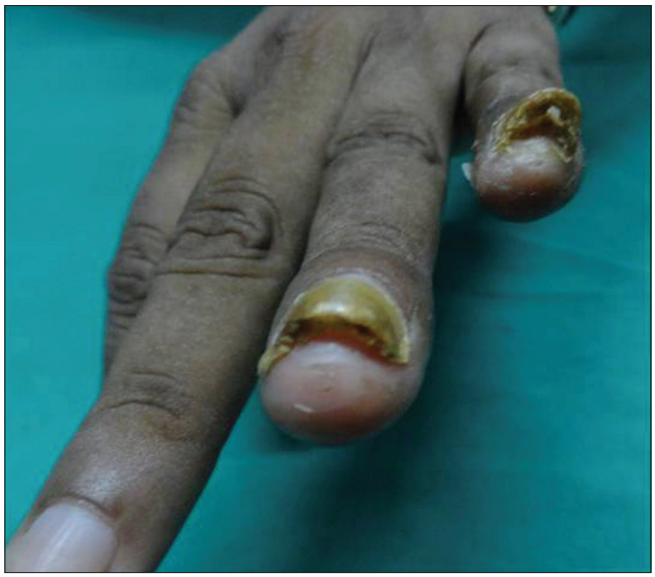

Figure 2a: Subungual warts: pretreatment

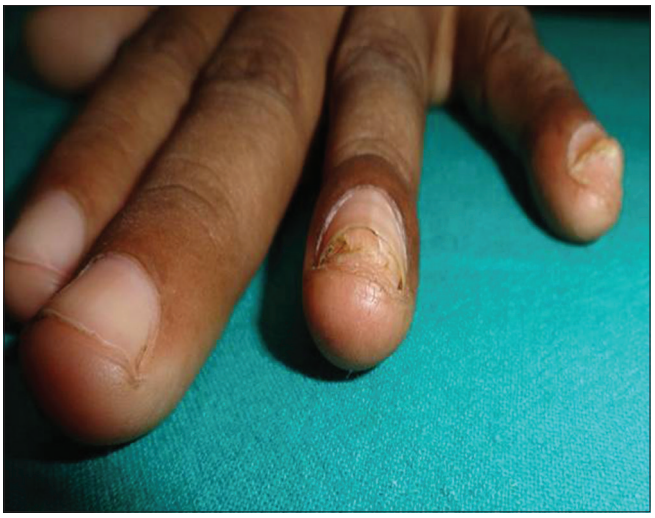

Figure 2b: Subungual warts: complete clearance of difficult-to-treat warts with three doses of *Bacillus* Calmette Guerin (at 12 weeks of treatment)

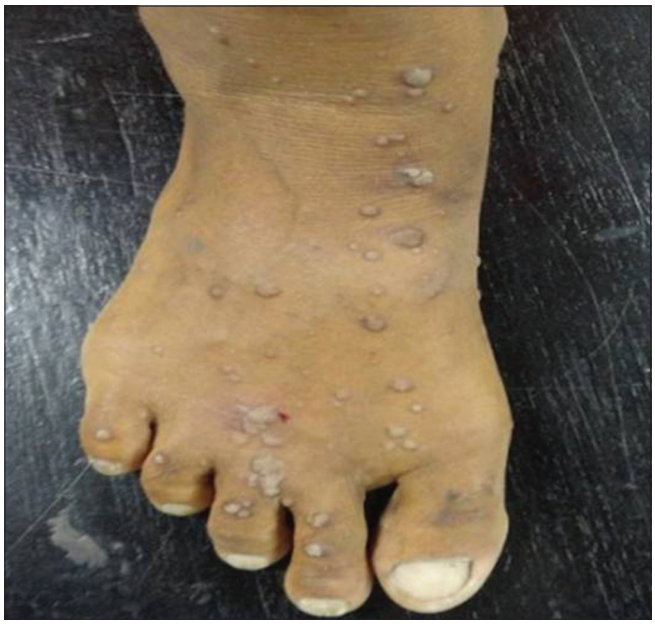

Figure 3a: Pretreatment photograph showing multiple warts (verucca plana) on foot

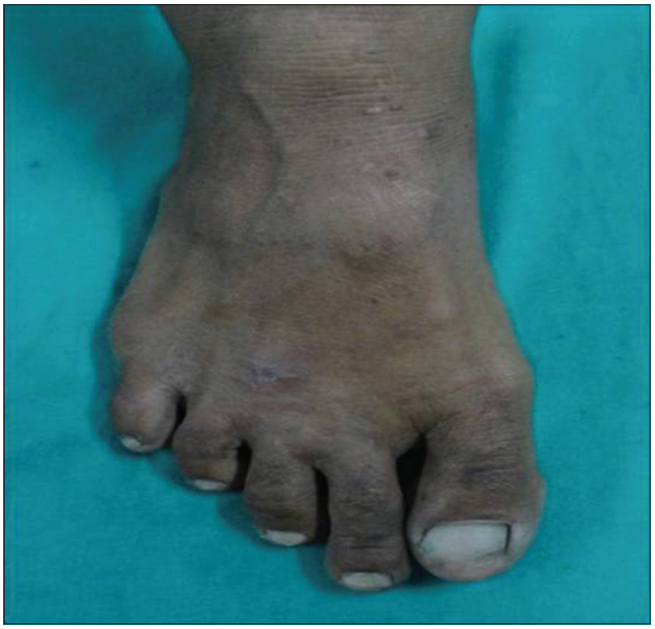

Figure 3b: Posttreatment photograph showing complete clearance of multiple warts (verucca plana) on foot with three doses of tuberculin purified protein derivative (at 12 weeks of treatment)

categories. Interestingly, complete responders had had significantly larger warts than had those who responded partially [Table 4]. No recurrences were seen in either of the two groups.

Bacillus Calmette–Guerin immunization scar was present in 30.3% of patients in the *Bacillus* Calmette–Guerin group and in 51.9% in the tuberculin purified protein derivative group [Table 1]. Subgroup analysis with the presence of a *Bacillus* Calmette–Guerin immunization scar as the grouping variable showed no significant differences in the reduction of the number of warts between the two subgroups at all follow-ups in the *Bacillus* Calmette–Guerin group [Table 5]. A similar subgroup analysis in the tuberculin purified protein derivative group showed a significant difference of the number of warts at baseline itself (P = 0.029); analysis of covariance with the baseline as covariate yielded no significant differences between these subgroups [Table 6].

The quality of life (assessed by the Dermatology Life Quality Index) was comparable (P = 0.667) in both the treatment arms at baseline. The index had improved significantly from baseline at the end of the study with both *Bacillus* Calmette–Guerin (P = 0.004) and purified protein derivative (P = 0.005), but an intergroup comparison showed no significant difference between the two treatment groups (P = 0.482).

Adverse events were observed more frequently in the *Bacillus* Calmette–Guerin group. More patients complained of pain during injection in this group, though this was not statistically

Table 1: Clinicodemographic profile of patients included

Parameters	BCG group (*n*=33)	Tuberculin PPD group (*n*=27)	*P* (between groups)
Age (years)			
Mean±SD	28 ± 10.74	32.37 ± 12.48	0.150
Range	16–55	18–58	
Sex (male:female)	20:13	17:10	0.936
Income (above poverty line:below poverty line	24:9	19:8	0.688
Residence (rural:urban)	10:23	11:16	0.950
Duration of illness (months), mean±SD	11±13.64	13.15 ± 8.85	0.591
Size of warts at baseline (cm), mean±SD	0.54±0.32	0.71 ± 0.45	0.132
Type of wart			
Verruca vulgaris	15	19	0.116
Verruca plana	6	4	
Palmoplantar wart	12	4	
BCG immunization scar (%)	10 (30.30)	14 (51.85)	0.673

P value is from Student's unpaired *t*-test for age and duration of illness and size of warts, Fisher's exact test for sex distribution, income, BCG immunization scar. BCG: Bacillus Calmette-Guerin, PPD: Purified protein derivative, SD: Standard deviation

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

Visit	BCG group (*n*=33)	Tuberculin PPD group (*n*=27)	*P* (between groups)
Baseline			
Mean±SD	13.94±17.02	15.70±12.65	0.262
95% CI	7.91-19.97	10.95-26.60	
First follow-up			
Mean±SD	8.36±11.34*	11.59±10.31*	0.057
95% CI	4.34-12.38	7.70-20.15	
Second follow-up			
Mean±SD	6.85±11.61*	8.96±8.84*	0.028
95% CI	2.73-10.97	5.95-19.09	
Third follow-up			
Mean±SD	5.07±10.77*	7.96±8.01*	0.013
95% CI	1.25-8.89	5.64-18.95	
P (within groups)	<0.001	<0.001	

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. *Significant reduction from baseline. SD: Standard deviation, CI: Confidence interval, BCG: *Bacillus* Calmette-Guerin, PPD: Purified protein derivative, ANOVA: Analysis of variance

Table 3: Comparison of complete clearance of warts in the two treatment groups

Visit	BCG group (*n*=33) (%)	Tuberculin PPD group (*n*=27) (%)	*P* (between groups)
First follow-up	1 (3.03)	0	1.000
Second follow-up	10 (30.3)	0	0.005
Third follow-up	16 (48.48)	5 (18.52)	0.028

P value between groups by Fisher's exact test. BCG: *Bacillus* Calmette-Guerin, PPD: Purified protein derivative

significant (P = 0.796). An abscess occurred at the injection site in one patient in the *Bacillus* Calmette–Guerin group. Scar formation was also found to be higher in the *Bacillus* Calmette–Guerin group (nearly 36%), but this too was not statistically significant (P = 0.620) [Table 7]. One patient in the *Bacillus* Calmette–Guerin group developed two scars during therapy. Laboratory parameters were within normal limits and comparable between the groups. None of the patients experienced any serious adverse event during the period of the trial.

Discussion

Immunotherapy for warts employs the ability of the immune system to recognize certain viral, bacterial and fungal antigens that induce a delayed-type hypersensitivity reaction in a previously sensitized individual, not only to the antigens themselves but also against the wart virus, which increases the ability of the immune system to recognize and clear the human papillomavirus.[2,11] Injection of the antigen results in peripheral blood mononuclear cell proliferation, promoting Th1 cytokine responses, particularly interferon-gamma and interlekuin-2. This results in activation of cytotoxic T cells and natural killer cells that help to eradicate human papillomavirus-infected cells.[12] It is also proposed that antigen immunotherapy can stimulate tumor necrosis factor-α and interlekuin-1 release, downregulating gene transcription of human papillomavirus.[13] The ability of the antigen to change the cytokine milieu to a Th1 response pattern triggering a cell-mediated immune response against the human papillomavirus seems to be the cornerstone of immunotherapy.

Immunotherapy addresses the limitations of ablative therapy in that it enhances the cell-mediated immune response that clears the virus-infected tissue irrespective of whether it is visible or not. It might also be able to target warts situated away from the site of the immunotherapeutic injection and therefore help in treating multiple warts, warts on inaccessible sites or sites where ablative therapy is difficult (e.g., subungual or periungual regions).

Agents used for intradermal/intralesional immunotherapy include extracted proteins (e.g., tuberculin), bacterial agents (e.g., *Bacillus* Calmette–Guerin, *Mycobacterium w* vaccine), fungal agents (e.g., *Candida albicans*, *Trichophyton*), viral agents (measles, mumps and rubella, and autoinoculation of warty tissue).[7-9] Some investigators favor determining the sensitization status of the individual with a presensitization test while others feel that this approach is not practical in view of patient compliance and increased costs.[14] India being a country where the prevalence of tuberculosis is very high (249/100,000 population) with an estimated 40% of the population infected with *M. tuberculosis* and where routine immunization against tuberculosis is in practice, it can be argued that the Indian population is widely sensitized to *M. tuberculosis*.[15] We therefore chose a related antigen (*Bacillus* Calmette–Guerin or tuberculin purified protein derivative), eliminating the need of a sensitization test.

Both forms of immunotherapy appeared effective in our study, with significant responses seen 4 weeks onward. Our findings also indicate that both can be advocated irrespective of the patients' *Bacillus* Calmette–Guerin immunization status. However we did find that *Bacillus* Calmette–Guerin was more effective than tuberculin purified protein derivative in terms of complete clearance and reduction in numbers of warts, perhaps because of greater numbers of cross-reacting epitopes being present on the whole

bacterial antigen of *M. bovis* (in *Bacillus* Calmette–Guerin) than in the protein extract of *M. tuberculosis* (in tuberculin purified protein derivative).

Reduction in wart numbers continued even after the three dosages were complete (as evident by the follow-up visit 4 weeks after the last injection). Complete cure was obtained in patients with larger warts, while the duration or type of warts did not appear to make a difference in our study. It may be hypothesized that larger warts have larger viral loads and hence greater epitope sharing with the cross-reacting antigen. However some previous studies had different results, with larger warts, those present for longer durations and plantar warts showing less favorable outcomes.[16,17]

Table 4: Comparison between complete responders (both with *Bacillus* Calmette-Guerin or purified protein derivative) and noncomplete responders

Parameters	Complete response (*n*=21)	Partial response (*n*=39)	*P* (between groups)
Age (mean±SD)	28.19±9.53	31.26±12.66	0.393
Sex (male:female)	13:8	23:15	0.861
Duration of illness (months), mean±SD	11.5±16.59	12.13±8.25	0.119
Size of lesions at baseline (cm). mean±SD	0.72±0.37	0.57±0.40	0.023
Type of warts			
Verruca vulgaris	11	22	0.714
Verruca plana	3	7	
Palmoplantar wart	7	9	

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. SD: Standard deviation

Scarring, which occurred in many of our cases, can cause discontent among patients; they should be made aware of this possible adverse effect before beginning therapy. Unexposed parts of the body (e.g., thighs) can be chosen for injections in those who are concerned about scarring. The study was limited by the fact that patients could not be followed up for more than 4 weeks. We also could not estimate cytokine levels or peripheral blood mononuclear cell responses to *Bacillus* Calmette–Guerin/tuberculin purified protein derivative injections due to infrastructural constraints. It needs to be mentioned that one vial of *Bacillus* Calmette–Guerin when reconstituted can be used for ten patients and has to be utilized within 4 h. It is therefore advisable to club patients for *Bacillus* Calmette–Guerin immunotherapy on one particular day to prevent wastage. This limitation is absent with tuberculin purified protein derivative.

Conclusion

Both *Bacillus* Calmette–Guerin and tuberculin purified protein derivative given intradermally at 4-weekly intervals show positive responses and are well-tolerated therapeutic options for viral warts. *Bacillus* Calmette–Guerin was found to be more effective than tuberculin purified protein derivative though it has the limitations of causing more pain and scarring. Response starts occurring 4 weeks after the first injection and continues even after completion of the three-dose schedule. Since the injections are given at a site away from the lesions being treated, this modality is suited for multiple lesions and for lesions in inaccessible and difficult-to-treat sites, such as the subungual or periungual regions.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

Table 5: Among patients receiving *Bacillus* Calmette-Guerin, comparison of the number of warts at follow-up visits in those having *Bacillus* Calmette-Guerin immunization scar versus those not having it

Visit	BCG immunization scar present (*n*=10)	BCG immunization scar absent (*n*=23)	*P* (between groups)
Baseline (mean±SD)	21.20±24.83	10.78±11.60	0.357
First follow-up (mean±SD)	10.80±11.53	7.30±11.35	0.281
Second follow-up (mean±SD)	8.90±12.49	5.96±11.38	0.638
Third follow-up (mean±SD)	5.80±10.11	4.75±11.25	0.638

P value between groups determined by Mann-Whitney U-test. SD: Standard deviation, BCG: *Bacillus* Calmette-Guerin

Table 6: Among patients receiving tuberculin purified protein derivative, comparison of the number of warts at follow-up visits in those having *Bacillus* Calmette-Guerin immunization scar versus those who are not having it

Visit	BCG immunization scar present (*n*=10)	BCG immunization scar absent (*n*=23)	*P* (between groups)
Baseline (mean±SD)	21.2±24.83	10.78±11.60	-
First follow-up (mean±SD)	10.80±11.53	7.30±11.35	0.867
First follow-up (corrected mean, SE)	11.71, 0.96	11.47, 0.10	
Second follow-up (mean±SD)	8.90±12.49	5.96±11.38	0.751
Second follow-up (corrected mean, SE)	9.26, 1.28	8.65, 1.33	
Third follow-up (mean±SD)	5.80±10.11	4.75±11.25	0.785
Third follow-up (corrected mean, SE)	8.22, 1.29	7.69, 1.35	

P value between groups taking baseline number of lesions as covariate determined by ANCOVA adjusted by Bonferroni correction. SD: Standard deviation, SE: Standard error, BCG: *Bacillus* Calmette-Guerin, ANCOVA: Analysis of covariance

Table 7: Adverse events

Adverse events	BCG group (*n*=33) (%)	Tuberculin PPD group (*n*=27) (%)	*P* (between groups)
Pain during injection			
Baseline	15 (45.45)	11 (40.74)	0.796
First follow-up	3 (0.09)	1 (0.04)	0.620
Second follow-up	2 (0.06)	1 (0.04)	1.000
Abscess			
First follow-up	0	0	
Second follow-up	1 (0.03)	0	1.000
Third follow-up	0	0	
Scarring			
First follow-up	12 (36.34)	4 (14.81)	0.620
Second follow-up	12 (36.34)	4 (14.81)	0.620
Third follow-up	12 (36.34)	4 (14.81)	0.620

P value between groups obtained by Fisher's exact test. BCG: *Bacillus* Calmette-Guerin, PPD: Purified protein derivative

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