Abstract

Background: Cutaneous warts present a therapeutic challenge because of recurrence and multiplicity and may become a frustrating condition for both patients and physicians. In the past few years, there has been an increase in intralesional immunotherapy for recurrent multiple warts not only because of its encouraging results in the treatment but also due to its ability to clear distant warts and preventing recurrence. Objective: The objective of this study was to evaluate the efficacy and safety of intralesional bacillus Calmette–Guerin (BCG) vaccine immunotherapy in the treatment of recurrent multiple warts. Materials and Methods: This study included 40 adult patients with multiple recurrent extragenital warts of different sizes, numbers, and duration, with or without distant warts. Patients were injected intralesionally with 0.1 ml BCG vaccine into the largest wart at a 3-week interval, directly without a presensitization skin test, until complete clearance or for a maximum of three sessions. Follow-up was done every month for 3 months to detect any recurrence. Results: Out of the 40 patients enrolled in the study, 34 patients completed the treatment protocol of three injections and 3 months of follow-up and six patients discontinued for various reasons. Complete clearance of the lesions was achieved in 25 (73.53%) patients, partial clearance in 8 (23.53%) patients, and no response in 1 (2.94%) patient. Complete response was demonstrated in 75% of those presenting with distant warts. Therapy-related side effects were mild in the form of pain during injection, itching, erythema at the site of injection, and flu-like symptoms. None of the patients with complete response showed recurrence of lesions in a 3-month follow-up period. Conclusion: Intralesional BCG immunotherapy is a safe, effective, and promising treatment modality for recurrent multiple warts.

Key Words: Bacillus calmette–guerin immunotherapy, cutaneous warts, immunotherapy

Introduction

Verrucae (warts) are benign proliferations of the skin, commonly encountered in day-to-day practice, often multiple and recurrent, resulting from an infection caused by human papillomavirus (HPV), an epitheliotropic DNA virus. Spontaneous resolution of warts has been reported in 65%–78% of patients within 2 years. In spite of currently available interventions including topical agents such as podophyllotoxin, trichloroacetic acid, formaldehyde, 5-fluorouracil, tretinoin, salicylic acid, and destructive therapies such as cryosurgery, laser ablation, intralesional bleomycin, electrofulguration, and surgical excision, they represent a therapeutic challenge to physicians and patients alike.

The cell-mediated immunity seems to play an important role in the control and clearance of HPV infection, for which various immunomodulators have been used such as oral cimetidine, levamisole, zinc sulfate, topical imiquimod, and contact sensitizers such as diphencyprone (DCP), dinitrochlorobenzene (DNDB), and squaric acid dibutyl ester (SADBE). There are some newer trends for immunotherapy in the treatment of warts, and recently, intralesional immunotherapy by different agents such as skin test antigens (mumps, Candida, and Trichophyton), bacillus Calmette–Guerin (BCG) vaccine, tuberculin purified protein derivative (PPD), measles, mumps and rubella (MMR) vaccine, and Mw (Mycobacterium indicus pranii) popularly

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known as Mw) vaccine has been tried and proved to be effective in the treatment of warts.[5–11]

Intralesional immunotherapy not only utilizes the ability of the immune system to mount a delayed-type hypersensitivity response to certain viral, bacterial, and fungal antigens but also to the HPV. Although the exact mechanism of immunotherapy is obscure, infiltration of CD4 T-lymphocytes and macrophages in wart lesions and activation of CD4 lymphocytes and an increase in Th1 cytokines such as interleukin-2 (IL-2), tumor necrosis factor-alpha (TNF-α), and interferons (IFN-α, β, and γ) have antiviral effects on HPV, through the downregulation of gene transcription and activation of cytotoxic and natural killer cells. Unique feature of immunotherapy is that it not only clears the treated wart but also the distant warts, unlike traditional therapies.[11,12]

BCG vaccine, being cheap, could be a very useful modality of wart treatment in poor and developing economies.[13] In light of the above facts, we undertook the study to evaluate the efficacy and safety of BCG vaccine in the treatment of warts.

Materials and Methods

A total of 40 adult patients with multiple recurrent extragenital cutaneous warts of different sizes and duration, with or without distant warts, were enrolled in the study. Distant warts were defined as warts at different anatomic sites away from the treated warts. Patients with acute febrile illness, any wart therapy in the previous month before enrolment, history of asthma or allergic skin disorders, pregnancy, lactation, and with iatrogenic or primary immunosuppression were excluded from the study. The diagnosis of warts was made clinically, and the patients were advised not to use any other wart therapy during the study period. All the patients were explained about the study, nature of the disease, and the intervention.

After obtaining written informed consent from all patients, baseline characteristics of warts, including number, site, size, duration, and the presence or absence of distant warts, were noted and photographic documentation was done at the beginning of the study and at each follow-up visit. All patients with previous BCG scar were injected with 0.1 ml of BCG vaccine (Serum Institute of India Ltd., 1 ml vial) into the largest wart intralesionally using an insulin syringe, held parallel to the skin surface with the bevel facing upward. Injections were given at a 3-week interval for three treatment sessions or till complete clearance is achieved. Response to treatment was evaluated by the decrease in the size of warts and sequential photographic comparison. The response was considered to be complete if there was complete disappearance of the warts and return of normal skin markings, partial response if the warts had regressed in size by 25%–99%, and no response if there was 0%–25% decrease in wart size. Immediate and late adverse effects of the BCG vaccine were evaluated after each treatment session. Follow-up was done every month for 3 months to detect any recurrence after clearance of warts. Patients who did not respond completely were offered other modalities of treatment on completion of 3-month follow-up period (after last BCG injection).

Institutional Ethics Committee approval was obtained and the protocol was submitted to the Clinical Trials Registry India (CTRI), India. The study was commenced after obtaining the approval of CTRI (CTRI/2014/08/004834).

Results

A total of 40 patients received intralesional BCG vaccine of which six patients after first dose discontinued for various reasons, including failure to follow-up (two patients) and side effects of the procedure such as flu-like symptoms (four patients) and hence were not included in the study.

Thus, 34 patients completed the study [Table 1]. All patients were men, in the age range from 18 to 46 years with a mean age of 25.5 years. The mean duration of the disease was 7.8±4.06 (range: 1–15) months. The head-and-neck region was the most common site (n=19, 55.8%), followed by the upper (35.3%) and lower limbs (8.82%). Distant warts were observed in 35.3% (12) patients. Nineteen (55.88%) patients received at least one previous therapy with topical agents such as salicylic acid, retinoic acid, trichloroacetic acid, oral zinc therapy, and destructive modalities such as cryotherapy, electrocautery, curettage, laser therapy, and surgical excision.

Complete response [Figures 1-3] was seen in 73.53% (25) patients, partial response in 23.53% (8) patients, and no response in one patient [Table 2]. Out of those patients

| Number of patients (%) |
|------------------------|
| Site of warts           |
| Head and neck (beard)   | 19 (55.88) |
| Upper limb             | 12 (35.3)  |
| Lower limb             | 3 (8.82)   |
| Number of warts         |
| 1-5                    | 5 (14.7)   |
| 6-10                   | 12 (35.3)  |
| >10                    | 17 (50)    |
| Size (mm)              |
| 1-5                    | 8 (23.53)  |
| 6-10                   | 14 (41.17) |
| >10                    | 12 (35.30) |
| Distant warts (n)      | 12 (35.30) |
| Previous treatment     | 19 (55.88) |
presenting with distant warts, complete clearance was observed in 75% (9 of 12).

Side effects were mild and insignificant. Tolerable immediate pain during injection occurred in all patients and was the most common adverse effect. Other local reactions such as erythema in 3 (8.8%), edema in 2 (5.9%), and itching in 13 (38.2%) patients were also seen at the site of injection. All patients had flu-like symptoms within 12 h of injection in the form of high-grade fever, malaise, joint pain, and weakness, which were managed by nonsteroidal anti-inflammatory drugs that resolved within 24–48 h. Indeed, 4 of original 40 recruited patients discontinued after first injection because of this side-effect. Other delayed side effects observed at the site of injection were ulceration (5.9%), scarring (14.7%), nodule/granuloma formation (11.8%), hypopigmentation (5.9%), and BCGitis (2.9%). After the 3-month follow-up period, none of the patients with complete response showed the recurrence of warts.

**Discussion**

Recurrent multiple warts represent a therapeutic challenge which is frustrating for both the patients and physicians. Although several therapeutic modalities have been used in the treatment of warts, a universally efficacious approach with low recurrence rate has yet to be explored. In immunocompetent patients, extragenital warts are known to resolve spontaneously, showing the prominence of cell-mediated immunity evidenced by infiltration with CD4 T lymphocytes and macrophages and raised Th1 cytokines (IL-2, TNF-α, and IFN-α, β, and γ) in wart lesions. Keratinocytes are both the main target of HPVs as well as main source of inflammatory cytokines. Thus, by manipulating the local immunological milieu, therapeutic response may be achieved against latent as well as clinical cutaneous HPV infection. Although a significant portion of common warts has been proposed to resolve spontaneously within 2 years, about one-third do not resolve and become recalcitrant despite repeated treatments.

As the immune system seems to play a role in control and clearance of warts, various immunotherapeutic modalities have been used including oral levamisole, cimetidine, zinc sulfate, etretinate, and topical/injectable preparations such as contact sensitizers (e.g.- SADBE, DNBC, DCP), IFN-α, and imiquimod have been used, but limited data are available to support their use.

Use of intralesional injections of various antigens such as *Candida* antigen, mumps, *Trichophyton* antigens, tuberculin PPD, BCG, MMR, and Mw vaccine, is another approach, which is being increasingly investigated for the treatment of multiple warts, for their nonspecific vaccine-like immunostimulant effect against HPV, especially for inducing clearance of lesions distant to the site of application.

Horn et al. compared the efficacy of skin test antigens (mumps, *Candida*, and *Trichophyton*), IFN-α-2b, and placebo injection in a randomized controlled trial. More than 75% clinical resolution was observed in 54% of the patients injected with antigen alone and 68%

| Table 2: Clinical response in patients with warts |
|-----------------------------------------------|
| **Clinical response** | **Number of patients (%)** |
| Complete response (100%) | 25 (73.53) |
| Partial response (25-99%) | 8 (23.53) |
| No response (0-25%) | 1 (2.94) |
of the patients injected with antigen plus IFN. Side effects seen included fever, myalgias, and injection site erythema and edema.

Daulatabad et al. in three case series of seven patients reported BCGitis in one and lymphadenopathy in three patients, which were treated with isoniazid and rifampicin in the Indian setting and highlighted the danger of BCG in endemic areas. Though in our study, side effects were not so common, only one patient developed BCGitis at the injection site.

Maronn et al. reported their 1-year experience with intrallesional Candida antigen therapy for both warts and molluscum contagiosum. In the wart group, 87% had complete resolution. In a recent uncontrolled study, intrallesional Candida albicans antigen immunotherapy was given to 34 patients, of which 56% showed a complete resolution of warts at all places on the body, while 38% failed to show any response.

In an open-label pilot study, Gupta et al. gave intrallesional Mycobacterium w vaccine immunotherapy for the treatment of anogenital warts and found near complete clearance in 88.9% of cases. In another open-label study, 83% of cutaneous warts treated with weekly intrallesional Mw vaccine resolved completely. The mean time to complete response was 9.7 weeks and distant wart clearance was noted in 70%. More recently, killed Mycobacterium indicus pranii (Mw) vaccine was evaluated in a study by Singh et al., in which complete clearance was reported in 54.5% patients, of which 86.3% patients showed a response in lesions distant to the site of injection. Therapy was relatively well tolerated.

In an Egyptian randomized placebo-controlled trial, Nofal and Nofal reported complete resolution of warts in 81.4% of patients treated with MMR vaccine as compared with 27.5% of patients in the placebo group. Injection site pain and flu-like symptoms were the only side effects noted in this study. Flu-like symptoms are immunological response to the vaccine and occur with most vaccines in which they are injected in the pediatric age group under the National Immunization Schedule. In another study by Nofal, complete clearance was observed in 63% of patients treated with intrallesional MMR vaccine, without presensitization skin test.

In an open-label uncontrolled study, tuberculin PPD was given to patients with difficult to treat warts, in which complete clearance was achieved in 76% patients after four sessions, while the remaining 24% patients were nonresponders. Adverse effects were erythema, edema, and pain at the site of injections.

BCG was introduced as a prophylactic agent against tuberculosis as well as in the treatment of alopecia areata and recurrent oral aphthosis. In most of the tuberculosis-endemic countries, BCG (live-attenuated vaccine derived from Mycobacterium bovis), is a part of the immunization schedule. The vaccine is considered to be safe and protects against the disseminated forms of tuberculosis. The incidence of BCG infection has been estimated to be around 1:10,000 to 1:100,000 population. These reactions may include the development of blister at the injection site, lymphadenopathy, or severe systemic dissemination. BCG-induced reactions have been categorized into local, regional, distant, or disseminated pattern. Conventionally, BCGitis refers to local and regional patterns, whereas BCGosis refers to the distant and disseminated pattern. These usually develop in children with an underlying immunodeficiency such as severe combined immunodeficiency. There is also risk of the development of lupus vulgaris at the vaccination site.

Topical application of BCG led to the clearance of genital warts within 6 weeks in six out of ten (60%) patients at a median follow-up of 9.2 months in one study and of condylomata acuminata in 80% of the studied patients after six applications, without recurrence after a 6-month follow-up period. Salem et al. also evaluated topical viable BCG vaccine for warts in children and noted a complete response in 65% patients with common warts and 45% with plane warts.

In an earlier single-blind placebo-controlled study, viable BCG was used as immunotherapy in warts, given as 0.1 ml intradermally over the upper arm in 81 patients. The result showed a complete response in 39.7% after 1–3 doses of BCG vaccination, which was significantly high when compared with a response of 13.7% after placebo treatment (P<0.001).

Encouraging results with intrallesional BCG immunotherapy were also seen in case reports of periungual wart, recallscitant wart of 5-year duration over the foot, and also medically resistant condylomata acuminata of the penis.

Munnangi compared BCG and MMR and showed superior efficacy and less side effects with MMR, though their regimen was different from our study and number of patients in each group were only 15.

Table 3 compares the dosing schedule, response rates, and side effects of various antigens/vaccines of previous immunotherapy studies with the present study. We achieved a complete response in 73.53% of patients, while partial response in 8 (23.53%) patients, and no response in one patient. Side effects were mild and insignificant. All patients had tolerable immediate injection site pain and flu-like symptoms within 12 h of injection in the form of high-grade fever, malaise, joint pain, and weakness, which were managed by nonsteroidal anti-inflammatory drugs that resolved within 24–48 h.
In our study, BCG immunotherapy appears to be a safe and promising modality for recurrent multiple cutaneous warts.

Although the exact mechanism of action of BCG vaccine is obscure, experimental evidence has shown that it stimulates macrophages, T- and B-lymphocytes, natural killer cell function, and augment IL-1 production.\[^7,22,23\]

Intralesional immunotherapy has been shown to be associated with the release of different cytokines such as IL-2, IL-4, IL-5, IL-8, TNF-\(\alpha\), and IFN-\(\gamma\), which stimulate a strong immune response against HPV. It has also been reported that the antigen injection is associated with the proliferation of peripheral blood mononuclear cells that promotes T-helper 1 cytokine responses that further activate cytotoxic T-cells and natural killer cells to eradicate HPV-infected cells. A unique feature of immunotherapy is that this not only clears the treated wart but also clears distant warts, unlike traditional therapies.\[^5‑7,16\]

Small sample size and the absence of control group were the main limitations of our study. Double-blind controlled method would have increased the strength of the study.

Conclusion
Intralesional BCG immunotherapy is a promising, inexpensive, safe, and effective modality for recurrent multiple warts as it has high tolerability, widespread response, and sustained effect with low recurrence rates. Small sample size and the absence of a control group were the main limitations of our study. As most of the studies on immunotherapy are open-label trial, randomized controlled studies in a larger population are required to ascertain the optimal dosage, concentration, and duration of therapy of these agents, to provide maximum therapeutic benefit.

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Conflicts of interest
There are no conflicts of interest.

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| Study                      | Antigen/vaccine | Maximum number of sessions | Interval between two sessions (week/s) | Complete response rate (%) | Adverse effects                                      |
|----------------------------|-----------------|----------------------------|----------------------------------------|----------------------------|------------------------------------------------------|
| Johnson et al.\[^5\]       | Mumps or Candida| 3                         | 3                                      | 74                         | Immediate pain, pruritus                              |
| Sharquie et al.\[^7\]      | BCG vaccine     | 3                         | 4                                      | 40                         | Nil                                                  |
| Nofal and Nofal\[^6\]      | MMR vaccine     | 5                         | 2                                      | 85                         | Flu-like symptoms, erythema, edema, pain, and itching |
| Saoji et al.\[^10\]        | PPD             | 4                         | 2                                      | 76                         | Erythema, edema, pain                                |
| Singh et al.\[^10\]        | Mycobacterium    | 10                        | 2                                      | 55                         | Intradermal granuloma, pain paresthesia, atrophic scarring |
| Present study              | BCG vaccine     | 3                         | 3                                      | 74                         | Immediate pain, flu-like symptoms, erythema, edema, BCGitis |

BCG: Bacillus Calmette-Guerin, MMR: Measles, Mumps, Rubella, PPD: Purified protein derivative
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