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# Immunotherapy of Cutaneous Warts: A Novel Comparative Study Between Intralesional Vitamin D3 and Normal Saline, Eastern India

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## ABSTRACT

**Background:** Warts are common benign skin lesions caused by human papillomavirus (HPV). Recalcitrant warts—those that are unresponsive to conventional therapy—pose a significant treatment challenge. Immunotherapy using intralesional agents, such as vitamin D3, has emerged as a promising alternative. The study aimed to evaluate and compare the efficacy and safety of intralesional vitamin $D_3$ versus normal saline in the treatment of cutaneous warts.

**Methods:** This was a double-blinded randomized controlled trial (RCT) study that included 60 patients with recalcitrant warts, randomized into two groups: Group A received intralesional Vitamin D3 (600,000 IU/ml) and Group B received normal saline. Injections are administered every 4 weeks for a maximum of 4 sessions, followed by 3 months of follow-up. The total study duration was one year.

**Results:** Group A showed significantly higher complete clearance rates (73.3%) compared to Group B (13.3%). The therapeutic response was evident in most cases after the second session. Adverse effects in the Vitamin D3 group were mild and self-limiting.

**Conclusion:** Intralesional Vitamin D3 is a safe, effective, and well-tolerated treatment for recalcitrant warts, outperforming normal saline in both clearance rate and patient satisfaction.

**Key-words:** Cutaneous warts, Immunotherapy, Intralesional Vitamin D3, Normal saline, Double-blinded randomized controlled trial, Recalcitrant warts, Dermatology

## INTRODUCTION

Cutaneous warts, also known as verrucae, are benign epidermal proliferations caused by infection with human papillomavirus (HPV), a DNA virus with over 100 distinct genotypes.

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The virus infects keratinocytes through microabrasions, inducing abnormal proliferation that leads to lesions that may appear on any site of the skin or mucosa. Although these lesions are frequently self-limiting in immunocompetent individuals, they can persist for prolonged periods and become recalcitrant, causing significant psychosocial distress and physical discomfort to affected individuals [1].

Conventional therapies for treating warts include destructive modalities such as cryotherapy, electrocautery, chemical cauterisation (using trichloroacetic acid or salicylic acid), radiofrequency

 

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ablation, and laser therapy. These methods aim to remove the visible lesion but do not address the underlying viral infection or the host's immune status. Consequently, they are associated with pain, a risk of scarring, pigmentary changes, and a high recurrence rate. Additionally, these modalities are often not suitable for multiple or difficult-to-access lesions, particularly in cosmetically sensitive areas or pediatric patients [2].

Given these limitations, immunotherapy has emerged as a promising therapeutic alternative. Immunotherapy aims to stimulate the host immune system to recognize and eliminate HPV-infected keratinocytes not only at the treated site but also at distant lesions. It offers several advantages: it is minimally invasive, has lower recurrence rates, and may provide long-term immunity against re-infection [3].

Among various immunotherapeutic agents, intralesional Vitamin D3 has attracted attention due to its ability to modulate immune responses. Vitamin D3 is known to enhance innate immunity by increasing the expression of antimicrobial peptides, such as cathelicidin and β-defensin, while also modulating adaptive immunity by influencing the function of T cells and macrophages. Its intralesional use in dermatological conditions, such as alopecia areata, keloids, and warts, has shown promising results, particularly in recalcitrant cases. The ease of availability, low cost, and safety profile further support its clinical utility [4].

Normal saline, though inert, is often employed as a control in clinical trials to account for the placebo effect and spontaneous resolution of warts. Its inclusion allows for a more accurate assessment of the therapeutic efficacy of the active agent by eliminating procedural bias, such as the mechanical trauma of injection [5].

Despite emerging evidence supporting the efficacy of intralesional immunotherapy, there remains limited comparative data specifically between Vitamin D3 and normal saline in recalcitrant warts. The current study aims to evaluate and compare the efficacy and safety of intralesional Vitamin D3 and normal saline in the treatment of recalcitrant cutaneous warts. It also assesses the number of treatment sessions required, the onset of clinical response, adverse effects, and recurrence during follow-up, to identify a more effective and patient-friendly therapeutic option for these difficult-to-treat lesions.

## MATERIALS AND METHODS

**Study Design and Setting-** This was a double-blinded randomized controlled trial (RCT) study. Patients were unaware of the injections they were getting. There were two investigators. Investigator 1 administered the injections and Investigator 2 assessed and analysed the results, who was unaware of the type of injection the patients were getting. The study spanned 1 year and included patients with recalcitrant cutaneous warts. Patients were diagnosed clinically and confirmed with the help of Dermoscopy.

**Inclusion and Exclusion Criteria-** Patients aged between 18 and 60 years with clinically diagnosed cutaneous warts, unresponsive to previous treatment for at least 6 months, were included. Exclusion criteria were:

- Pregnancy and lactation
- Immunosuppressed status or autoimmune diseases
- History of hypersensitivity to Vitamin D3
- Use of systemic immunomodulators in the past 3 months
- Presence of active infection or inflammation at the injection site

**Sample Size and Group Allocation-** A total of 60 patients were enrolled in the study. The sample size was determined based on previous studies evaluating treatment efficacy in recalcitrant warts. Patients were randomly divided into two groups of 30 each using simple random sampling:

**Group A (Vitamin D3 Group)-** Received intralesional injection of 0.2–0.5 ml of Vitamin D3 (600,000 IU, 15 mg/ml) per lesion.

**Group B (Control Group)-** Received intralesional injection of an equal volume of normal saline.

**Procedure-** After obtaining informed consent, intralesional injections were administered using a 27-gauge insulin syringe into the largest wart. Injections were given at four-week intervals, with a maximum of four sessions per patient. Paring of the thick keratotic surface was performed before injection when required. Patients were evaluated at each visit and followed up monthly for three months post-treatment.

**Assessment Criteria-** Clinical response was evaluated at baseline, after two weeks, and four weeks post-treatment using predefined grading criteria.

 

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Blinding was maintained at the level of the assessing physician to reduce bias:

- Patients were not informed about their group allocation, i.e., whether they received Vitamin D3 or normal saline.
- The treating physician was aware of the treatment being administered, as the physical consistency of Vitamin D3 and normal saline differs.
- The observer physician, who assessed the treatment response, was blinded and did not know which treatment each patient had received.

  This ensured double blinding, thereby improving the objectivity of outcome assessment.

**Statistical Analysis-** Data were analyzed using appropriate statistical software. Categorical variables were expressed in percentages and compared using the Chi-square test. A p-value<0.05 was considered statistically significant.

**Ethical Approval-** The study protocol was approved by the Institutional Ethics Committee of IPGME&R, Kolkata (Approval No.: IPGME&R/IEC/2022/282).

**RESULTS**

A total of 60 patients with recalcitrant cutaneous warts were included in the study and randomly assigned to two groups. Group A received intralesional Vitamin D3 injections, while Group B received intralesional normal saline. Each group included 30 patients. Both groups were comparable at baseline in terms of age, gender distribution, duration of lesions, and number of warts.

At the end of four treatment sessions and three months of follow-up, treatment response was significantly better in Group A than in Group B ($p$<0.001). In Group A, 22 patients (73.3%) showed complete clearance of warts, while only 4 patients (13.3%) in Group B achieved the same outcome. Partial response, defined as 50–99% reduction in size or number, was noted in 6 patients (20%) in Group A and 8 patients (26.7%) in Group B. No response (<50% improvement) was reported in only 2 patients (6.7%) in the Vitamin D3 group, compared to 18 patients (60%) in the saline group. The distribution of responses is detailed in Table 1.

**Table 1:** Comparison of Treatment Response Between Group A and Group B

| Response Category | Definition | Group A (n=30) | Group B (n=30) | p-value |
|---|---|---|---|---|
| Complete Response | 100% clearance of all warts | 22 (73.3%) | 4 (13.3%) | <0.001 |
| Partial Response | 50–99% reduction in number/size | 6 (20.0%) | 8 (26.7%) | 0.37 (NS) |
| No Response | <50% reduction in number/size | 2 (6.7%) | 18 (60.0%) | <0.001 |

Early signs of improvement were observed in most patients from Group A after the second injection. Among the 22 patients in Group A who achieved complete clearance, 8 did so within two sessions, 10 within three sessions, and 4 required all four sessions (Table 2). This suggests that while some cases responded rapidly, continued treatment up to four doses provided additional benefit.

**Table 2:** Number of Sessions Required for Complete Clearance in Group A

| Sessions Required | Number of Patients (n=22) | Percentage of Responders | Cumulative Clearance (%) |
|---|---|---|---|
| 2 Sessions | 8 | 36.4% | 36.4% |
| 3 Sessions | 10 | 45.5% | 81.9% |
| 4 Sessions | 4 | 18.1% | 100% |

Adverse effects were minimal in the Vitamin D3 group. Six patients (20%) experienced mild pain at the injection site, and two patients (6.7%) developed transient erythema or swelling. None of the patients in the saline group reported any local or systemic side effects (Table 3). All adverse events were self-limiting and did not require discontinuation of therapy.

 

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**Table 3:** Adverse Effects Reported During the Study

| Adverse Effect | Description | Group A (n=30) | Group B (n=30) |
|---|---|---|---|
| Pain at Injection Site | Transient, mild-to-moderate, <24 hrs duration | 6 (20.0%) | 0 (0.0%) |
| Erythema / Swelling | Localized, self-resolving within 48 hrs | 2 (6.7%) | 0 (0.0%) |
| Systemic Symptoms | Fever, malaise, or allergic reaction | 0 (0.0%) | 0 (0.0%) |

Clinical progression of a patient treated with intralesional Vitamin D3 (D3 group). Image 'a' shows multiple verrucae over the dorsum of both hands-on day 0 (pre-treatment). Image 'b' shows significant reduction in wart size and number after the second session. Image 'c' demonstrates complete clearance with smooth skin at the 6-month follow-up (Fig. 1a).

Clinical progression of a patient treated with intralesional normal saline (N1 group). Image 'a' shows multiple warts on the dorsum of the feet at baseline. Image 'b' reveals minimal improvement after two sessions. Image 'c' shows persistence of lesions with no significant clearance even at 6-month follow-up (Fig. 1b).

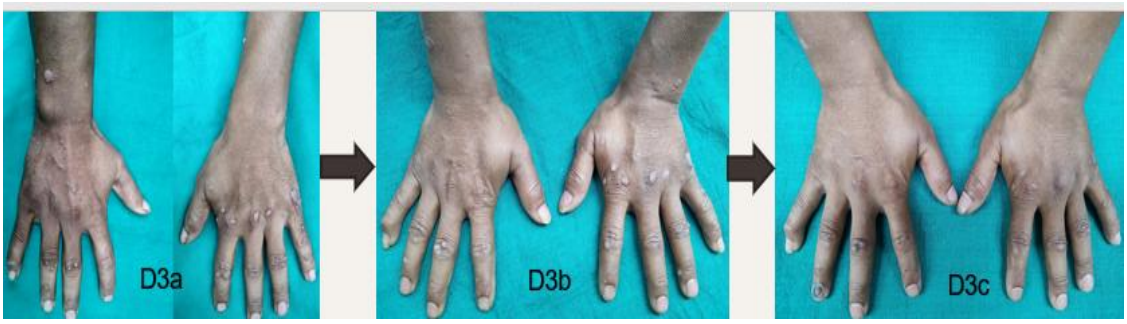


**Fig. 1a:** D3 showing response to injection vit-D3 (*a=day 0, b=after 2nd session, c=at 6th month follow-up*)

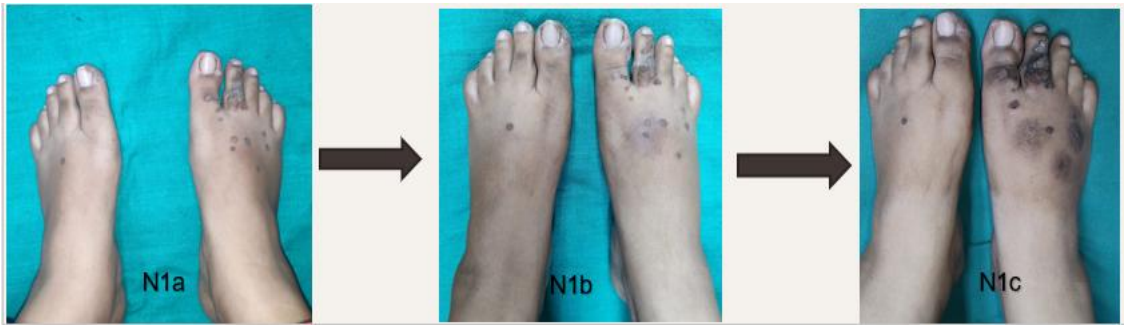


**Fig. 1b:** N1 showing response to injection normal saline (*a=day 0, b=after 2nd session, c=at 6th month follow-up*)

## DISCUSSION

Warts are benign proliferations of the epidermis caused by various strains of the human papillomavirus (HPV), commonly encountered across all age groups. Although many warts resolve spontaneously, a significant subset becomes recalcitrant, meaning they are resistant to conventional treatment options. In such cases, standard destructive therapies such as cryotherapy, electrocautery, and topical keratolytics often fail to provide long-term clearance. They are associated with pain, scarring, high recurrence rates, and patient dissatisfaction [6].

Immunotherapy has emerged as a promising alternative approach, particularly for recalcitrant and multiple lesions. It aims to harness the body's immune system to clear infected keratinocytes and potentially provide systemic clearance, including distant and subclinical lesions. Several agents have been explored for this purpose, including BCG vaccine, MMR vaccine, Candida antigen, and Vitamin D3 [7].

Vitamin D3 plays a dual role in skin health—beyond its classical involvement in calcium homeostasis, it exerts notable immunomodulatory effects. It enhances the innate immune response by upregulating antimicrobial peptides such as cathelicidin and defensins. It also

 

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modulates adaptive immunity by suppressing pro-inflammatory cytokines and regulating T-cell proliferation and differentiation. These properties have been explored in various dermatoses, including psoriasis, alopecia areata, and warts [8].

In the present study, a significant therapeutic response was observed in the Vitamin D3 group, with 73.3% of patients showing complete clearance of warts. This aligns with previous studies, such as that by Raghukumar *et al*., who reported complete resolution in 72% of cases using intralesional Vitamin D3 [9]. Another study by Nofal *et al.* demonstrated similar outcomes, with up to 80% of treated individuals achieving full clearance [10]. These results suggest that Vitamin D3 induces a sustained immune response sufficient to eradicate HPV-infected cells and prevent recurrence.

In contrast, only 13.3% of patients in the normal saline group showed complete clearance. This low response rate emphasizes the limited therapeutic value of mechanical trauma or placebo in recalcitrant wart cases. It also validates the role of Vitamin D3 as an active agent, rather than the response being merely due to the injection technique.

The onset of response in most patients treated with Vitamin D3 was observed after the second injection. Among those who achieved complete clearance, the majority responded by the third session. This progressive trend in response is consistent with the time required for immune activation and supports a protocol involving at least three sessions for optimal results. Minimal adverse effects were reported in the Vitamin D3 group, limited to transient pain and mild erythema at the injection site. No systemic effects were noted, indicating the treatment's safety and tolerability, particularly important in pediatric and immunocompromised populations [11].

Photographic documentation supported these outcomes visually, with patients treated using Vitamin D3 demonstrating complete resolution of hand warts within three sessions. These clinical responses are consistent with prior studies reporting high clearance rates following Vitamin D3 immunotherapy [5,6,12]. In contrast, patients who received normal saline injections exhibited minimal to no improvement even after six months of follow-up, underscoring the limited therapeutic value of placebo or injection trauma alone [9].

This study is strengthened by its randomized design and objective photographic assessments. However, limitations include the relatively small sample size and the short duration of follow-up, which may not capture long-term recurrences—a limitation also noted in similar studies using Vitamin D3 [10,13]. Further large-scale studies with extended follow-up are recommended to validate the durability of the treatment response and explore its application in other HPV-related skin conditions, as explored in broader comparative immunotherapy research [7,14].

Overall, the findings support the efficacy, safety, and practicality of intralesional Vitamin D3 in managing recalcitrant warts, offering a patient-friendly, minimally invasive, and cost-effective alternative to destructive modalities, especially in multiple or resistant lesions [2,4,15].

## CONCLUSIONS

We concluded that Intralesional Vitamin D3 was found to be a safe, well-tolerated, and significantly more effective treatment for recalcitrant cutaneous warts compared to normal saline. It demonstrated a high complete clearance rate, early onset of response, and minimal adverse effects. The treatment was not only efficacious at the local site but also showed potential for resolving distant lesions, suggesting a broader systemic immunomodulatory effect. Given its simplicity, low cost, and minimal invasiveness, intralesional Vitamin D3 can be considered a strong alternative to conventional destructive methods, particularly in patients with multiple, resistant, or cosmetically sensitive lesions.

Future studies should aim to evaluate the long-term durability of response, recurrence rates, and the immunological basis of action through larger, multicenter randomized trials. Additionally, exploring the role of Vitamin D3 in combination immunotherapy or its use in immunocompromised individuals and other HPV-related dermatoses may further expand its clinical utility.

## CONTRIBUTION OF AUTHORS

**Research concept–** Sudip Mandal, Subhamoy Neogi, Olympia Rudra

**Research design–** Sudip Mandal, Subhamoy Neogi, Olympia Rudra, Tirthankar Gayen

Supervision– Olympia Rudra, Kakali Mridha

**Materials–** Sudip Mandal, Subhamoy Neogi, Olympia Rudra

**Data collection–** Sudip Mandal, Subhamoy Neogi, Kakali

 

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Mridha

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**Writing article–** Sudip Mandal, Subhamoy Neogi

**Critical review–** Olympia Rudra, Tirthankar Gayen, Kakali Mridha, Gobinda Chatterjee

**Article editing–** Olympia Rudra, Tirthankar Gayen, Kakali Mridha, Gobinda Chatterjee

**Final approval–** Kakali Mridha, Gobinda Chatterjee

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