STUDY PROTOCOL AND STATISTICAL ANALYSIS PLAN

Intralesional Therapies for Cutaneous Viral Warts: A Comparative Analysis of Vitamin D_3 and Acyclovir

Official Title: Intralesional Therapies for Cutaneous Viral Warts: A Comparative Analysis of Vitamin D_3 and Acyclovir

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Multicenter Randomized Controlled Trial

Site 1 (Lead Site): Department of Dermatology, Pakistan Railway Hospital
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Site 2: Riphah International Hospital, Sector A DHA Phase 5, Islamabad
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1. Study Synopsis

Study Title	Intralesional Therapies for Cutaneous Viral Warts: A Comparative Analysis of Vitamin D3 ar
Study Design	Prospective, single-blinded, multicenter, randomized controlled trial
Study Sites	Site 1: Dermatology Department, Pakistan Railway Hospital Site 2: Riphah International Hospital, DHA Phase 5, Islamabad
Principal Investigator	Dr. Nadia Ghazanfar (Pakistan Railway Hospital)
Site Investigator / Co-PI	Dr. Fatima Sajid (Riphah International Hospital)
Study Duration	3 months follow-up period
Sample Size	40 participants (20 per group)
Population	Patients aged 12 years and above with clinically diagnosed cutaneous warts
Intervention (Group A)	Intralesional Acyclovir (70 mg/mL)
Comparator (Group B)	Intralesional Vitamin D3 (200,000 IU; 5 mg/mL)
Primary Outcome	Clinical response at week 8 (complete clearance, partial response, or no response)
Secondary Outcomes	Recurrence rate, adverse effects, number of sessions to clearance
Randomization	Simple randomization with sealed opaque envelopes
Blinding	Single-blind (outcome assessor blinded)

2. Introduction and Background

Cutaneous warts are benign epithelial proliferations caused by human papillomavirus (HPV) infection. They are among the most common dermatological conditions, affecting individuals of all ages. Warts can present as common (verruca vulgaris), plantar, flat, or periungual types, and while often self-limiting, they can be persistent, recurrent, and cosmetically distressing.

Current treatment modalities include destructive therapies (cryotherapy, electrocautery, laser), topical agents (salicylic acid, imiquimod), and intralesional therapies. However, no single treatment offers a consistently high cure rate, and recurrence remains a significant clinical challenge. Intralesional therapies have gained attention due to their potential to stimulate local and systemic immune responses against HPV.

Intralesional vitamin D3 has been proposed as an immunomodulatory agent that can enhance the host immune response against HPV-infected keratinocytes. Intralesional acyclovir, an antiviral agent primarily used against herpes viruses, has also been explored for its potential efficacy against cutaneous warts. This multicenter study aims to compare the effectiveness and safety of these two intralesional therapies across two clinical sites.

3. Study Objectives

3.1 Primary Objective

To compare the clinical efficacy of intralesional acyclovir versus intralesional vitamin D3 in the treatment of cutaneous viral warts at week 8.

3.2 Secondary Objectives

- To compare the recurrence rate between the two treatment groups
- To evaluate the safety profile and adverse effects of each treatment
- To compare the number of treatment sessions required for complete clearance

4. Study Design

This is a prospective, single-blinded, multicenter, randomized controlled trial with two parallel arms. The study is conducted at two sites: the Dermatology Department of Pakistan Railway Hospital (lead site) and Riphah International Hospital, DHA Phase 5, Islamabad. The follow-up period is three months.

4.1 Randomization

Eligible participants at each site are randomly allocated into two equal groups (Group A and Group B) using a simple randomization technique. Allocation concealment is ensured using sealed opaque envelopes prepared centrally and distributed to both sites.

4.2 Blinding

The study is single-blinded. Outcome assessment is performed by an independent investigator who is blinded to group allocation at each site. Participants and treating physicians are aware of the assigned intervention.

4.3 Multicenter Coordination

Both sites follow an identical study protocol, including the same inclusion and exclusion criteria, treatment procedures, dosing schedules, outcome assessments, and data collection instruments. The principal investigator (Dr. Nadia Ghazanfar) oversees the overall conduct of the study, while the site investigator (Dr. Fatima Sajid) is responsible for day-to-day operations at the second site. Regular communication between sites ensures protocol adherence and data quality.

5. Study Population

5.1 Inclusion Criteria

- Age 12 years and above
- Clinically diagnosed cutaneous warts (common, plantar, flat, or periungual)
- Both treatment-naive patients and those with recalcitrant warts
- Willingness to comply with follow-up visits

5.2 Exclusion Criteria

- Pregnant or lactating women
- Immunocompromised patients
- History of hypersensitivity to vitamin D3 or acyclovir
- Receiving systemic immunosuppressive or antiviral therapy
- Secondary infection at injection site
- Any topical or destructive treatment for warts in the previous three months

5.3 Sample Size

A total of 40 participants will be enrolled across both sites, with 20 participants allocated to each group.

6. Interventions

6.1 Group A: Intralesional Acyclovir

Patients in Group A receive intralesional acyclovir (70 mg/mL), prepared by reconstituting a 250 mg vial with 3.5 mL of distilled water.

6.2 Group B: Intralesional Vitamin D3

Patients in Group B receive intralesional vitamin D3 (200,000 IU; 5 mg/mL).

6.3 Dosing Protocol

Lesion Size	Injection Volume
≤0.5 cm	0.2 mL
0.5 – 1 cm	0.3 – 0.5 mL
1 – 1.5 cm	0.5 – 1 mL

Injections are administered at baseline and repeated every 2 weeks for a maximum of four sessions, with follow-up until three months. The same dosing protocol is followed at both study sites.

7. Study Procedures and Schedule

7.1 Baseline Assessment

At baseline, detailed patient biodata is recorded, including age, sex, duration, type, number, size, and site of lesions. Standardized digital photographs are taken before initiation of therapy.

7.2 Treatment Visits

Injections are administered at baseline (week 0), week 2, week 4, and week 6 (maximum four sessions). At each follow-up visit, the following are assessed:

- Change in size and number of lesions
- Treatment response
- Occurrence of any adverse events
- Standardized digital photographs

7.3 Follow-Up

Patients are followed up until three months from baseline to monitor for recurrence and any late adverse effects.

8. Outcome Measures

8.1 Primary Outcome

Clinical response at week 8, categorized as:

- **Complete clearance:** 100% resolution of the wart lesion
- **Partial response:** Reduction in size or number of lesions without complete clearance
- **No response:** No change or increase in size or number of lesions

8.2 Secondary Outcomes

- Early recurrence of warts in participants achieving complete clearance
- Adverse effects (pain, erythema, swelling, ulceration, scarring)
- Number of treatment sessions required for complete clearance

9. Statistical Analysis Plan

9.1 Software

All data will be analyzed using IBM SPSS Statistics version 26.

9.2 Descriptive Statistics

Normality of continuous variables will be assessed using the Shapiro-Wilk test. Normally distributed variables will be expressed as mean +/- standard deviation, while non-normally distributed variables will be summarized as median and interquartile range (IQR). Categorical variables will be presented as frequencies and percentages.

9.3 Baseline Comparisons

Baseline continuous variables will be compared between groups using the independent t-test or Mann-Whitney U test, as appropriate. Categorical variables will be compared using the Chi-square test or Fisher's exact test.

9.4 Efficacy Analysis

Treatment response at week 8 will be analyzed using the Chi-square test. Efficacy will be assessed by comparing complete response versus partial/no response between groups.

9.5 Secondary Analyses

Among patients achieving complete clearance, the number of treatment sessions required will be compared using the Mann-Whitney U test.

9.6 Site Effect Analysis

To assess potential site effects in this multicenter trial, baseline characteristics and treatment outcomes will be compared across sites. If significant differences are identified, site will be included as a covariate in secondary analyses.

9.7 Significance Level

A p-value less than 0.05 will be considered statistically significant for all analyses.

10. Safety Monitoring

Patients at both sites will be monitored at each visit for adverse effects including pain, erythema, swelling, ulceration, or scarring. All adverse events will be documented systematically using standardized case report forms. Any serious adverse event will warrant discontinuation of treatment and appropriate medical management. Serious adverse events will be reported to the principal investigator and the ethics committee within 24 hours.

11. Study Organization and Responsibilities

11.1 Principal Investigator

Dr. Nadia Ghazanfar
Department of Dermatology, Pakistan Railway Hospital
Email: nadia.ghazanfar@riphah.edu.pk

Responsibilities: Overall study oversight, protocol development and amendments, regulatory submissions, data integrity, final analysis and reporting, coordination between sites.

11.2 Site Investigator / Co-Principal Investigator

Dr. Fatima Sajid
Riphah International Hospital, Islamabad Expy, Sector A DHA Phase 5, Islamabad
Email: fatima.sajid@riphah.edu.pk

Responsibilities: Day-to-day conduct of the study at Site 2, participant recruitment and enrollment, treatment administration, data collection, adverse event reporting, and ensuring protocol adherence at the site.

11.3 Multicenter Coordination

Regular meetings (at least monthly) will be held between the principal investigator and site investigator to review enrollment progress, protocol deviations, adverse events, and data quality. Both sites will use identical case report forms, treatment protocols, and outcome assessment procedures to ensure consistency.

12. Ethical Considerations

The study was approved by the institutional ethical review committee. This single ethics approval covers both study sites. Written informed consent will be obtained from all participants prior to enrollment. For participants aged 12-17 years, assent will be obtained from the participant and written informed consent from a parent or legal guardian. Confidentiality of patient data will be maintained throughout the study at both sites.

13. Data Management

All data will be collected on standardized case report forms at both sites. Digital photographs will be stored securely with coded identifiers. Data from both sites will be entered into a centralized SPSS database managed by the principal investigator and verified for accuracy. Patient identifiers will be kept separate from study data to ensure confidentiality. Data from Site 2 will be transmitted securely to the lead site for central analysis.

14. References

References to be listed as per the final published manuscript.