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Original Article

Dermatology Section

Intralesional Bleomycin in Periungual Warts: A Prospective Randomised Controlled Study

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ABSTRACT

Introduction: Periungual Warts (PW) are difficult to treat because of their challenging location, high recurrence rate and resistance to treatment. Bleomycin is a cheaper and readily available drug that has potential efficacy in treating warts, although limited data on its use are available in the literature.

Aim: To study the efficacy and safety of Intralesional (IL) bleomycin in treating PW.

Materials and Methods: A prospective randomised controlled study was conducted at the Department of Dermatology, SCB Medical College and Hospital, Odisha, India, from September 2019 to August 2020. A total of 56 patients with PW were randomised equally by block randomisation into two groups: Group A and Group B. Group A received 1 U/mL IL bleomycin, while Group B was given dH_2O. Follow-up assessments were conducted at four, twelve and 24-week intervals. All follow-up visits included photographic documentation to confirm the cure rates before and after treatment. To evaluate the treatment's safety and effectiveness, the number and size of wart lesions, as well as injection side-effects, were noted at baseline and during each follow-up visit. Data analysis was performed using Statistical Package for Social Sciences (SPSS) version 26.0 (SPSS Inc., Chicago, IL). The Chi-square test was used to compare the study and control groups and to conduct follow-up analysis. Statistical significance was set at a level of ≤0.05, with a confidence interval of 95%.

Results: Group A and B patients had 54 and 42 wart lesions, respectively. The complete cure rate in Groups A and B was 74.1% (40/54 warts) and 4.8% (2/42 warts), respectively, after four weeks. After the second follow-up, at twelve weeks, the cure rate was significantly higher in the experimental group: 97.3% versus 4.76% (p=0.00001). All cases during the IL injection experienced pain at the injection site and haemorrhagic eschar was observed in every case within the first week of the IL injection. No significant systemic adverse effects were noted.

Conclusion: Intralesional bleomycin is highly efficacious and safe for the treatment of warts. Systemic side-effects are negligible, making it an effective treatment option for clearing warts in difficult areas, such as PW.

Keywords: Bleomycin sulphate, Placebo, *Verruca vulgaris*

INTRODUCTION

Warts (*Verruca vulgaris*) refer to benign proliferative lesions, which may occasionally develop into verrucous carcinoma, caused by the *Human Papillomavirus* (HPV). Only a few subtypes (HPV-1, 2 and 4) are capable of infecting the specific sites of mucous and epithelial layers of skin [1,2]. Depending on the subtypes of HPV, cutaneous warts are classified into different types, including common warts, genital warts, flat warts, deep palmoplantar warts, filiform warts and digitiform warts [3,4]. Approximately 10% of the world's population is affected by warts, marking them as a widespread viral infection [3,5]. PW grow near or under the nails, making them more challenging to treat due to their location. These warts begin as pinhead-sized, shiny, smooth and typically undetectable particles. They develop into pea-sized, rough and often dark-brown, grey, or black, horny growths within a matter of weeks or months. These warts may split open, swell and become tender [6].

Treatment options for PW mainly include chemical destruction with salicylic acid, physical destruction via hyperthermia, cryotherapy, electrocautery, laser therapy, immune therapy with vitamin D or Measles, Mumps and Rubella (MMR), chemotherapy with agents such as imiquimod or 5-fluorouracil and IL bleomycin [5,7]. Managing multiple warts and those located in hard-to-reach areas presents challenges, as they tend to be resistant to common therapeutic modalities and recurrences are frequently observed after treatment [8]. Topical regimens or oral therapies often carry the adverse effects of delayed relief, pain and tissue damage, along with a high recurrence rate [5].

Bleomycin belongs to a broad spectrum of glycopeptide antibiotics derived from Streptomyces verticillus and was initially approved as an anticancer agent by the FDA in 1975 [9,10]. Bleomycin possesses antitumour, antibacterial and antiviral activity, which may be attributed to its ability to bind with Deoxyribonucleic Acid (DNA), leading to DNA strand scission and the elimination of purine and pyrimidine bases [11,12]. Bleomycin is inactivated by the enzyme bleomycin hydrolase, which is generally present in all body tissues but is found in very small amounts in the skin. Consequently, after intralesional injection, a significant amount of active drug remains available at the site, with even a small quantity being sufficient for wart treatment. IL bleomycin has been proposed as a potential treatment modality for warts and has been used since the 1970s in Western countries [13,14].

Currently, there is limited evidence regarding the efficacy of IL bleomycin for treating PW. Some reports indicate that the cure rate is higher than with other therapies [15-17]. The present research aims to evaluate the efficacy and safety of IL injection of bleomycin for the treatment of PW.

MATERIALS AND METHODS

A prospective randomised controlled study was conducted in the Department of Dermatology, SCB Medical College and Hospital, Odisha, India, from September 2019 to August 2020 involving patients with PW. The study was performed according to the Declaration of Helsinki-ethical principles for medical research involving human subjects. The study commenced after obtaining approval

from the Institutional Ethical Committee (IEC no: 940/14.10.19). Informed written consent was obtained from all recruited patients.

Sample size calculation: A convenient sampling method was employed. Consecutive patients meeting the inclusion criteria during the study period were enrolled in the study.

Inclusion and Exclusion criteria: Patients aged over 18 and under 60 years, diagnosed with PW (either single or multiple) and without any history of previous treatment who provided written consent were included in the research. Exclusion criteria encompassed patients who did not consent to the study, pregnant or lactating women, patients with Reynaud's phenomenon, pulmonary fibrosis, scleroderma, or any chronic systemic diseases such as renal, hepatic and cardiovascular disorders were excluded from the study.

Study Procedure

A total of 56 outpatients presenting with PW were enrolled in the study. A total of 23 participants were excluded for not meeting the inclusion criteria.

Each patient was required to provide informed consent and undergo a comprehensive history-taking process that included details such as name, age, sex, duration of warts/disease, progression, number of past interventions and family history of similar lesions.

Therapeutic procedures: A bleomycin vial (containing 15 mg of dry bleomycin powder), distilled water, lignocaine 2% solution and an insulin syringe were all required. Patients were randomised into two groups using the block randomisation method (Group A and Group B), with 28 patients in each group. Patients in Group A received IL bleomycin, while patients in Group B received distilled water (placebo).

To prepare the bleomycin stock solution (3 mg/mL), a 15 mg bleomycin vial was diluted with 5 mL of distilled water and stored at 4-8°C for 60 days. Just before injection, a combination of two parts 2% lignocaine and one part bleomycin stock solution was prepared in a 26 G insulin syringe to achieve a final concentration of 1 mg/mL (1 U/mL). Each wart and the surrounding skin areas were cleaned with isopropyl alcohol. The callus surrounding the wart was removed with superficial paring. The new solution was injected intralesionally until the lesions blanched. The amount of injection required was determined by the size of the warts. The total volume injected during a single treatment session was limited to 2 mL, with each injection into a single wart restricted to 1 mL.

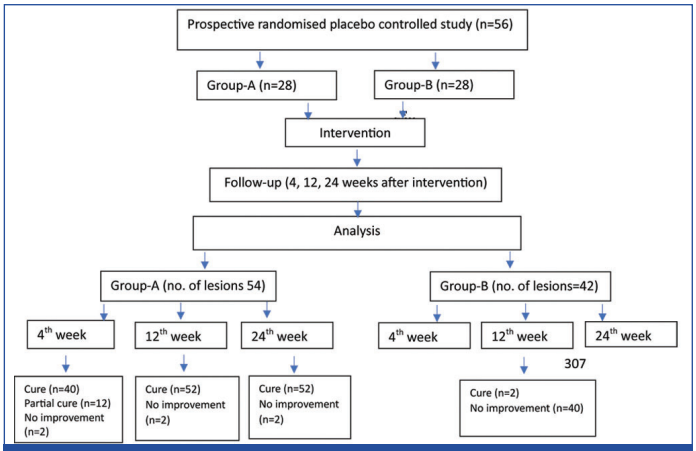
In the control group, distilled water was injected using the same method as the bleomycin solution. Patients in both groups were scheduled for follow-up visits at four, twelve and 24 weeks. If necessary, treatment was repeated in the test group at the end of the fourth week for clinical evaluation or if warts recurred. Side-effects of the injection, such as pain, oedema, oozing, crusting and Raynaud's phenomenon, were recorded at each follow-up for future assessment.

Patients were followed-up while maintaining photographic records during all visits at 4, 12 and 24 weeks to validate pre- and post-treatment rates. The treatment response was categorised by observing the number of wart lesions as completely clear, partially cured, or showing no improvement, by comparing baseline photographs with follow-up photographs. Similarly, injection side-effects were recorded at baseline and during each follow-up visit to assess the efficacy of the treatment.

The study flow diagram is provided in [Table/Fig-1].

STATISTICAL ANALYSIS

Data analysis was performed using SPSS version 26 (SPSS, Inc., Chicago, IL). The Chi-square test was used to compare the study and control groups, as well as for follow-up analysis. Statistical significance was set at a p-value of ≤0.05, with a confidence interval of 95%.



[Table/Fig-1]: Study flow diagram.

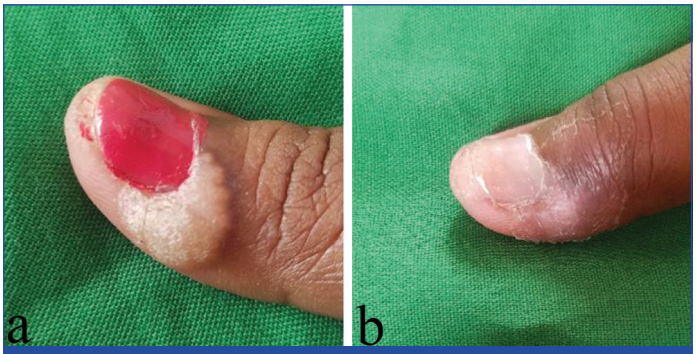
RESULTS

A total of 56 patients were included in the study, divided into Group A and Group B (1:1, each having 28 patients), with 54 and 42 wart lesions, respectively. The ages of the patients ranged from 18 to 50 years, with a mean age of 30.5±6.84 years in Group A and 27.71±7.70 years in Group B. In total, 32 patients (57%) were male and 24 patients (43%) were female. The duration of the warts ranged from two months to two years, with a mean duration of 12 months. All of these data for the 56 patients are presented in [Table/Fig-2]. The baseline parameters (age, sex and sub-distribution of warts) between the two groups were statistically comparable and no significant statistical difference was observed.

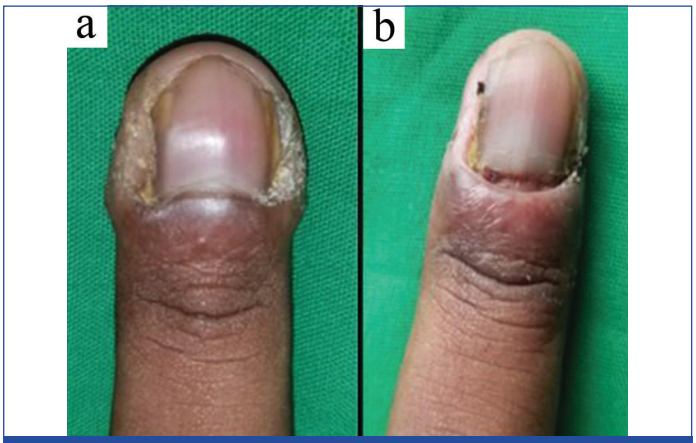
Serial number	Group A			Group B		
	Age (in years)/ gender	Duration of disease in months	Number of warts	Age (in years)/ gender	Duration of disease in months	Number of warts
1	18/F	12 mnth	2	21/M	9 mnth	2
2	30/M	13 mnth	3	31/M	15 mnth	1
3	32/M	11 mnth	1	20/M	12 mnth	1
4	35/M	15 mnth	2	19/F	13 mnth	3
5	37/F	12 mnth	3	18/F	11 mnth	1
6	39/F	9 mnth	3	23/M	12 mnth	2
7	24/M	7 mnth	1	42/F	9 mnth	1
8	27/M	17 mnth	1	23/M	15 mnth	2
9	23/M	6 mnth	2	25/F	8 mnth	1
10	23/M	18 mnth	1	23/F	16 mnth	4
11	22/F	6 mnth	3	30/M	4 mnth	1
12	24/F	19 mnth	2	27/M	24 mnth	3
13	26/M	3 mnth	1	31/M	19 mnth	1
14	38/F	20 mnth	2	26/F	3 mnth	2
15	39/M	2 mnth	3	39/M	20 mnth	1
16	29/F	20 mnth	2	22/F	3 mnth	1
17	42/M	24 mnth	2	32/M	19 mnth	2
18	50/F	3 mnth	1	22/M	5 mnth	1
19	19/F	16 mnth	1	23/F	18 mnth	1
20	23/M	8 mnth	1	21/F	6 mnth	2
21	23/M	15 mnth	3	25/M	17 mnth	1
22	23/F	9 mnth	1	21/F	9 mnth	1
23	37/M	11 mnth	4	19/F	7 mnth	2
24	39/M	13 mnth	1	46/M	11 mnth	1
25	41/F	12 mnth	3	21/F	15 mnth	1
26	38/M	12 mnth	2	37/M	12 mnth	1
27	43/M	14 mnth	1	49/M	13 mnth	1
28	24/M	9 mnth	2	20/F	14 mnth	1

[Table/Fig-2]: Data of all 56 patients.

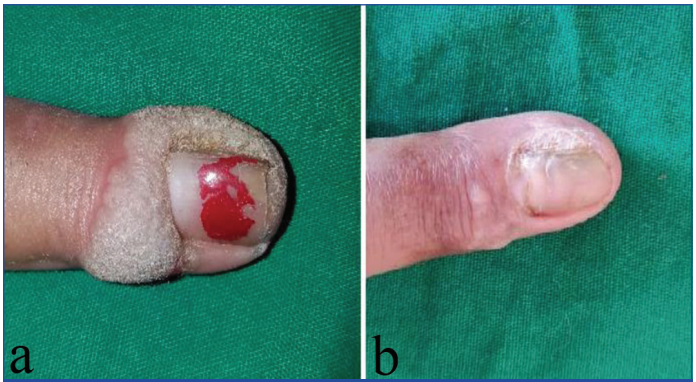
Of the 54 warts in Group A that received IL bleomycin, 52 (96.3%) showed complete resolution. At the first follow-up, four weeks after the initial bleomycin injection, 40 (74.1%) warts had achieved complete clearance [Table/Fig-3,4], 12 had partial clearance with size reduction compared to the baseline photograph [Table/Fig-5] and two warts showed no improvement. Bleomycin was administered a second time to these fourteen warts. At the second follow-up, 52 warts were completely cured. After 24 weeks of follow-up, only two warts did not respond to treatment. In Group B, only two warts cleared after six months of follow-up out of 42 warts that received IL distilled water. All follow-up data for the two groups are shown in [Table/Fig-5]. This demonstrates a clear difference in results between the two groups.



[Table/Fig-3]: a) Pretreatment without IL bleomycin; b) Post-treatment with IL bleomycin after four weeks of follow-up showing complete wart clearance.



[Table/Fig-4]: a) Pretreatment without IL bleomycin; b) Post-treatment with IL bleomycin after four weeks of the treatment period showing complete clearance of wart.

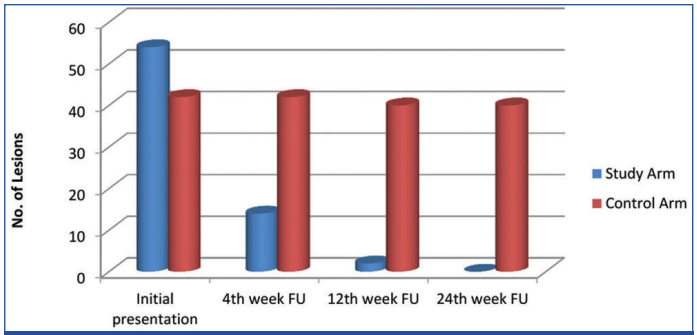


[Table/Fig-5]: a) Pretreatment without IL bleomycin; b) Post-treatment with IL bleomycin after four weeks follow-up showing partial wart clearance.

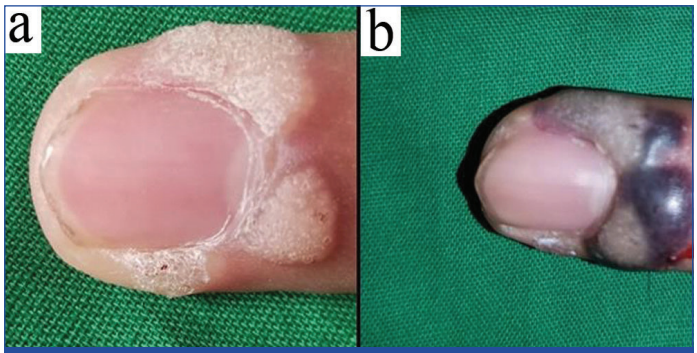
For further confirmation evaluated through statistical analysis, a statistically significant difference was observed intragroup in the number of lesions cleared after each treatment follow-up between Groups A and B ($p<0.001$) [Table/Fig-6-8]. The most common side-effect was pain at the time of injection, which persisted for 2-3 days in some patients in both groups. All patients in Group A formed haemorrhagic bullae or eschar 4-7 days after the bleomycin injection [Table/Fig-9,10]. In Group A, 18 patients (64%) experienced hyperpigmentation [Table/Fig-11] and only 2 patients (7%) had mild scarring [Table/Fig-12]. None of the patients had systemic effects, Raynaud's phenomenon, or nail dystrophy. However, severe side-effects or systemic toxicity were not observed in any of the patients.

Lesions	Study arm (n=28)	Control arm (n=28)	Chi-square statistic value	p-value*
Total no. of warts at baseline	54	42	-	-
At 4 weeks	14	42	-	-
At 12 weeks	2	40	80.43	0.00001
At 24 weeks	2	40	80.43	0.00001

[Table/Fig-7]: Comparison of number of lesions present for treatment periods (4, 12 and 24 weeks) between Group A (Study group) and B (Control group).
*Chi-square test



[Table/Fig-8]: Bar diagram comparing the number of lesions present for treatment periods (4, 12 and 24 weeks) between groups A and B.



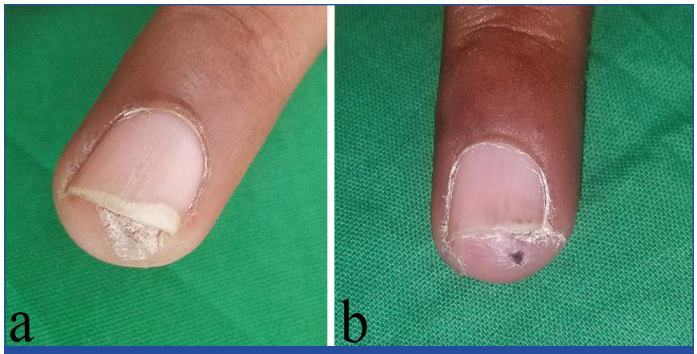
[Table/Fig-9]: a) 1st session treatment with IL bleomycin; b) Post-treatment with IL bleomycin after four days development of haemorrhagic bullae.

DISCUSSION

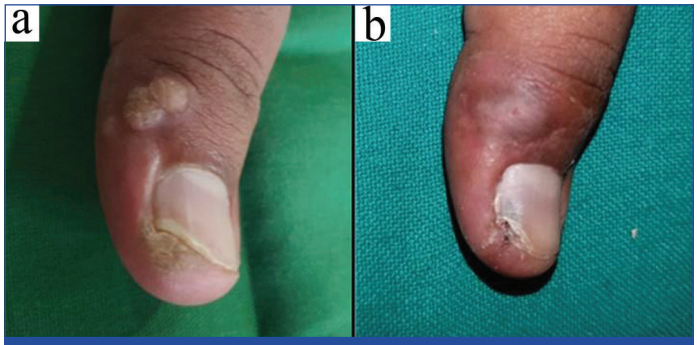
In the present research, the cure rate in Groups A and B was 74.1% (40/54 warts) and 4.8% (2/42 warts), respectively, after four weeks. After the second follow-up at twelve weeks, the cure rate was significantly higher in the experimental group, at 97.3% compared to the control group at 4.76%. All cases during the IL injection experienced pain at the injection site and haemorrhagic eschar was

Follow-up duration and improvement of warts	Group A (n=54 warts)			Group B (n=42 warts)		
	At 4 weeks (after 1 injection)	At 12 weeks (after 2 injections)	At 24 weeks (after 3 injections)	At 4 weeks (after 1 injection)	At 12 weeks (after 2 injections)	At 24 weeks (after 3 injections)
Number of warts completely cleared.	40	52	52	0	2	2
Number of warts partially cleared.	12	0	0	0	0	0
Number of warts not cleared.	2	2	2	42	40	40

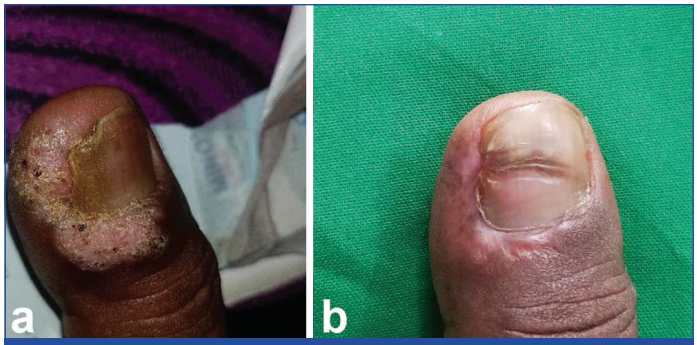
[Table/Fig-6]: Improvements of warts in each follow-up in groups A and B.



[Table/Fig-10]: a) Pretreatment without IL bleomycin; b) Post-treatment with IL bleomycin after four weeks of treatment period with clearance of wart and some remnant of haemorrhagic eschar.



[Table/Fig-11]: a) Pretreatment without IL bleomycin; b) Post-treatment with IL bleomycin after 12 weeks of treatment period showing complete clearance of warts and hyperpigmentation of post-treatment area.



[Table/Fig-12]: a) Pretreatment without IL bleomycin; b) Post-treatment with IL bleomycin after 24 weeks of treatment period showing complete clearance of warts and scarring of post-treatment.

observed in every case within the first week of the IL injection. No significant systemic adverse effects were reported.

The PW were very distressing and adversely affected patients' quality of life due to their higher rate of residual or recurrence despite multiple lines of treatment. Warts may fail to clear even after repeated follow-ups with various current treatment modalities, such as 75% salicylic acid, cryotherapy, photodynamic therapy, CO_2 laser, etc., [18].

Bleomycin is a cytotoxic antitumour antibiotic discovered by Umezawa H et al., in 1965 in the soil near a Japanese coal mine and it appears to be a promising treatment option for various cancers [19]. IL bleomycin is used as a repurposing regimen for many dermatological diseases, including vascular anomalies, vascular malformation, haemangioma, telangiectasia, cutaneous malignancies, malignant melanoma, basal cell carcinoma, leishmaniasis cutis and warts, among others [20].

The IL bleomycin has been reported to be efficacious in various types of wart lesions with minimal side-effects [13-15,21,22]. The previous study by Soni P et al., a placebo-controlled clinical survey, treated 82 warts with IL bleomycin and eight warts with normal saline as a placebo, demonstrating a 96.10% cure rate for palmoplantar warts and a 100% rate for PW [16]. Similarly, the present study showed a 96.3% cure rate in PW within 12 weeks of treatment.

Due to a lack of standardisation, different studies have employed bleomycin solutions at varied concentrations. In the present study, a concentration of 1 U/mL of bleomycin was administered. Previous research has used concentrations such as 0.15% and 0.05% to treat warts safely without side-effects [23]. Bleomycin at 3 Units/mL intralesionally was administered by Singal A and Grover C, resulting in a 100% cure rate [13]. In the current study, the concentration of the stock solution was reduced by diluting it with two parts of 2% lignocaine, which also acts as a local anaesthetic and reduces localised pain during and after the injection.

Dhar SB et al., compared IL bleomycin with cryotherapy and reported that bleomycin was 1.23 times more effective than cryotherapy, with a clearance rate of 86.4% for bleomycin compared to 72.3% for cryotherapy [21]. The present study also indicated that fewer treatment sessions were required than with other therapeutic procedures. In the current research, pain was identified as an adverse effect in 100% of patients, whereas it was reported in 86% of patients previously [13].

Hyperpigmentation was observed in 64% of patients in the present study; this finding is consistent with previous studies [13]. The current study found IL bleomycin to be safe and efficacious in clearing PW with a concentration of 1 mg/mL of bleomycin. At the 12 week follow-up visit, complete improvement was observed in 52 (96.3%) PW lesions. During the drug administration, pain persisted for 2-3 days in both groups. Hyperpigmentation was noted in 18 patients (64%) and mild scarring was observed in 2 (7%) patients.

Limitation(s)

The study had a small sample size, a shorter follow-up period and the patients were not compared to alternative treatment modalities.

CONCLUSION(S)

In the present research, a significant percentage of PW were cured completely and none of the subjects experienced any serious side-effects. IL injection of bleomycin is an affordable treatment with a low risk of recurrence and better adherence among patients with PW. No specific setup or machinery is necessary for wart treatment with bleomycin. More randomised, double-blind studies with adequate sample sizes and longer follow-up durations are required to further establish the safety and efficacy of IL bleomycin in treating viral warts.

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- Manual Googling: Jan 30, 2025
- iThenticate Software: Feb 01, 2025 (13%)

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