Original Article

Comparative Efficacy of Intralesional MMR and Vitamin D_3 Immunotherapy in Cutaneous Warts: A Randomized Controlled Trial with Clinical and Dermoscopic Assessment

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Abstract

Background: Cutaneous warts are common benign epidermal proliferations caused by human papillomavirus. Conventional destructive therapies are associated with pain, scarring, and recurrence. Intralesional immunotherapy and dermoscopic assessment have emerged as effective alternatives for treatment and monitoring. The aim is to compare the efficacy and safety of intralesional Measles-Mumps-Rubella (MMR) vaccine and intralesional Vitamin D3 in the treatment of cutaneous warts using clinical and dermoscopic assessment. **Material and Methods:** Prospective randomized controlled study conducted in the Department of Dermatology, Venereology and Leprology, Teerthanker Mahaveer Medical College and Research Centre, Moradabad. One hundred twenty-two patients with clinically diagnosed cutaneous warts were randomized equally into two groups. Group A received intralesional Vitamin D3 and Group B received intralesional MMR vaccine at 3-week intervals for a maximum of four sessions. Clinical and dermoscopic evaluations were performed at baseline and follow-up visits. Statistical Analysis Used: Data were analyzed using SPSS version 26.0. Appropriate descriptive and inferential statistical tests were applied, with $p<0.05$ considered statistically significant. **Results:** The mean age was 32.15±11.66 years, and 57.4% of participants were males. Both treatment modalities demonstrated significant clinico-dermoscopic improvement, including reduction in papillomatous surface changes, thrombosed capillaries, and vascular structures, with favorable clearance rates and minimal adverse effects. **Conclusion:** Intralesional MMR vaccine and Vitamin D3 are effective, safe, and economical treatment options for cutaneous warts. Dermoscopy provides an objective, non-invasive tool for monitoring therapeutic response and lesion resolution.

Keywords: Cutaneous warts; Human papillomavirus; Intralesional immunotherapy; MMR vaccine; Vitamin D3; Dermoscopy.

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Introduction

Cutaneous warts are common benign epidermal proliferations caused by human papillomavirus (HPV) infection and remain a frequent therapeutic challenge because of recurrence, treatment-associated discomfort, and variable response rates. Intralesional immunotherapy has emerged as an effective treatment modality by enhancing cell-mediated immunity against HPV. Both intralesional measles-mumps-rubella (MMR) vaccine and Vitamin D_3 have demonstrated promising efficacy and safety in recent studies. However, comparative data incorporating objective dermoscopic assessment remain limited. Therefore, this study was undertaken to compare the efficacy and safety of intralesional MMR vaccine and Vitamin D_3 in the treatment of cutaneous warts using clinical and dermoscopic evaluation.[1-15]

Materials and Methods

This prospective, parallel-group, randomized controlled trial was conducted in the Department of Dermatology, Venereology and Leprosy, Teerthanker Mahaveer Medical College and Research Centre (TMMC&RC), Moradabad, Uttar Pradesh, India, to compare the efficacy and safety of intralesional Vitamin D_3 and intralesional Measles-Mumps-Rubella (MMR) vaccine in the treatment of cutaneous warts. Patients attending both the outpatient and inpatient dermatology services with clinically diagnosed cutaneous warts were screened for eligibility. The trial was prospectively registered with the Clinical Trials Registry of India (CTRI/2024/11/077359), and the study protocol was approved by the Institutional Ethics Committee and the College Research Committee before commencement of the study. No changes were made to the study methodology, eligibility criteria, interventions, or outcome

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measures after initiation of the trial.

Patients aged 12 years or older with clinically diagnosed cutaneous warts who were willing to participate and provided written informed consent were included in the study. Patients who had used warty topical or systemic treatment within four weeks, had history of hypersensitivity to Vitamin D_3, MMR vaccine and/or any vaccine component, had an acute febrile illness or active bacterial infection, were immunocompromised patients or were receiving immunosuppressive medications, were pregnant mothers or lactating mothers, or had a history of keloid were not included in the study.

The number of participants considered 122 was sufficient for the study to have 80% statistical power in detecting a difference in treatment response between the two treatment groups at a 95% confidence level while accounting for a 10% loss for follow-up patients. In this regard, a total of 122 eligible participants were enrolled and randomized into two groups, one 61 participants who received intralesional Vitamin D_3 (Group A) and the other 61 participants who received intralesional MMR vaccine (Group B). Allocation sequence was generated by a computer-generated random number sequence and simple randomisation without blocking or stratification was used. Allocation concealment was achieved by sequentially numbered, opaque, sealed envelopes with treatment allocation details, opened only after enrolment of eligible participants were included. An investigator uninvolved in the recruitment of participants and the assessment of outcomes generated the random allocation sequence; the principal investigator enrolled and allocated eligible participants to treatment using the concealed allocation sequence. There were no a priori analysis plans, nor were there any stopping guidelines.

All participants gave their written informed consent and were then subjected to a detailed history taking, thorough dermatological examination, standardized clinical photography and dermoscopic analysis performed with a Dermolite dermoscope (DL5) in both polarized and non-polarized mode. For Group A, Vitamin D_3 (600,000IU; 15 mg/mL) was injected intralesionally. After local infiltration with lignocaine 2-5 mL, up to 0.5 mL of the Vitamin D_3 was locally infiltrated, using a 31 gauge insulin syringe in the largest representative wart. Intralesional MMR vaccine (Tresivac®, Serum Institute of India, Pune, India) was given to the participants in Group B. The vaccine was rehydrated following the manufacturer's instructions and up to 0.5 mL was administered intralesionally into the largest representative wart with a 31-gauge insulin syringe. In both treatment groups the volume injected was scaled based on wart size and treatment sessions were repeated every three weeks up to four or until complete lesion clearance occurred. The main results were to compare the percentage of patients with complete clinical and dermoscopic clearances after receiving Vitamin D_3 intralesional therapy versus intralesional MMR vaccine. Secondary outcome measures were anthropometric changes of the warts, dermoscopic changes during treatment, time to complete clearance of lesions, adverse effects and recurrence during follow-up. Clinical and dermoscopic evaluations were conducted at baseline and at each treatment visit while all subjects were followed monthly for three months post-therapy. Responses to treatment were marked using a predetermined 4-point scale where Score 3 meant complete clinical and dermoscopic resolution, Score 2 meant complete clinical resolution but partial dermoscopic resolution, Score 1 meant more than 50% clinical improvement without any complete dermoscopic improvement, and Score 0 meant no clinical or dermoscopic improvement. No modifications were made to the predefined primary or secondary outcome measures after commencement of the study.

Because of the nature of the interventions, blinding of participants and the treating investigator was not feasible. Nevertheless, dermoscopic images and standardized clinical photographs were independently evaluated by a dermatologist who remained blinded to treatment allocation throughout the study. Although both interventions were administered intralesionally using identical syringes and comparable injection techniques, complete blinding could not be achieved because of differences in the appearance and preparation of the two therapeutic agents.

All statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) software version 26.0 (IBM Corp., Armonk, NY, USA). Quantitative variables were expressed as mean ± standard deviation, whereas qualitative variables were presented as frequencies and percentages. Comparisons between treatment groups were carried out using the independent Student's t-test for normally distributed continuous variables, the Mann–Whitney U test for non-normally distributed continuous variables, and the Chi-square test or Fisher's exact test for categorical variables, as appropriate. All statistical tests were two-tailed, and a p value of less than 0.05 was considered statistically significant. No subgroup analyses were pre-specified; however, exploratory analyses were performed where appropriate to evaluate associations between baseline characteristics and treatment response.

Results

A total of 122 patients with clinically diagnosed cutaneous warts were assessed for eligibility, fulfilled the inclusion criteria, and were enrolled in the study. Following simple randomization, 61 participants were allocated to receive intralesional Vitamin D_3 (Group A) and 61 participants to receive intralesional MMR vaccine (Group B). All randomized participants received the allocated intervention, completed the treatment protocol, and were available for both clinical and dermoscopic assessments. No participant was lost to follow-up, withdrew consent, discontinued treatment, or was excluded from the final analysis. Consequently, all 122 randomized participants were analyzed according to their originally assigned treatment groups, in accordance with the study protocol (Figure 1). Participant recruitment was carried out during the approved study period following clearance from the Institutional Ethics Committee and College Research Committee, and the trial was completed as planned without premature termination or implementation of stopping criteria.

The study population comprised 122 patients with cutaneous warts, with ages ranging from 15 to 65 years. The mean age of the participants was 32.15 ± 11.66 years, with a median age of

30 years, and the largest proportion of patients (40.2%) belonged to the 21–30-year age group. Males constituted 57.4% (n = 70) of the study population, while females accounted for 42.6% (n = 52). Baseline demographic and clinical characteristics, including age, sex, marital status, and number of warts, were comparable between the intralesional Vitamin D_3 and MMR groups, with no statistically significant differences observed, thereby confirming baseline homogeneity of the study groups [Table 1]. All 122 randomized participants were included in both efficacy and safety analyses, with 61 participants in each treatment arm, and all analyses were performed according to the original treatment allocation.

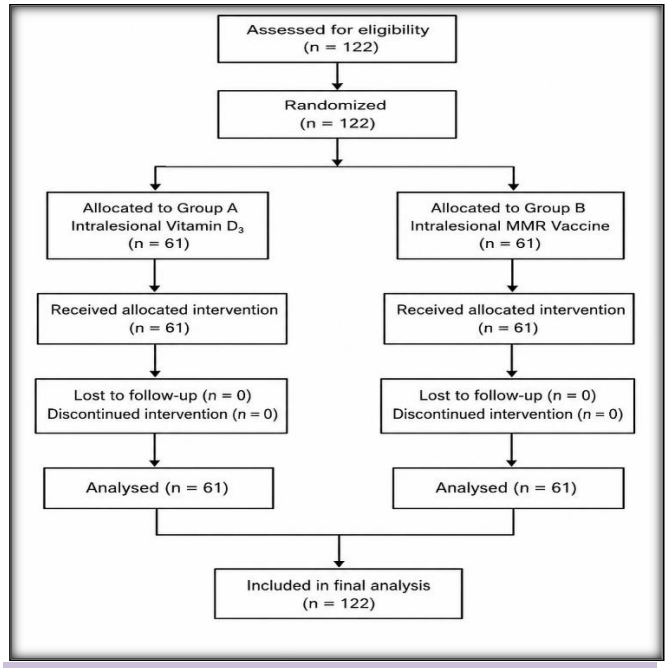

Figure 1: Flow Diagram

Evaluation of the primary outcome demonstrated that, following completion of treatment, complete clinical response (Score 3) was achieved in 36 participants (29.8%), apparent clinical cure (Score 2) in 43 participants (35.5%), partial response (Score 1) in 25 participants (20.7%), and no clinical response (Score 0) in 17 participants (14.0%). Comparison of treatment outcomes between the two study groups revealed no statistically significant difference in clinical response following either the third treatment session ($\chi^2 = 0.350$, df = 3, p = 0.950) or the fourth treatment session ($\chi^2 = 0.227$, df = 3, p = 0.973), indicating that both intralesional Vitamin D_3 and intralesional MMR vaccine produced comparable rates of clinical improvement and lesion clearance [Table 2].

Dermoscopic evaluation also demonstrated substantial therapeutic improvement in both treatment groups. Complete dermoscopic clearance was observed in 80 participants (65.6%), whereas residual dermoscopic findings persisted in 42 participants (34.4%). At week 12, complete dermoscopic clearance was achieved in 70.5% of patients treated with intralesional Vitamin D_3 compared with 60.7% of those receiving intralesional MMR vaccine. Reduction in vascular structures was observed in 80.3% and 72.1% of participants, respectively, while restoration of normal skin markings occurred in 77.0% of the Vitamin D_3 group and 68.9% of the MMR group. Likewise, disappearance of thrombosed dots and other characteristic dermoscopic wart features was progressively documented throughout treatment, reflecting gradual lesion regression in both groups [Table 3; Figures 1 and 2].

Longitudinal analysis demonstrated significant improvement in wart response scores over successive treatment sessions irrespective of treatment modality. Non-parametric analysis using the Wilcoxon signed-rank test revealed statistically significant improvement during the treatment period ($Z = -2.958$, p = 0.003), while similar findings were confirmed by the Sign test (p = 0.002). Exploratory session-wise analyses further demonstrated progressive improvement in both clinical and dermoscopic response scores with each successive treatment session in both groups. Although complete dermoscopic clearance appeared numerically higher among participants receiving intralesional Vitamin D_3, no statistically significant difference in overall therapeutic efficacy was observed between the two treatment modalities, and no subgroup demonstrated a significantly different treatment response.

Both treatment modalities were well tolerated throughout the study. Adverse events were mild, transient, and self-limiting, with injection-site pain representing the most frequently reported complaint in both groups. No participant discontinued therapy because of adverse effects, and no serious adverse events were encountered during the study period. Furthermore, during the three-month post-treatment follow-up, no recurrence of cutaneous warts was documented in either treatment group, indicating sustained therapeutic response following completion of intralesional immunotherapy.

Table 1: Baseline Demographic and Clinical Characteristics of Study Participants According to Treatment Group (N = 122)

Characteristic	Intralesional MMR (n = 61)	Intralesional Vitamin D_3 (n = 61)	p value
Age (years), Mean ± SD	32.93 ± 11.22	31.36 ± 12.12	0.458
Sex, n (%)			0.586*
Male	32 (52.5%)	29 (47.5%)	
Female	29 (47.5%)	32 (52.5%)	
Marital status, n (%)			0.145*
Married	37 (60.7%)	29 (47.5%)	
Unmarried	24 (39.3%)	32 (52.5%)	
Number of warts, n (%)			0.213*
Single	13 (21.3%)	8 (13.1%)	
Multiple	48 (78.7%)	53 (86.9%)	

Abbreviations: SD, standard deviation; MMR, Measles Mumps Rubella vaccine.
Statistical tests: Independent t-test for age; Chi-square test (*) for categorical variables.

Table 2: Clinical Response at Week 12 According to Treatment Group (N = 122)

Clinical Response	MMR Group (n = 61)	Vitamin D₃ Group (n = 61)	Total (N = 122)	p value
Score 0 (No response)	9 (14.8%)	8 (13.1%)	17 (14.0%)	
Score 1 (Partial response)	12 (19.7%)	14 (23.0%)	26 (21.3%)	
Score 2 (Apparent clinical cure)	22 (36.1%)	21 (34.4%)	43 (35.2%)	
Score 3 (Complete response)	18 (29.5%)	18 (29.5%)	36 (29.5%)	
Total	61 (100%)	61 (100%)	122 (100%)	0.973

Abbreviations: MMR, Measles Mumps Rubella vaccine.
Statistical test: Chi-square test ($\chi^2 = 0.227$, df = 3, p = 0.973).

Table 3: Dermoscopic Response at Week 12 According to Treatment Group (N = 122)

Dermoscopic Parameter	MMR Group (n = 61)	Vitamin D₃ Group (n = 61)	Total (N = 122)
Vascular reduction	44 (72.1%)	49 (80.3%)	93 (76.2%)
Dot disappearance	40 (65.6%)	46 (75.4%)	86 (70.5%)
Skin line reappearance	42 (68.9%)	47 (77.0%)	89 (73.0%)
Complete dermoscopic clearance	37 (60.7%)	43 (70.5%)	80 (65.6%)
Residual dermoscopic features	24 (39.3%)	18 (29.5%)	42 (34.4%)

Values are presented as number (%).

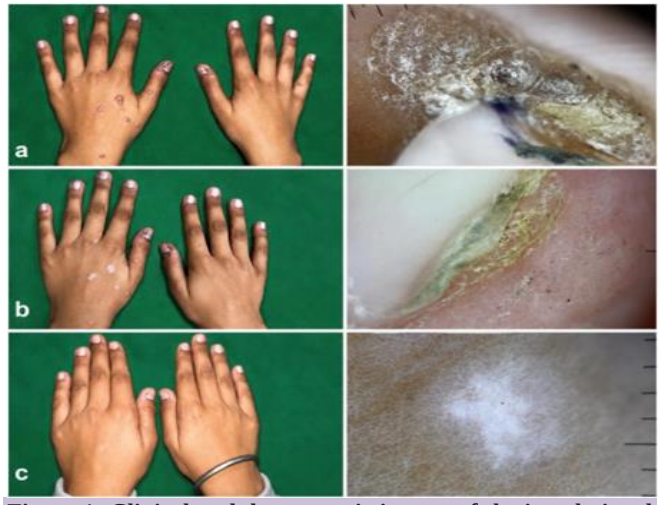

Figure 1: Clinical and dermoscopic images of the intralesional MMR group showing sequential treatment response: (a) baseline, (b) second visit with wart regression, and (c) third visit showing near-complete clinical and dermoscopic clearance.

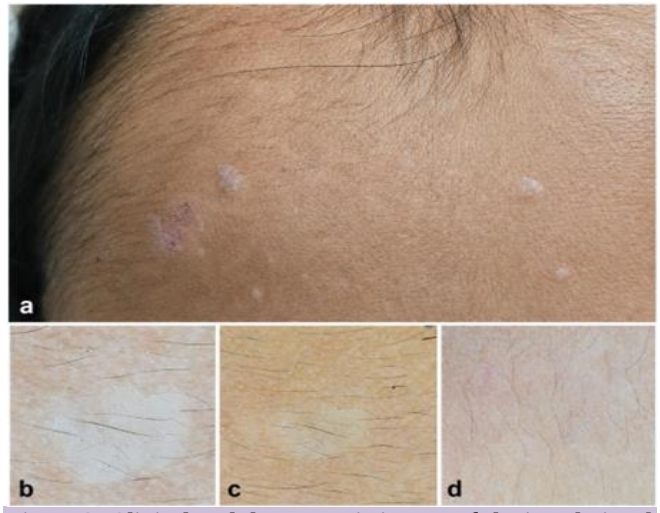

Figure 2: Clinical and dermoscopic images of the intralesional vitamin D₃ group showing treatment response: (a, b) baseline clinical and dermoscopic findings, (c) second visit with partial regression, and (d) third visit showing further improvement.

Discussion

The present prospective randomized controlled trial compared the efficacy and safety of intralesional Vitamin D₃ and intralesional Measles-Mumps-Rubella (MMR) vaccine in the treatment of cutaneous warts using both clinical and dermoscopic assessment. All 122 patients were successfully randomized equally in the two treatment groups and there was no loss to follow up. Progressive clinical and dermoscopic improvement was observed in both treatment arms, while almost half of the patients reached complete clinical and dermoscopic clearance at the end of the treatment. The overall therapeutic efficacy between the intralesional Vitamin D₃ and intralesional MMR vaccine was not significantly different with complete dermatoscopic clearance numerically higher in the Vitamin D₃ group. The present results suggest that both immunotherapeutic treatments represented good options for the treatment of cutaneous warts and dermoscopy is valuable as an additional tool for the objective assessment of the effect of treatment and documenting the regression of the lesions.[1,8,14,15]

Most of the respondents in this study were aged 21- to 30-years, with a mean age of 32.15 ± 11.66 years while the males occupied a higher proportion in the study population. The population profile agrees with data from other reports by Al-Sabak et al., Chaudhary et al., and Baniya et al., who also found that warts occur more commonly in young adults (with a slight male predominance) and may be related to more exposure to the sun, a higher prevalence of outdoor activities and repeated minor trauma that may allow the HPV to be inoculated.[7,9,14]

Progressive improvement was observed with each treatment in both intralesional treatments; almost 20% of patients reached apparent clinical cure or complete clinical resolution at the end of treatment. Importantly, no difference was seen in the response to the therapy between the Vitamin D₃ group and the MMR group, indicating similar efficacy of the two immunotherapeutic treatments. Similar conclusions were drawn by Mohta et al. and Joshi et al. who found that neither treatment was statistically significantly inferior, and found that Vitamin D₃ and intralesional MMR vaccine are equally effective at clearing warts.[1,8]) Sallam et al. concluded, however, that whether it is intralesional MMR vaccine or Vitamin D₃, both are effective immunotherapeutic treatment options for cutaneous warts.[15]) Baniya et al.

demonstrated that there is no statistically significant difference in clinical efficacy of either intralesional MMR vaccine or intralesional Vitamin D_3, further supporting these conclusions.

Therapeutic effect of intralesional MMR vaccine is thought to be mediated by the induction of delayed-type hypersensitivity (DTH) responses that results in boosting the production of Th1 cytokines, activation of macrophages and enhanced recognition and destruction of HPV-infected keratinocytes. Intralesional Vitamin D_3 also has immunomodulatory activity by acting on vitamin D receptors found on immune cells and keratinocytes, which stimulate epidermal proliferation/differentiation, elevation of AMP production and regulation of innate and adaptive immunity, all of which may lead to regression of distant untreated warts via systemic immune activation. Recent studies by Ali and Jalal, Al-Sabak et al., and Ghaseminejad-Raeini et al. have demonstrated favorable therapeutic outcomes with intralesional Vitamin D_3, findings that are in agreement with the present study.[3,7,10]

One of the principal strengths of the present study was the incorporation of dermoscopy as an objective, non-invasive tool for therapeutic monitoring. Sequential dermoscopic examination demonstrated progressive reduction in vascular structures, disappearance of thrombosed capillaries and hemorrhagic dots, restoration of normal dermatoglyphics, and eventual complete dermoscopic clearance of lesions. In some patients, these dermoscopic changes occurred before clinical normalization, which means that dermoscopy could help in evaluating the therapeutic effect early with the purpose of recognizing the presence of subclinical disease before the lesions disappear. Al Rudaisat and Cheng as well as Cha et al. also reported similar findings in their papers, which emphasized that dermoscopy was useful in monitoring wart regression and predicting treatment success.[2,12]

The treatment had a very good safety profile for both treatments. These adverse events were not severe, did not last long and did not result in any long-term harm; the most common complaints were pain at the site of injection in both treatment groups. There were no serious adverse events experienced in this study period and no one stopped therapy due to side effects. The results of this study also align with previous trials of intralesional immunotherapy, which have also shown very low rates of treatment-related morbidity and no reports of warts reoccurring over a 3-month period.[1,8,15]

The duration of follow-up was relatively short, but no recurrence has been noted suggesting the immune-mediated clearance of the HPV infection was sustained, similar to previous studies that have investigated wart immunotherapies.[1,14,15]

There are some limitations in the present study. The results from this single centre study might not be fully representative of a wider group of people. Late recurrences may have been missed in the 3-month follow-up period. Furthermore, there was no HPV genotyping and immunological marker analysis performed; therefore, the effect of these on treatment response cannot be assessed. Although the images were assessed blindly to exclude observer bias, it is not possible to blind participants and treating investigators. These findings warrant larger multicentric randomized trials with longer follow-up durations, HPV genotyping, and detailed immunological evaluation for validation and a further establishing of these immunotherapeutic modalities' comparative efficacy.

In conclusion, the results of the current study showed that intralesional Vitamin D_3 and intralesional MMR vaccine are therapeutic and efficient methods which are generally well tolerated, safe and cost effective for the management of cutaneous warts. The absence of statistically significant difference between the treatment group further strengthens the use of either modality for routine clinical practice and in tertiary care settings, while patients with various ages, education levels, sex, and working groups provided objective evidence of treatment responses via dermoscopy which allowed for early detection of treatment-related changes, assessment of residual disease, and confirmation of complete lesion resolution.[2,12] However, multicentric studies with larger and more diverse populations, and longer follow-up periods are required to further develop the external validity and to determine the diagnostic importance of dermoscopy in the monitoring of intralesional immunotherapy in warts.

Conclusion

Intralesional Vitamin D_3 and intralesional Measles-Mumps-Rubella (MMR) vaccine were equally effective, well tolerated and safe in the treatment of cutaneous warts. The results of the two treatment methods were similarly progressive clinically and dermoscopically, and there was no statistical difference in overall treatment results. Dermoscopy was a useful, objective and non-invasive tool to help identify residual disease and was often used to show improvement before complete clinical resolution, offering a useful tool for monitoring the treatment response. In the case of multiple, recurrent or difficult warts to treat, both intralesional Vitamin D_3 and intralesional MMR vaccine appear viable therapeutic options worth considering due to their efficacy, low recurrence, minimal adverse effects, ease of administration and cost effectiveness. These results should be confirmed by further multicentric randomized controlled trials with longer follow-up, HPV genotyping, and immunological evaluation to support and validate the use of intralesional immunotherapy in the dermatological daily practice.

Limitations: There are some limitations in the present study. The results from this single-center study may not be applicable to broader populations. The number of late recurrences may have been underestimated as the follow-up period was relatively short (three months). Furthermore, HPV typing and immunological marker evaluations were not conducted, limiting the evaluation of their effects on response to treatment. Last, possible observer bias could have occurred due to the unblinded study design. These findings need to be confirmed in larger multicenter studies with longer follow-up.

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Conflicts of interest

There are no conflicts of interest.

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