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Original Article

# Efficacy of imiquimod 5% cream for persistent human papillomavirus in genital intraepithelial neoplasm

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**Abstract**

*Objective*: To assess the clinical response to imiquimod 5% cream in patients with persistent human papillomavirus (HPV) infection after treatment of genital intraepithelial neoplasia.
*Materials and Methods*: Imiquimod 5% cream was applied to treat 76 women with persistent HPV after surgical therapy for cervical or vaginal intraepithelial neoplasia (CIN or VAIN). One sachet of cream was placed in the cervical *os* and vagina with an applicator twice weekly for 8 weeks. Repeated HPV evaluation and Papanicolaou (Pap) smear and/or biopsy were performed 3 months following treatment completion.
*Results*: In total, 58 of the 76 patients (76.3%) were clear of HPV infection and had normal Pap smears after administration of imiquimod cream. Although atypia or mild dysplasia was noted in 15 of the 18 patients (83.3%) with persistent HPV infection after imiquimod cream treatment, the degree of severity was noticeably less than the initial diagnosis in most of these patients. Persistent HPV positivity was observed in 12 of the 64 patients (18.8%) with CIN and 6 of the 12 patients (50.0%) with VAIN.
*Conclusion*: Topical imiquimod 5% cream may be beneficial in most cases of genital intraepithelial neoplasia, especially CIN, with persistent HPV following surgical treatment.


*Keywords:* cervical intraepithelial neoplasia; gyne-oncology; human papillomavirus; imiquimod 5% cream; vaginal intraepithelial neoplasia

## Introduction

The standard therapy for genital intraepithelial neoplasia is surgical, either ablative or excisional. However, these two forms of therapies merely treat the lesion and not the etiological factor, which is mostly correlated to the underlying human papillomavirus (HPV). It is reported that patients with histologically proven complete removal of cervical intraepithelial neoplasia (CIN) may have a recurrence [1,2]. Recently pooled long-term follow-up data indicate that treated women are still at an increased risk for subsequent invasive cervical cancer, compared to the general population, for at least 10 years and maybe even up to 20 years after treatment [3]. The risk of recurrent CIN is higher in women older than 50 years [4], which is consistent with the observation that viral persistence increases with age [5]. Thus, the persistence of HPV infection after treatment of genital intraepithelial neoplasia should be considered as a greater risk factor of relapse or progression to a more invasive lesion.

Several epidemiological studies have indicated that persistently infected women with oncogenic HPV types are more likely to develop cervical neoplastic lesions [6]. Chua and Hjerpe found that among recurring cases, a 92% HPV prevalence was noted at the first follow-up smear 3 months after cervical conization [7]. It is also reported that patients with persisting HPV infection after treatment demonstrated a statistically significant higher rate of recurrences than patients in whom HPV was successfully eradicated. None of the patients in whom HPV infection was eradicated developed recurrent disease [7,8]. Therefore, in addition to genital cytology and colposcopic examination, HPV testing could

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potentially become a valuable tool used to monitor the therapeutic results for genital intraepithelial neoplasia treatment and to discriminate patients with a higher risk of disease recurrence.

As aforementioned, current standard therapies have a limited impact in preventing recurrence. Another important issue in the control of genital lesions is the treatment of established HPV infections and HPV-associated diseases. Imiquimod is a low-molecular-weight imidazoquinoline heterocyclic amine that acts as an immune response modifier and induces cytokines to fight the causative agent of genital lesions, the HPV [9–11]. Cytokines are known to stimulate the induction of natural killer cells, which exhibit a cytotoxic action to the HPV-infected and carcinogenic cells [12,13]. Although imiquimod 5% cream has been used widely for the treatment of condyloma acuminate of the vulva, there have been sporadic reports with a small number of patients using the drug for the management of high-grade vulvar intraepithelial neoplasia (VIN II/III) [14–16], as well as for vaginal intraepithelial neoplasia (VAIN) [17,18], with acceptable rates of disease regression. In addition, recent studies have demonstrated that the immune system plays an important role in HPV disease regression [19]. Imiquimod has revealed immunomodulating, antiviral, and antitumor activities in animal studies [9]. Therefore, the rationale for this type of therapy is based on the conjecture that multicentric lesions of the lower genital tract are strongly related to the HPV [20].

The purpose of the present study is to assess the efficacy of topical imiquimod in patients with persistent HPV infection after treatment of genital intraepithelial neoplasia and to evaluate the recurrence during subsequent long-term follow-up.

## Materials and methods

We reviewed surgical cases with available records in the Department of Obstetrics and Gynecology at Keelung Chang Gung Memorial Hospital from January 2006 to December 2009. Those women with persistent HPV infection following both surgical treatment for CIN or VAIN and treatment with imiquimod 5% cream (Aldara; 3M Health Care Limited, Loughborough, UK) thereafter were evaluated. Treatment was off-label because vaginal and/or cervical use is not a listed indication for imiquimod 5% cream. The use of vaginal or cervical imiquimod 5% cream was undertaken simply to treat patients and was not done as a research project. However, a general consent form was signed by each patient prior to treatment. In addition, the retrospective study was approved by the Chang Gung Memorial Hospital Ethical Medicine Committee.

The preoperative diagnoses were taken from the patients' colposcopically directed biopsies. For those whose initial biopsy showed CIN II or III, the diagnoses were further confirmed by the pathological specimens resected by conization. Local $CO_2$ vaporization of the vagina or cervix was performed for 10 of the patients with VAIN I, two with VAIN II and 44 with CIN I. Therapeutic cold knife conization was done for five patients with CIN II and 15 with CIN III, which was confirmed with the complete resection of cervical lesions in all of these patients. Investigation of HPV infection was performed 1 month prior to and after surgical therapy.

Cervical or vaginal sampling for HPV DNA was performed with the Digene cervical sampler. DNA specimens were collected by inserting the Digene brush 1–1.5 cm into the cervical *os*, until the largest outer bristles of the brush touched the ectocervix or upper vagina. Three full rotations were done, performed in a counter-clockwise direction. The brush was then inserted into the bottom of the transport tube, with its shaft snapped off at the score line and the tube was capped securely. The Hybrid Capture II HPV DNA test (Digene Corporation, Gaithersburg, MD, USA) was used for the detection of HPV. This hybridization antibody capture assay uses chemiluminescence to quantitatively detect the presence of low risk (6, 11, 42, 44) and high/intermediate risk (16, 18, 31, 33, 35, 45, 51, 52, 56) HPV types in cervical or vaginal swabs. According to the manufacturer's instructions, the clinical relative sensitivity is 90.1% and the clinical relative specificity is 95.6%.

Because the positive HPV tests were noted after local treatment, the patients sought topical treatment via imiquimod 5% cream. One sachet of cream (0.25 g) was placed in the entire cervix and/or entire vagina with an applicator twice weekly for 8 weeks. The procedure was performed by Dr Chen. Three months after the imiquimod treatment was administered, the patients had follow-up Papanicolaou smears and HPV tests done. The patients were also asked if they experienced any discomfort during or after the cream treatment.

Fisher's exact tests were used to evaluate the association of menopausal status, location of lesions, surgical method and lesion grade with HPV clearance/persistence, after treatment using imiquimod cream.

## Results

A total of 76 patients with CIN or VAIN, who had persistent HPV infection after surgical therapy, participated in this study. After topical imiquimod cream treatment, no expression of HPV was confirmed in 58 (76.3%) of the patients. Of the 18 patients with persistent HPV infection, one patient had CIN III, three had CIN II after conization, eight had CIN I and six had VAIN I after local $CO_2$ vaporization (Table 1).

Table 1
Patients with persistent HPV infection versus patients with eliminated HPV infection after topical treatment with imiquimod cream.

| Histology | Persistent HPV | No HPV | Total |
|---|---|---|---|
| | $n = 18$ (23.7%) | $n = 58$ (76.3%) | $n = 76$ |
| CIN III | 1 | 14 | 15 |
| CIN II | 3 | 2 | 5 |
| CIN I | 8 | 36 | 44 |
| VAIN II | 1 | 1 | 2 |
| VAIN I | 5 | 5 | 10 |

Although atypia or mild dysplasia was noted in 15 of the 18 patients (83.3%) with persistent HPV infection after imiquimod cream treatment, the degree of severity was noticeably less than the initial diagnosis in most of these patients. After treatment, atypia was found in 10 of the patients (1 with CIN III, 1 with CIN II, 3 with CIN I, 1 with VAIN II and 4 with VAIN I) and CIN I was noted in two patients with CIN II and three with CIN I.

Univariate Fisher's exact tests showed a significantly higher proportion of persistent HPV infection in the patients with vaginal lesions after treatment with imiquimod cream ($p = 0.029$). Menopausal status, pretreatment surgical methods and lesion grade were not associated with HPV clearance/persistence (Table 2).

## Discussion

Although different conservative methods for the treatment of genital preinvasive lesions have been developed and extensively applied, the persistence of HPV infection after treatment and its relationship with the lesion evolution has become an important issue. The present review demonstrated that after imiquimod 5% cream treatment, clearance of HPV infection occurred in 76.3% of the patients with CIN or VAIN. Imiquimod cream seems to be less effective in patients with VAIN than in those with CIN. Although such efficacy is encouraging, several factors concerning these results still should be examined.

Recent studies have been developed so as to evaluate the feasibility of using imiquimod to treat patients with VIN, VAIN, and CIN. Most of these studies have focused on the histological regression of the lesion, but not on the clearance of the HPV infection like our study does. Diaz-Arrastia et al [21] treated eight patients with genital intraepithelial neoplasia, in which previous standard therapies had failed, with topical imiquimod 5%. Included in these eight cases were two patients with cervical dysplasia, two with vaginal dysplasia and four with vulvar dysplasia. They found six of the patients to be responsive, both partially and completely, to treatment. Three patients had recurrences but all were successfully re-treated with imiquimod 5% cream. In addition to the effects on the regression of the lesion, they demonstrated an increase of 2′,5′-oligoadenylate synthetase RNA expression after imiquimod treatment, which may potentially be an important mechanism of action of imiquimod on antiviral activity. In a study where topical imiquimod was used to treat 25 patients with VIN and who tested positive for HPV DNA [22], they revealed that HPV had been cleared in 15 of the patients (60%). In addition, the research noted a strong association between viral clearance and histological regression. This is compatible to our results, in which HPV clearance reached up to 76.3% and no persistent nor abnormal histological changes were noted in those HPV cleared by imiquimod. The efficacy of imiquimod in clearing HPV infection seems to be elevated in our study. Although there were controversial results regarding the use of surgical treatment to eradicate the HPV infection [8,23], further investigation is required to examine whether the preimiquimod surgical treatment may have strengthened the effects of the imiquimod or not.

Table 2
Possible factors affecting topical treatment with imiquimod cream.

| | Persistent HPV, *n* (%) | No HPV, *n* (%) | *p* |
|---|---|---|---|
| Menopause | | | 0.088 |
| Yes | 12 (66.7) | 26 (44.8) | |
| No | 6 (33.3) | 32 (55.2) | |
| Location of lesion | | | 0.029 |
| Cervix | 12 (66.7) | 52 (89.7) | |
| Vagina | 6 (33.3) | 6 (10.3) | |
| Surgical method | | | 0.4528 |
| Conization | 4 (22.2) | 16 (27.6) | |
| $CO_2$ laser | 14 (77.8) | 42 (72.4) | |
| Lesion grade | | | 0.5767 |
| LSIL | 13 (72.2) | 41 (70.9) | |
| HSIL | 5 (27.8) | 17 (29.1) | |

HSIL = high-grade squamous intra-epithelial lesion;
LSIL = low-grade squamous intra-epithelial lesion.

Although the use of imiquimod in the cervix has been questioned due to the difference in the anatomic and imiquimod characteristics of the external skin and vagina, the beneficial effects of imiquimod on the cervix have been reported [21,24]. In the present review, the effect of imiquimod on HPV clearance was significantly better on CIN than on VAIN. Although HPV had only cleared in six (50%) of the 12 patients with VAIN, the histological regression of VAIN could reach 100%, except for the five patients with atypia after imiquimod treatment. Similar results were also noted in other studies [17,18], except they did not reveal the effect of treatment on HPV clearance. Buck et al [17] demonstrated that 36 (86%) of the 42 patients with VAIN I were clear of any VAIN lesion after receiving one imiquimod course (each course consists of a single administration weekly for 3 weeks); five patients were cleared after two treatment courses and one patient after three courses. In another study of seven patients with positive HPV and VAIN II/III, six patients were found to have either VAIN I or simple HPV infection and one patient had regression lesion from VAIN III to VAIN II after imiquimod treatment [18]. Because the case numbers in all of these studies, including our review, are too minute, further investigation is still needed to determine and confirm whether the effects of imiquimod on CIN would be superior to that on VAIN. However, imiquimod may have an effect on the HPV clearance and regression of the genital intraepithelial neoplasia.

It is not known why some patients responded to imiquimod whereas others did not. It has been considered that the persistence of HPV might be associated and correlated with several factors, including the presence of high-risk types, viral load, age, number of sexual partners, sex hormone regulation and host immunity [25]. van Seters et al [22] evaluated the effects of imiquimod on vulvar dysplasia, in which HPV had cleared in 14 of the patients with HPV type 16 and one with type 33. They demonstrated that there was no significant association between HPV type and viral outcome. In this review, we did not examine the HPV type, but the HPV load did not

affect the effects of imiquimod (data not shown). In addition, the effect on HPV clearance was also not related to menopausal status, surgical method and/or histological grading of the lesion. Because imiquimod acts as an immune response modifier, further evaluation is needed to determine whether the immune response of patients to imiquimod plays an important role or not and why patients would have differing responses to the treatment.

This review differs from other studies in the respect that these patients received standard surgical therapy prior to imiquimod treatment. Thus, a major limitation is that there are no randomized controlled trials. Another limitation is that a lack of response to imiquimod treatment does not seem to be associated with a higher risk of lesion progression, but that may be related to the duration of the follow-up. Therefore, from this review, it can be concluded that the effect of combined imiquimod treatment and standard surgical therapy for CIN and VAIN and the efficacy of imiquimod for HPV clearance are worth further investigation. However, longer follow-up periods are needed to make any conclusion regarding the effectiveness, recurrence rates and recurrence free interval duration of this treatment.

## References


[1] Paraskevaidis E, Kalantaridou SN, Paschopoulos M, Zikopoulos K, Diakomanolis E, Dalkalitsis N, et al. Factors affecting outcome after incomplete excision of cervical intraepithelial neoplasia. Eur J Gynecol Oncol 2003;24:541–3.

[2] Milojkovic M. Residual and recurrent lesions after conization for cervical intraepithelial neoplasia grade 3. Int J Gynaecol Obstet 2002;76:49–53.

[3] Soutter WP, Sasieni P, Panoskaltsis T. Long-term risk of invasive cervical cancer after treatment of squamous cervical intraepithelial neoplasia. Int J Cancer 2006;118:2048–55.

[4] Flannelly G, Bolger B, Fawzi H, De Lopes AB, Monaghan JM. Follow up after LLETZ: could schedules be modified according to risk of recurrence? BJOG 2001;108:1025–30.

[5] Castle PE, Schiffman M, Herrero R, Hildesheim A, Rodriguez AC, Bratti MC, et al. A prospective study of age trends in cervical human papilloma virus acquisition and persistence in Guanacaste, Costa Rica. J Infect Dis 2005;191:1808–16.

[6] Villa LL. Human papillomaviruses and cervical cancer. Adv Cancer Res 1997;71:321–41.

[7] Chua KL, Hjerpe A. Human papillomavirus analysis as a prognostic marker following conization of the cervix uteri. Gynecol Oncol 1997;66:108–13.

[8] Bodner K, Bodner-Adler B, Wierrani F, Kimberger O, Denk C, Grünberger W. Is therapeutic conization sufficient to eliminate a high-risk HPV infection of the uterine cervix? A clinicopathological analysis. Anticancer Res 2002;22:3733–6.

[9] Lacey CJ, Fairley I. Medical therapy of genital human papilloma virus-related disease. Int J STD AIDS 1995;6:399–407.

[10] Tyring SK, Arany I, Stanley MA, Tomai MA, Miller RL, Smith MH, et al. A randomized controlled molecular study of condyloma acuminate clearance during treatment with imiquimod. J Infect Dis 1998;178:551–5.

[11] Miller RL, Gerster JF, Owens ML, Slade HB, Tomai MA. Imiquimod applied topically: a novel immune response modifier and new class of drug. Int J Immunopharmacol 1999;21:1–14.

[12] Testerman TL, Gerster JF, Imbertson LM, Reiter MJ, Miller RL, Gibson SJ, et al. Cytokine induction by the immunomodulators imiquimod and S-27609. J Leukoc Biol 1995;58:365–72.

[13] Gibson SJ, Imbertson LM, Wagner TL, Testerman TL, Reiter MJ, Miller RL, et al. Cellular requirements for cytokine production in response to the immunomodulators imiquimod and S-27609. J Interferon Cytokine Res 1995;15:537–45.

[14] van Seters M, Fons G, van Beurden M. Imiquimod in the treatment of multifocal vulvar intraepithelial neoplasia 2/3. Results of a pilot study. J Reprod Med 2002;47:701–5.

[15] Jayne CJ, Kaufman RH. Treatment of vulvar intraepithelial neoplasia 2/3 with imiquimod. J Reprod Med 2002;47:395–8.

[16] Todd RW, Etherington IJ, Luesley DM. The effect of 5% imiquimod cream on high-grade vulvar intraepithelial neoplasia. Gynecol Oncol 2002;85:67–70.

[17] Buck HW, Guth KJ. Treatment of vaginal intraepithelial neoplasia (primarily low grade) with imiquimod 5% cream. J Low Genit Tract Dis 2003;7:290–3.

[18] Haidopoulos D, Diakomanolis E, Rodolakis A, Voulgaris Z, Vlachos G, Intsaklis A. Can local application of imiquimod cream be an alternative mode of therapy for patients with high-grade intraepithelial lesions of the vagina? Int J Gynecol Cancer 2005;15:898–902.

[19] Arany I, Tyring SK, Brysk MM, Stanley MA, Tomai MA, Miller RL, et al. Correlation between pretreatment levels of interferon response genes and clinical responses to an immune response modifies (Imiquimod) in genital warts. Antimicrob Agents Chemother 2000;44:1869–73.

[20] van Beurden M, ten Kate FJ, Smits HL, Berkhout RJ, de Craen AJ, van der Vange N, et al. Multifocal vulvar intraepithelial neoplasia grade III and multicentric lower genital tract neoplasia is associated with transcriptionally active human papillomavirus. Cancer 1995;75:2879–84.

[21] Diaz-Arrastia C, Arany I, Robazetti SC, Dinh TV, Gatalica Z, Tyring SK, et al. Clinical and molecular responses in high-grade intraepithelial neoplasia treated with topical imiquimod 5%. Clin Cancer Res 2001;7:3031–3.

[22] van Seters M, van Beurden M, ten Kate FJ, Beckmann I, Ewing PC, Eijkemans MJ, et al. Treatment of vulvar intraepithelial neoplasia with topical imiquimod. N Engl J Med 2008;358:1465–73.

[23] Ruge S, Felding C, Skouby SO, Lundvall F, Hørding U, Norrild B. $CO_2$ laser vaporization in the treatment of cervical human papillomavirus infection in women with abnormal Papanicolaou smears. Gynecol Obstet Invest 1992;33:172–6.

[24] Travis LB, Weinberg JM, Krumholz BA. Successful treatment of vulvar intraepithelial neoplasia with topical imiquimod 5% cream in a lung transplanted patient. Acta Derm Venerol 2002;82:475–6.

[25] Chua KL, Hjerpe A. Persistence of human papillomavirus (HPV) infections preceding cervical carcinoma. Cancer 1996;77:121–7.