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Original Article

Pidotimod plus recombinant human interferon α-2b suppository boosts HPV clearance in high-risk patients following loop electrosurgical excision procedure

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Abstract: Objective: To assess human papillomavirus (HPV) negative conversion, vaginal microecological recovery, and serum inflammatory factor levels in patients with high-risk HPV infection treated with pidotimod (PDT) plus recombinant human interferon α-2b (rh-IFN-α2b) suppository after loop electrosurgical excision procedure (LEEP). Methods: A total of 97 patients with high-risk HPV infection who underwent LEEP from March 2020 to May 2023 were retrospectively selected. Among these cases, 45 treated with rh-IFN-α2b suppository were assigned as control group, while the other 52 treated with PDT + rh-IFN-α2b suppository were assigned as combined group. HPV negative conversion, side effects (fever, nausea, abdominal discomfort, and vaginal burning sensation), vaginal microecology recovery (vaginal pH, Nugent score), and serum inflammatory markers (interleukin [IL]-4, IL-12, and interferon [IFN]-γ) were compared between the two groups. Furthermore, univariate and multivariate analyses were performed to identify factors associated with failure to achieve negative conversion. Results: The combined group demonstrated a higher HPV negative conversion rate, treatment response rate, and vaginal microecological recovery rate (lower vaginal pH and Nugent score) compared to the control group. Also, post-treatment IL-4 levels were lower, while IL-12 and IFN-γ were higher in the combined group. The side effects in the two groups were similar. Univariate and multivariate analyses revealed that HPV type and treatment modality were associated with HPV negative conversion failure, but were not independent predictors. Conclusions: PDT plus rh-IFN-α2b suppository can effectively improve HPV negative conversion, accelerate vaginal microecology recovery, and modulate serum inflammatory responses in high-risk HPV patients after LEEP.

Keywords: High-risk HPV, LEEP, pidotimod, recombinant human interferon α-2b suppository, serum inflammatory markers

Introduction

Cervical carcinoma (CC), the second most common malignancy that poses a serious threat to women's physical and mental health, is etiologically associated with persistent infection with high-risk (HR) human papillomavirus (HPV) [1, 2], primarily types 16 and 18. These infections can affect the cervix, vulva, vagina, anus and other sites, leading to precancerous lesions and cancer [3, 4]. As indicated Epidemiological data estimate nearly 600,000 new cases and 320,000 deaths from CC globally each year [5]. Risk factors for HPV infection include immunosuppression, a history of sexually transmitted infections, and multiple sexual partners. Most HPV-infected patients remain asymptomatic in the early stages [6]. Loop electrosurgical excision procedure (LEEP) is a widely used surgical modality for early-stage CC and can effectively reduce HR-HPV persistence. However, it is often followed by a high rate of postoperative HPV infection [7]. Therefore, optimizing post-LEEP intervention to enhance recovery and restore vaginal microecology is crucial for reducing CC risk and mortality.

Recombinant human interferon α-2b (rh-IFN-α2b) possesses the same biological function as IFN-α2b, including immune enhancement, antitumor, and anti-virus effects [8]. It inhibits HPV RNA replication and promotes tissue regeneration, improving vaginal immune function [9, 10]. While short-term use of rh-IFN-α2b is effective,

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relapse can occur after discontinuation [11]. Pidotimod (PDT), a synthetic compound with immunostimulatory properties, regulates both adaptive and innate immune responses [12]. PDT has shown efficacy in treating recurrent respiratory infection in children and improving immune function, particularly the Th1/Th2 balance [13]. When combined with vitamin C, PDT can reduce the recurrence of HPV-related diseases by enhancing immunity [14]. This study aims to investigate the clinical benefits of combining rh-IFN-α2b suppositories with PDT for HR-HPV patients post-LEEP, providing a therapeutic strategy for improving patient outcome.

Patients and methods

Case selection

This retrospective study included 97 patients who underwent LEEP for HR-HPV at our hospital between March 2020 and May 2023. Of these, 45 cases treated with rh-IFN-α2b suppository were assigned to the control group and the other 52 treated with PDT + rh-IFN-α2b suppository were assigned to the combined group. No significant inter-group difference was found in general data (P>0.05). The research was approved by the Ethics Committee of The Second Affiliated Hospital of Shaanxi University of Chinese Medicine.

Inclusion criteria: Female patients diagnosed with HR-HPV by cervical exfoliative cytology, positive for HPV DNA, and showing grade II cervical intraepithelial neoplasia on pathology; Presence of cervical condylomatous lesions; No recent use of other drugs; Complete sexual history and clinical data.

Exclusion criteria: Gonorrhea, mycoplasma, trichomonas infection, or other infections; Cervical carcinoma; Women in lactation or pregnancy; History of recurrent HPV infection; Cardiac, renal, or pulmonary dysfunction; Mental illness or cognitive dysfunction.

Intervention methods

The control group received rh-IFN-α2b suppository treatment. After vaginal cleaning, patients were administered 1 rh-IFN-α2b suppository (Wuhan Amyjet Technology Co., Ltd., ENZ-PR-T192-0010) once a night, every other day. The combined group received PDT in addition to rh-IFN-α2b suppositories. Patients in this group took 0.8 g PDT (Wuhan Amyjet Technology Co., Ltd., P437650) orally once daily, alongside the rh-IFN-α2b suppository regimen. Both groups underwent treatment for 8 weeks. During this period, patients were instructed to refrain from sexual activity, avoid bathing, and avoid consuming irritating foods.

Data collection and outcome measurement

HPV negative conversion, side effects (fever, nausea, abdominal discomfort, and vaginal burning sensation), vaginal microecological recovery, vaginal pH, Nugent score, and serum inflammatory markers (interferon [IFN]-γ, interleukin [IL]-4, and IL-12) were comparatively analyzed.

HPV negative conversion: HPV-DNA was tested in follow-up patients. Negative conversion was defined as the absence of all HR-HPV subtypes on vaginal and cervical examination. Effective conversion: negative conversion of some HR-HPV subtypes, with ≥1 subtype remaining positive; Ineffective conversion: persistent positivity of all HR-HPV types.

Side effects: The incidence of side effects such as fever, nausea, abdominal discomfort, and vaginal burning sensation after treatment was counted.

Vaginal microecology recovery: Vaginal secretions were collected for pH detection and Gram staining. Recovery was defined by a vaginal pH ≤4.5, bacterial density between 10-999 by oil immersion lens, Lactobacillus dominance, a Nugent score ≤3, and negative catalase and sialidase tests. Cases not meeting these criteria were considered unrecovered.

Nugent score: Vaginal swab smears were subjected to Gram staining. The Nugent score, based on the morphology and quantity of different microflora, indicates bacterial vaginosis: a score <4 is normal, 4-6 suggests possible bacterial vaginosis, and >6 indicates bacterial vaginosis.

Serum inflammatory markers: Fasting cubital venous blood (3 mL) was collected before and after treatment. Serum levels of IL-4, IL-12, and IFN-γ were measured using enzyme-linked im-

Table 1. Comparison of patient general information between the two groups

Factor	n	Control group (n=45)	Combined group (n=52)	χ^2/t	P
Age (years)	97	40.38±10.64	43.15±11.50	1.225	0.224
Parity (times)	97	1.49±0.59	1.69±0.78	1.406	0.163
Pausimenia				0.135	0.714
Yes	21	9 (20.00)	12 (23.08)		
No	76	36 (80.00)	40 (76.95)		
HPV type				1.021	0.312
16/18	17	6 (13.33)	11 (21.15)		
Others	80	39 (86.67)	41 (78.85)		
Number of types of HPV infection				3.709	0.157
1	77	32 (71.11)	45 (86.54)		
2	18	12 (26.67)	6 (11.54)		
≥3	2	1 (2.22)	1 (1.92)		

Note: HPV, human papillomavirus.

Table 2. Comparison of HPV negative conversion rate between the two groups

Factor	Control group (n=45)	Combined group (n=52)	χ^2	P
Negative conversion	21 (46.67)	35 (67.31)	4.212	0.040
Effective	11 (24.44)	12 (23.08)		
Ineffective	13 (28.89)	5 (9.62)		
Response rate	32 (71.11)	47 (90.38)	5.929	0.015

Note: HPV, human papillomavirus.

munosorbent assay (ELISA). The instructions of the corresponding human ELISA kits (SenBeiJia Biological Technology Co., Ltd., SBJ-H0293, SBJ-H0282, and SBJ-H0184) were strictly followed during the testing.

Primary outcomes included HPV negative conversion, vaginal microecology recovery, and serum levels of inflammatory markers. Secondary outcomes included the side effects, vaginal secretion pH, and the Nugent score.

Statistical methods

SPSS 21.0 was used for data analyses. Continuous variables were presented as mean ± SEM. Inter-group comparisons were performed using independent sample t-tests, while within-group comparisons were conducted using paired t-tests. Categorical variables, such as HPV negative conversion and side effects, were expressed as n (%), with between-group comparisons made using the χ^2 test. A binary logistic regression model was used for multivariate analysis of factors contributing to ineffective HPV negative conversion. A *P* value less than 0.05 was considered significant.

Results

Comparison of general information between the two groups

There were no significant differences between the control and combined groups in terms of age, parity, menopause status, HPV type, or number of HPV types (all P>0.05; **Table 1**).

Comparison of HPV negative conversion rate between the two groups

The HPV negative conversion rate was 67.31% in the combined group, significantly higher than 46.67% in the control group (P<0.05). Also, a higher overall response rate was observed in the combined group (90.38% vs. 71.11%; P< 0.05), as shown in **Table 2**.

Univariate analysis of factors affecting HPV negative conversion

Univariate analysis indicated that HPV types and treatment modalities were significantly associated with ineffective HPV negative conversion (P<0.05; **Table 3**).

Table 3. Univariate analysis of factors influencing HPV negative conversion

Factor	n	Ineffective negative conversion group (n=18)	Effective negative conversion group (n=79)	χ^2	P
Age (years)				2.454	0.117
<40	43	5 (27.78)	38 (48.10)		
≥40	54	13 (72.22)	41 (51.90)		
Parity (times)				0.021	0.884
<2	50	9 (50.00)	41 (51.90)		
≥2	47	9 (50.00)	38 (48.10)		
Pausimenia				1.779	0.182
Yes	21	6 (33.33)	15 (18.99)		
No	76	12 (66.67)	64 (81.01)		
HPV type				6.978	0.008
16/18	17	7 (38.89)	10 (12.66)		
Others	80	11 (61.11)	69 (87.34)		
Number of types of HPV infection				4.838	0.089
1	77	11 (61.11)	66 (83.54)		
2	18	6 (33.33)	12 (15.19)		
≥3	2	1 (5.56)	1 (1.27)		
Treatment modalities				5.929	0.015
rh-IFN-α2b	45	13 (72.22)	32 (40.51)		
PDT+rh-IFN-α2b	52	5 (27.78)	47 (59.49)		

Note: HPV, human papillomavirus; rh-IFN-α2b, recombinant human interferon α-2b; PDT, pidotimod.

Table 4. Multivariate analysis of factors influencing HPV negative conversion

Factor	β	SE	Wald	P	Exp (β)	95% CI
HPV types	1.099	0.626	3.080	0.079	3.002	0.880-10.249
Treatment modalities	1.042	0.608	2.937	0.087	2.835	0.861-9.337

Note: HPV, human papillomavirus.

Table 5. Comparison of side effects between the two groups

Factor	Control group (n=45)	Combined group (n=52)	χ^2	P
Fever	1 (2.22)	2 (3.85)		
Nausea	1 (2.22)	1 (1.92)		
Abdominal discomfort	1 (2.22)	1 (1.92)		
Vaginal burning	1 (2.22)	2 (3.85)		
Total	4 (8.89)	6 (11.54)	0.183	0.669

Multivariate analysis of factors affecting HPV negative conversion

The factors identified by the univariate analysis (HPV types and treatment modalities) were used as independent variables in a binary logistic regression model, with ineffective HPV negative conversion (set as 1) and effective conversion (set as 0) as the dependent variable. Multivariate analysis revealed that neither HPV type nor treatment modality was an independent factor for ineffective negative conversion (P>0.05) (**Table 4**).

Comparison of side effects of medication between the two groups

The incidence of fever, nausea, abdominal discomfort, and vaginal burning sensation was similar between the two groups, with no significant difference in overall incidence of adverse events (8.89% vs. 11.54%; P>0.05) (**Table 5**).

Comparison of vaginal microecology recovery between the two groups

The vaginal microecological recovery rate was significantly higher in the combined group com-

Table 6. Comparison of vaginal microecology recovery between the two groups

Factor	Control group (n=45)	Combined group (n=52)	χ^2	P
Normal or recovered	29 (64.44)	45 (86.54)	6.510	0.011
Unrecovered	16 (35.56)	7 (13.21)		

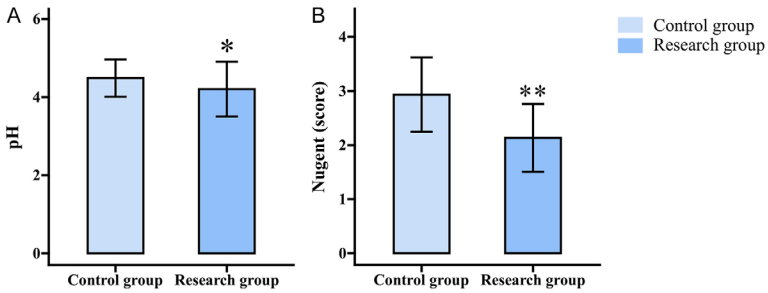

Figure 1. Comparison of vaginal secretion pH and Nugent scores between the two groups. A. pH value of vaginal secretions; B. Nugent scores. Note: *P<0.05, **P<0.01, vs. control group.

pared to the control group (86.54% vs. 64.44%, P<0.05), as shown in **Table 6**.

Comparison of vaginal secretion pH and Nugent score between the two groups

The combined group exhibited significantly lower vaginal secretion pH values and Nugent scores compared to the control group (P<0.05; **Figure 1**).

Comparison of serum levels of inflammatory markers between the two groups

Inflammatory markers IL-4/12 and IFN-γ were measured to assess the influence of the treatments. No significant inter-group differences were observed before treatment (P>0.05). After treatment, IL-4 levels decreased significantly in both groups, while IL-12 and IFN-γ increased significantly (P<0.05). Notably, the combined group had lower IL-4 and higher IL-12 and IFN-γ levels compared to the control group after treatment (all P<0.05). The details are shown in **Figure 2**.

Discussion

High-risk (HR) HPV infections are strongly associated with more than 99% of cervical cancers, with HPV 16/18 accounting for up to 80% of global cases [15, 16]. Persistent HR-HPV infection of the genital mucosa leads to cell proliferation, genome instability, and, if untreated, potential malignant transformation [17]. Current treatment options for HR-HPV, alongside LEEP, include carbon dioxide therapy, laser therapy, cryotherapy, and antiviral drugs [18]. However, due to the latency of HR-HPV types and limited treatment options, recurrence is common, and treatment outcomes are often suboptimal. As such, there is a need to explore and optimize new treatment strategies.

In this study, the combined group showed obviously higher HPV negative conversion rate (67.31% vs. 46.67%) and overall treatment response rate (90.38% vs. 71.11%) compared to the control group, indicating that the combination of rh-IFN-α2b suppository and PDT effectively enhances HPV negative conversion and treatment response in HR-HPV patients after LEEP. rh-IFN-α2b, a broad-spectrum antibacterial drug, inhibits viral replication by stimulating cells to produce antiviral proteins [19]. However, its clinical efficacy is limited by its short half-life and low drug utilization rate, necessitating combination with other treatments [8]. PDT's therapeutic effects may stem from its ability to drive cytokine release and T cell proliferation, thereby stabilizing HPV-related immune disorders [20, 21].

Univariate and multivariate analyses showed that while HPV types and treatment modalities were associated with ineffective negative conversion, neither was an independent causal factor. In terms of safety, the incidences of fever, nausea, abdominal discomfort, and vaginal burning sensation were similar between the two groups, suggesting that the combination of rh-IFN-α2b suppository and PDT does not increase side effects and is well-tolerated, consistent with the research of Sun et al. [22]. Local administration of rh-IFN-α2b promotes macrophage phagocytosis and enhances lymphocyte killing, improving the vaginal microecological environment and reducing adverse reactions [23]. PDT's safety has been established for treating persistent asthma and recurrent respiratory infections [24, 25].

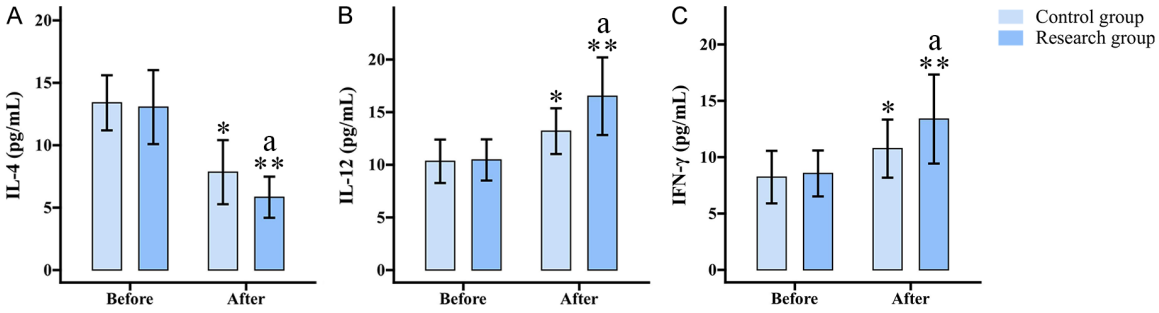

Figure 2. Comparison of serum levels of inflammatory markers between the two groups. A. IL-4; B. IL-12; C. IFN-γ. Note: *P<0.05, **P<0.01, vs. before treatment; aP<0.05 vs. control group. IL-4, interleukin-4; IL-12, interleukin-12; IFN-γ, interferon-γ.

Regarding vaginal microecology recovery, the combined group showed significantly higher recovery rates (86.54% vs. 64.44%) and lower vaginal secretion pH and Nugent scores, indicating that rh-IFN-α2b + PDT enhances vaginal microecological recovery. Serum inflammatory markers also showed favorable changes, with lower IL-4 and higher IL-12 and IFN-γ post-treatment compared to baseline and the control group, suggesting that the combination treatment improves the inflammatory environment in HR-HPV patients after LEEP. This aligns with Ding et al.'s study, where combining rh-IFN-α2b with traditional Chinese medicine improved HPV negative conversion and reduced serum inflammation while maintaining safety [26].

This study had several limitations. First, the small sample size (less than 100 cases) may have introduced bias in data collection. Second, the lack of follow-up data restricted our ability to assess the long-term effects of rh-IFN-α2b suppository plus PDT therapy. Future studies with follow-up assessment would provide valuable insight into the sustained effect of this treatment. Third, the study did not evaluate patients' emotional status and quality of life, which could reveal additional benefits of this therapeutic approach. We aim to address these limitations and refine our research methodology in future research.

Conclusion

In summary, this study demonstrated the remarkable clinical advantage of rh-IFN-α2b suppository plus PDT for HR-HPV patients after LEEP. The combination therapy notably improved the HPV negative conversion rate, treatment efficacy, vaginal microecological restoration, and serum inflammatory environment, with good safety and tolerability. This approach offers an optimized choice for HR-HPV patients after LEEP, serving as a valuable reference for their future management.

Disclosure of conflict of interest

None.

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