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Article

The clinical efficacy and influencing factors of HpD-photodynamic therapy in the treatment of high-grade vaginal squamous intraepithelial lesions

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Abstract: Objective: To investigate the efficacy and safety of HpD-photodynamic therapy in the treatment of high-grade vaginal squamous intraepithelial lesions. **Methods:** Fifteen patients diagnosed by pathology as vaginal HSIL were selected from January 2020 to September 2022. PDT was performed by 630 nm laser 48 h–72 h after intravenous injection of 2.0 mg/kg–2.5 mg/kg hematoporphyrin derivative. The power density is 100 mW/ cm^2, the irradiation time is 30 min, and the energy density is 180 J/cm^2. The curative effect was evaluated by histopathological biopsy and HPV examination 3–6 months after operation. The numerical pain rating scale (NPRS) system was used to record the intraoperative and postoperative pain. Patient's satisfaction was self-evaluated during the operation, the adverse reactions and complications were observed at 1 week, 1 month and 3–6 months after operation. **Results:** 15 patients were treated using PDT. After 3–6 months，8 cases were reported complete remission (CR), 3 cases were reported partial remission (PR) and 4 cases were reported no remission. The effective rate was 73.3%. All 15 patients were high-risk HPV infected and the clearance rate was 26.7% (4/15). 6 patients（40%）had tolerable lower abdominal pain within 1 week after operation, 4 patients (26. 7%) had tolerable lower abdominal pain within 1 week after operation (NPRS was 0. 73 ± 1. 2 and 0. 47 ± 0. 8) and 1 patient had local photosensitive reaction. **Conclusions:** PDT is effective, safe and non-invasive in the treatment of high squamous intraepithelial lesions of the vagina. It is worthy of clinical promotion and application.

Keywords: photodynamic therapy; hematoporphyrin injection; high-grade squamous intraepithelial lesion; vagina

1. Introduction

Vaginal intraepithelial neoplasia (VaIN) is a group of diseases characterized by abnormal proliferation and atypical hyperplasia of squamous epithelium in the vagina, which is closely related to HPV virus infection, cervical lesions, etc. [1]. It is difficult to treat and prone to recurrence. VaIN can be classified into low-grade lesions and high-grade lesions based on pathology. Although its incidence is not high, some VaIN grades 2–3 can progress to invasive cervical cancer [2]. With the popularization of cervical cancer screening, the detection rate of VaIN has also increased significantly. For VaIN, the treatment methods include drug therapy, physical therapy, surgical treatment, and radiation therapy, but there is no unified guideline for its treatment. Photodynamic therapy (5-aminolevulinic acid, ALA-PDT), particularly using 5-aminolevulinic acid (ALA), has gained attention as a

promising non-invasive treatment modality for VaIN. ALA-PDT is a highly selective approach that utilizes a photosensitizer (such as ALA) that, when activated by light of a specific wavelength, generates reactive oxygen species capable of destroying abnormal cells. This treatment method has been widely applied in dermatological diseases, certain malignancies, and various non-tumor conditions. Its advantages include reduced invasiveness, targeted action, and minimal damage to surrounding healthy tissue [3]. Several studies have demonstrated the effectiveness of ALA-PDT in treating VaIN, showing promising clinical results with fewer adverse effects compared to traditional surgical interventions. Despite the clinical benefits of ALA-PDT, alternative photosensitizers, such as hematoporphyrin derivative (HpD), have also been explored in the treatment of VaIN. HpD-PDT has shown significant therapeutic potential, with good clinical outcomes in the management of vaginal high-grade squamous intraepithelial lesions (HSIL). HpD is a photosensitizer that, when exposed to light of appropriate wavelength, produces cytotoxic reactive oxygen species, which selectively target and destroy abnormal epithelial cells without harming the normal vaginal tissue. This characteristic makes HpD-PDT an attractive treatment option, especially for patients seeking a less invasive and tissue-sparing alternative to traditional therapies. In our study, we retrospectively analyzed the clinical data of 15 patients who underwent HpD-PDT for the treatment of VaIN. The patients included in this analysis were diagnosed with vaginal HSIL, and the treatment involved the application of HpD-PDT using a specific light source to activate the photosensitizer. The clinical outcomes were evaluated based on lesion regression, recurrence rates, and any associated side effects. The results of our study demonstrated that HpD-PDT was effective in achieving complete or partial regression of VaIN lesions, with minimal adverse effects. This supports the use of HpD-PDT as a viable option in the management of VaIN, offering a promising alternative to conventional treatment methods. In conclusion, this study highlights the clinical efficacy of HpD-PDT in the treatment of VaIN, particularly high-grade lesions, and emphasizes its potential role in the development of individualized treatment strategies for patients. Further clinical trials and longitudinal studies are needed to validate these findings and establish standardized protocols for the use of PDT in the management of VaIN.

2. Materials and methods

2.1. Materials

2.1.1. General information

There were 15 patients with vaginal HSIL, all female; the average age was (51.6 ± 9.3) years. The average disease course was (1.89 ± 0.65) months. Among them, 12 patients were diagnosed with HSIL at the vaginal stump after total hysterectomy and bilateral adnexectomy for uterine fibroids, cervical cancer or precancerous lesions; 3 patients had HSIL on the unilateral vaginal wall without a history of uterine lesions. Among the 15 patients, 1 had VaIN grade I-II, 1 had VaIN grade II, 7 had VaIN grade II-III, and 6 had VaIN grade III. According to the number of lesions in the histopathological results after vaginal microscopic biopsy, they were

divided into single lesion (1 site) and multiple lesions (≥ 2 sites), among which 9 had single lesion and 6 had multiple lesions. All 15 patients were complicated with high-risk human papillomavirus (HR-HPV) infection, with an average disease course of (7.85 ± 14.6) months. According to HR-HPV typing, 7 cases contained HPV type 16/18, and 8 cases were other HPV types. This study was approved by the hospital's ethics committee, and all patients voluntarily signed an informed consent form.

Inclusion criteria: (1) Ages ranging from 18 to 75 years old; (2) Those diagnosed with high-grade squamous intraepithelial lesion of the vagina by histopathological examination of biopsy under colposcopy and HR-HPV typing test; (3) No treatment received within the recent 1 month.

Exclusion criteria: (1) Those with vaginal infections, gonorrhea, non-gonococcal urethritis or acute reproductive tract inflammation; (2) Those who have taken glucocorticoids for a long time or have autoimmune diseases; (3) Those with severe liver or kidney function impairment; (4) Porphyria; (5) Pregnant or lactating women; (6) HIV patients.

2.1.2. Instrument

The PDT630II Semiconductor Laser Photodynamic Therapy Device, manufactured by Guilin Xingda Broadcasting and Television Medical Equipment Co., Ltd., is a state-of-the-art medical device designed for PDT. This therapy modality is widely used for various dermatological, oncological, and other medical applications, leveraging the power of light to activate photosensitizing agents in the body and selectively target abnormal or diseased tissues. The PDT630II features a laser with a wavelength of (630 ± 3) nm, which falls within the red-light spectrum known for its optimal interaction with a range of photosensitizers commonly used in PDT. This specific wavelength is effective in penetrating the skin and tissues, ensuring that the photodynamic reaction occurs at the desired target site. The device's output power ranges from 0 to 2 watts, offering flexibility in treatment intensity depending on the patient's needs and the area being treated. One of the key advantages of the PDT630II is its ability to provide both continuous and intermittent output modes. The continuous output mode is useful for steady, uniform light delivery, while the intermittent mode allows for pulsatile light application, which may be beneficial for certain therapeutic effects, including reduced heat buildup and optimized photosensitizer activation. This versatility in output modes helps tailor the therapy to specific clinical requirements. Additionally, the PDT630II is equipped with safety features such as built-in cooling systems to minimize discomfort during treatment and to protect surrounding healthy tissues from overheating. Its ergonomic design ensures ease of use for healthcare professionals, and the device is designed for durability and long-term performance. This makes the PDT630II an effective and reliable choice for clinical environments, contributing to the advancement of photodynamic therapy in modern medical treatments.

2.1.3. Major drugs

Hematoporphyrin derivative, produced by Chongqing Maylor Biopharmaceutical Co., Ltd., is a specialized photosensitizing agent commonly used in PDT. The product is typically formulated in a 5ml vial containing 25mg of the active compound, which is designed for clinical use in various therapeutic settings,

especially in oncology and dermatology. Hematoporphyrin derivatives are known for their ability to accumulate preferentially in malignant tissues and, upon exposure to specific wavelengths of light, produce reactive oxygen species that can effectively destroy cancerous or abnormal cells. The Hematoporphyrin derivative from Chongqing Maylor Biopharmaceutical Co., Ltd. is an essential component of PDT, a treatment modality that combines light energy with a photosensitizer to target and treat a variety of medical conditions, including certain types of cancer, acne, and other skin disorders. The agent is activated when exposed to light of a particular wavelength, usually in the red or near-infrared spectrum, which causes it to generate singlet oxygen and other cytotoxic agents that destroy the targeted cells. This specificity enables selective treatment of abnormal tissue while minimizing damage to healthy surrounding tissues. As with all photosensitizing agents, proper storage and handling are crucial for maintaining the efficacy and safety of the product. According to the manufacturer's instructions, the Hematoporphyrin derivative should be stored in a dark, tightly sealed container to protect it from light and degradation. Additionally, it must be kept at a temperature below 0°C to ensure the stability of the compound, preventing any premature activation or loss of effectiveness. When used correctly under the supervision of healthcare professionals, Hematoporphyrin derivative is a powerful tool in the treatment of certain cancers and other conditions requiring targeted therapy. Its controlled storage and precise application are vital for achieving optimal therapeutic outcomes and minimizing potential side effects.

3. Methods

3.1. Pre-operative examination

In this case, high-risk human papillomavirus (HR-HPV) subtypes were detected within 3 months prior to treatment using fluorescence quantitative polymerase chain reaction (qPCR). This advanced molecular technique allows for the precise identification and quantification of HR-HPV DNA, which plays a crucial role in the development of cervical dysplasia and cervical cancer. The presence of HR-HPV subtypes is a key factor in the diagnosis and management of cervical intraepithelial neoplasia (CIN), as these subtypes are strongly associated with the progression of precancerous lesions to invasive carcinoma. The patient was diagnosed with CIN 2, a moderate form of cervical dysplasia, through a combination of diagnostic methods. Colposcopy, an essential procedure for visualizing the cervix, revealed abnormal tissue changes indicative of CIN 2. A biopsy was subsequently performed under colposcopic guidance, providing tissue samples for histopathological examination, which confirmed the diagnosis of CIN 2. Additionally, PCR detection was used to identify the presence of HR-HPV DNA in the cervical tissues, further confirming the viral etiology of the lesion and the patient's risk for progression to cervical cancer. Three weeks after the biopsy and following the patient's menstrual cycle, photodynamic therapy (PDT) was administered. PDT is an effective treatment for CIN, utilizing a photosensitizing agent activated by light to selectively destroy abnormal cells. The timing of PDT, performed 3–7 days after menstruation, is critical as it allows for optimal tissue conditions and minimizes the risk of bleeding or other complications following the biopsy. This approach ensures a more favorable

treatment outcome, targeting the dysplastic cells while preserving healthy surrounding tissue.

3.2. HPV detection method

The fluorescence qPCR method was used to detect high-risk human papillomavirus (HR-HPV) subtypes, including HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 66, and 82. This method is highly sensitive and specific, allowing for the precise identification of HPV DNA within cervical or other relevant tissues. The test relies on the amplification of HPV DNA using fluorescence-labeled probes, with the fluorescence signal directly proportional to the amount of viral DNA present. The specific operational steps for conducting the fluorescence quantitative PCR are detailed in the instructions for use of the reagent kit, which provide guidelines on reagent preparation, PCR conditions, and data interpretation. The PCR process involves DNA extraction from the patient sample, followed by amplification in a real-time PCR machine. The cycle threshold (Ct) value is then determined, with a Ct value ≤ 35 considered indicative of a positive result for HR-HPV infection. A positive result for HR-HPV, especially the high-risk types listed above, is significant in the clinical management of cervical lesions, as these HPV types are closely associated with the development of CIN and cervical cancer.

3.3. PDT treatment

All patients in this study were treated on an outpatient basis. Prior to the operation, each patient received an intravenous injection of 2.0 mg/kg–2.5 mg/kg of hematoporphyrin derivative (HpD). This photosensitizing agent was administered 48 h–72 h before the photodynamic therapy (PDT) to allow sufficient time for tissue accumulation of the drug. The PDT was performed in a cystostat position, which facilitates optimal access to the lesion site while ensuring patient comfort during the procedure. **Figure 1** shows the instruments used in the treatment. Routine disinfection of the external genitalia was performed to prevent infection. No anesthesia was required, as PDT is a minimally invasive procedure. A vaginal dilator was used to gently expand the vaginal canal, ensuring that the lesion site was fully exposed to the laser for optimal treatment. The laser light was transmitted through a microlens optical fiber and applied vertically to the lesion site. The treatment parameters were carefully adjusted to ensure effective irradiation. The diameter of the irradiation light spot was set between 3 cm to 4 cm, ensuring complete coverage of the lesion. For patients with a single lesion, one light spot was applied; for those with multiple lesions, multiple light spots were used to irradiate each individual lesion site. Post-treatment, patients were advised to avoid exposure to direct strong light on the whole-body skin for 1 month following HpD administration due to the photosensitizing nature of the drug. Additionally, to minimize the risk of complications and ensure proper healing, sexual activity and heavy abdominal exercises were prohibited for 3 months after PDT. A follow-up review was conducted 3 months after PDT to assess treatment efficacy and any potential side effects. This comprehensive approach aims to maximize therapeutic outcomes while minimizing risks for the patients.

Figure 1. Photodynamic therapy apparatus.

3.4. Follow-up

The patients were followed up 3 to 6 months after PDT. The clinical efficacy was determined by histopathology of biopsy under colposcopy and HR-HPV detection. The pain degree of patients during and after the operation was recorded by the Numerical Pain Rating Scale (NPRS) system, with a score ranging from 0 to 10. The higher the score, the greater the degree of pain. At the same time, the occurrence of adverse reactions such as intraoperative, 7 days after the operation, 1 month after the operation, 3 to 6 months after the operation skin photosensitivity reaction, vaginal bleeding, infection, scar, adhesion and stenosis was recorded. Satisfaction was investigated by self-assessment of patients.

4. Observation indicators

4.1. Criteria for evaluating the therapeutic effect of HSIL

Cured: Pathological biopsy indicates no intraepithelial lesion; Effective: Pathological biopsy indicates LSIL; Ineffective: Pathological biopsy indicates HSIL or lesion progression.

$$\text{Total } effective\ rate = \frac{Number\ of\ cured\ cases + Number\ of\ effective\ cases}{Total\ number\ of\ cases} \times 100\%$$

4.2. HPV clearance criteria

Cleared: HPV test is negative; Not cleared: HPV test is positive.

$$\text{Clearance } rate = \frac{Number\ of\ complete\ clearance\ cases}{Total\ number\ of\ cases} \times 100\%$$

4.3. Satisfaction evaluation

It is divided into three grades: satisfied, relatively satisfied and not satisfied.

$$\text{Total } Satisfaction\ Rate = \frac{Satisfied\ +\ Relatively\ Satisfied\ Cases}{Total\ number\ of\ cases} \times 100\%$$

5. Statistical methods

The data obtained from this study were analyzed using SPSS 18.0 statistical software, a widely used program for statistical analysis in clinical research. SPSS was employed to perform descriptive and inferential statistical analyses, allowing for a comprehensive understanding of the treatment outcomes and side effects associated with PDT in the cohort of patients. For continuous variables, such as NPRS scores and follow-up time, the data were expressed as mean ± standard deviation (SD), and comparisons between different time points or groups were made using paired or unpaired *t*-tests, depending on the study design. This method allowed for the evaluation of changes in pain levels, vaginal discharge, and other clinical parameters before and after treatment. The normality of the data distribution was assessed using the Shapiro-Wilk test, and if the data were not normally distributed, non-parametric tests such as the Mann-Whitney U test or Wilcoxon signed-rank test were applied. Categorical variables, such as the presence or absence of adverse effects (e.g., lower abdominal pain, vaginal discharge, photosensitivity), were analyzed using the chi-square test or Fisher's exact test, where appropriate, to determine the significance of differences between different groups or follow-up times. These tests helped to identify any associations between patient characteristics, treatment-related side effects, and clinical outcomes. The statistical significance level was set at $P < 0.05$, indicating that any result with a P value below this threshold was considered statistically significant. This stringent criterion helped ensure the reliability and validity of the findings, providing a robust basis for drawing conclusions about the safety and efficacy of PDT in the treatment of CIN in the patient population studied. All analyses were performed with a two-tailed test to account for both positive and negative associations.

6. Result

6.1. Follow-up situation

All 15 patients were followed up for 3 to 6 months after undergoing a single PDT procedure. Of the 15 patients, 9 were followed up at 3 months post-operation, while 3 patients had follow-up visits at 4 months post-operation. Three additional patients were followed up 6 months after the procedure, providing a broader range of outcomes over different time periods. The median follow-up time for all patients was 3 months, as summarized in **Table 1**. The follow-up period is critical for assessing the therapeutic efficacy of PDT, monitoring any side effects, and evaluating the resolution of lesions or recurrence of CIN. During these follow-up visits, patients were assessed for any ongoing symptoms, such as vaginal discharge, abdominal pain, or photosensitivity, and underwent additional diagnostic procedures like

colposcopy and biopsy if necessary. The relatively short follow-up period of 3–6 months is typical for evaluating early treatment outcomes and provides valuable data on the short-term effectiveness and safety of PDT in treating CIN and other cervical abnormalities.

Table 1. Vaginal stump lesion histopathological outcomes table.

Case	Diseased redion	Number of lesions	Before histopathological	After histopathological	HPV situation
1	Vaginal stump	3	VaIN II-III	VaIN I	Decline
2	Vaginal stump	1	VaIN I-II	Normal	Negative
3	Vaginal stump	1	VaIN III	Normal	Negative
4	Vaginal stump	2	VaIN II-III	VaIN I	Decline
5	Vaginal stump	1	VaIN III	Normal	Negative
6	Vaginal stump	1	VaIN III	VaIN I	Constant
7	Vaginal stump	2	VaIN II-III	VaIN I	Decline
8	Vaginal stump	1	VaIN III	Normal	Negative
9	Vaginal stump	1	VaIN III	Normal	Constant
10	Vaginal stump	3	VaIN II-III	VaIN II-III	Decline
11	Vaginal stump	1	VaIN II	VaIN I	Constant
12	Vaginal stump	2	VaIN II-III	VaIN II-III	Constant
13	Vaginal stump	1	VaIN III	VaIN III	Constant
14	Vaginal stump	1	VaIN III	Normal	Negative
15	Vaginal stump	2	VaIN II-III	Normal	Decline

6.2. HSIL efficacy

All 15 patients received one PDT treatment, with 7 of them being cured, 5 showing improvement, 3 being ineffective, and the effective rate being 80%.

6.2.1. Single lesion response

Among the 9 single lesion patients, 6 were cured (66.7%), 2 was effective (22.2%), and 1 were ineffective (11.1%), resulting in an overall effective rate of 88.9%.

6.2.2. Multiple lesion response

Among the 6 patients with multiple lesions, treatment outcomes were as follows: 1 patient (16.7%) was completely cured, with all lesions resolving completely. 3 patients (50%) showed effective results, with their multiple lesions reduced to low-grade squamous intraepithelial lesions (LSIL), indicating partial improvement. However, 2 patients (33.3%) had no significant improvement, classified as ineffective treatment. Based on these results, the overall effective rate of PDT in patients with multiple lesions was 66.7%, highlighting the therapy's potential for improving CIN in a substantial proportion of patients. Although PDT demonstrated effectiveness in reducing the severity of multiple lesions in the majority of cases, a subset of patients did not experience sufficient therapeutic response, suggesting the need for further treatment strategies or interventions in these cases.

6.3. HPV Clear the situation

15 patients were treated with PDT once, with 5 being cleared and 10 not being cleared, resulting in a total clearance rate of 33.3%. Among the patients who did not clear, 5 patients (50%) showed a decrease in HPV load. **Figure 2** shows the cleanup effect.

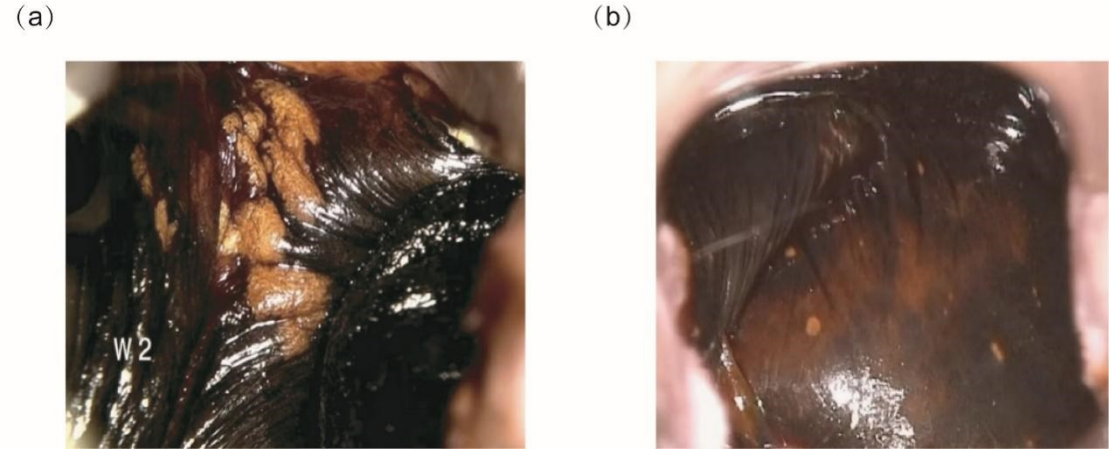

Figure 2. Before-and-after treatment comparison image.

6.4. Occurrence of adverse reactions

During PDT treatment, a cohort of 15 patients was monitored for treatment-related side effects and tolerability. Among these, 5 cases (33.3%) reported experiencing mild and tolerable lower abdominal pain, with a mean NPRS score of (0.95 ± 0.9) points. This pain was likely attributed to the localized effects of the PDT, as it is common for patients undergoing such therapies to report mild discomfort in the treatment area. The pain was not severe and was well-tolerated by most of the patients. In addition to abdominal discomfort, 10 cases (66.7%) experienced an increase in vaginal discharge during the treatment period. This increase in discharge is a known side effect of PDT, often related to the inflammatory response in the treated tissues, and persisted until the end of the treatment. The nature of this discharge was typically mucopurulent and resolved following the completion of the treatment course. Post-treatment, the majority of patients reported mild residual effects. Three patients (20%) experienced lower abdominal pain within one week after the PDT procedure, with a mean NPRS score of (0.56 ± 0.6) points. This pain was less intense compared to the initial discomfort during the therapy and resolved within a few days. Interestingly, all 15 patients continued to present with increased vaginal discharge, which is common following PDT, reflecting ongoing healing and cellular turnover in the cervix. This discharge gradually decreased and spontaneously resolved within 4 to 6 weeks after the operation. One patient (6.7%) developed a localized photosensitive reaction, likely due to inadequate light protection following the PDT procedure. The patient had not strictly adhered to the recommended avoidance of light, which can trigger photosensitivity reactions in patients treated with photosensitizing agents. However, after the patient followed the light-avoidance protocol more rigorously for an additional week, the photosensitive reaction subsided without further complications.

Importantly, no patients experienced serious adverse reactions such as bleeding, fever, infection, scarring, vaginal shortening, or stenosis, which are potential risks of invasive cervical treatments. Overall, the PDT procedure was well-tolerated, with only mild, self-limiting side effects reported, suggesting its safety and effectiveness as a treatment modality for CIN.

6.5. Satisfaction evaluation

All 15 patients participated in the satisfaction survey of PDT treatment. Among them, 10 were satisfied, 3 were relatively satisfied, and 2 were not satisfied. The total satisfaction rate was 86.7%.

7. Discussion

Photodynamic therapy (PDT) is a non-invasive and promising treatment method that utilizes the characteristic of photosensitizers accumulating selectively in abnormal tissues to treat abnormal tissues with high selectivity under the participation of light source and oxygen. Compared with traditional treatment methods, PDT has the characteristics of less trauma, preserving organ function, repeatable treatment, and high safety. Currently, PDT is mainly used in skin, mucous membrane lesions or tumors, due to its good therapeutic effects and the possibility of being combined with other treatment regimens, it has been applied in female genital warts, cervical intraepithelial neoplasia (SIL) and vulvar lesions, etc. in female reproductive tract diseases.

PDT involves two steps of drug administration and light exposure, through local or systemic administration, photosensitizers are specifically accumulated in the pathological tissue. When a specific wavelength of light is irradiated, the photosensitizer is activated, transitioning from the ground state singlet to a short-lived excited singlet [4]. The short-lived excited singlet photosensitizer can decay back to the ground state singlet through fluorescence or cross-systematically form a long-lived excited triplet [5]. The triplet photosensitizer can generate reactive oxygen species (ROS) within the tissue through Type I and Type II reactions. In Type I reactions, the triplet photosensitizer can interact directly with molecules in the tissue (i.e., lipids, proteins, DNA, and RNA) and transfer protons or electrons to form free radicals and free radical ions, which can react with oxygen to produce ROS. Excess ROS can induce cell apoptosis and necrosis [6]. In Type II reactions, the triplet photosensitizer can transfer electrons to molecular oxygen to generate singlet oxygen, which can also generate ROS. Type II reaction is based on the triplet-triplet annihilation phenomenon [7], where the energy of the triplet photosensitizer can be directly transferred to the triplet ground state oxygen, forming a singlet oxygen, which is a highly cellular toxic ROS that plays a leading role in PDT [8]. Therefore, Type II reaction is considered the main reaction in PDT.

PDT primarily destroys tumor cells through the following related mechanisms: (1) producing singlet oxygen and components with direct killing effects [8]; (2) PDT can damage the endothelial cells of the tumor vasculature and increase vascular permeability, stimulating thrombosis and leading to vascular occlusion, causing tumor tissue to undergo continuous hypoxia and cell death [9]; (3) PDT can promote

the release of inflammatory factors and mediators, activating the body's anti-tumor immune response [10].

Vaginal SIL is a type of refractory atypical squamous intraepithelial lesion that occurs in the vaginal epithelium. The vagina and cervix have a common embryonic origin, and many studies have linked HR-HPV infection with the occurrence of vaginal SIL. Laser treatment and radical surgery have good prognoses, but traditional treatment methods have long-term risks of vaginal contracture and sexual dysfunction in patients with widespread or scattered lesions, which can cause psychological damage to young women [11]. Yao et al. [12] treated 40 patients with persistent HR-HPV-infected vaginal LSIL with CO_2 laser (n = 20) or CO_2 laser combined with PDT (n = 20), and found that the complete pathological remission rates in the 2 groups were similar (65% vs. 85%); after follow-up for 1 year, the HR-HPV seroconversion rate was higher in the combined treatment group than in the CO2 laser group. Laser The combined treatment group had a higher rate of complete response (95% vs. 25%, $P < 0.001$), and no complications such as wound adhesion or scar narrowing were observed. Zhang et al. [11] treated 40 patients with surgery and 60 with ALA-PDT for HSIL of the vagina, and found that the complete response rates were similar in the 2 groups (93.3% vs. 82.5%) after follow-up for 3-6 months. The 2-year HPV clearance rates were also similar (77.55% vs. 64.52%). Similarly, other studies have shown that ALA-PDT is a feasible method for treating HR-HPV-infected patients with SIL of the vagina [13]. This indicates that PDT is an effective alternative treatment for patients with vaginal SIL, and combining it with CO2 laser therapy can eliminate the lesion while reducing the recurrence of the disease, warranting further investigation.

The results of this study show that after 1 PDT treatment for 15 patients, followed up for 3–6 months, the single lesion patients had an effective rate of 88.9%, while the multi-lesion patients had an effective rate of 66.7%. After the treatment, the patients only experienced increased vaginal discharge and tolerable pain. Some patients experienced photosensitivity, but no serious adverse reactions or complications occurred. The results indicate that PDT is a safe and effective treatment option for both single and multiple lesion HSIL of the vagina.

Currently, it is believed that HR-HPV infection is the main cause of VaIN [14]. HR-HPV infection is characterized by multifocality and multicentrality, and can simultaneously infect the cervix, vagina, and even the external genitalia. However, due to the occurrence of squamous epithelialization during the healing process after vaginal injury, if HR-HPV is infected, it is prone to multiply within cells, thereby inducing VaIN. In some studies, 59% of the patients were infected with HPV type 16 [15–17]. At the same time, the HPV load was found to be positively correlated with the severity of VaIN grading in patients with vaginal HSIL. In this study, the HR-HPV infection rate of patients with vaginal HSIL was 100%, among which the infection rate of HPV16 was 46.7%, similar to the reports in the literature. In this study, the clearance rate of HR-HPV was 26.7% after one PDT treatment within 3 to 6 months, among which the clearance rate of HPV in single lesions was 37.5%, and the clearance rate of HPV in multiple lesions was 14.3%. However, among the patients who did not clear the infection, there was 36.4% showed a decrease in HPV viral load. The results of this study indicated that PDT treatment had a significantly

higher clearance rate of HR-HPV than that of simple CO2 laser treatment (11%) [17]. However, in this study, the clearance rate of HR-HPV, whether single lesion or multiple lesions, was lower than the clearance rate of imiquimod cream treatment in the literature (63%) [18–20]. The author believes that this may be related to the multifocal nature of HR-HPV infection, that is, HR-HPV infection also exists in the vaginal wall outside the lesion area in the treatment area [21–23]. This suggests that in the future, it is necessary to collect HR-HPV samples from multiple points on the vaginal wall for confirmation; and based on this, expanding the irradiation range of PDT may increase the clearance rate of HR-HPV [24].

In conclusion, PDT treatment for vaginal HSIL is a minimally invasive, safe, and effective treatment method that can simultaneously remove the lesion and clear HR-HPV infection in some patients, improving the quality of life of patients and without obvious complications. It is worth clinical promotion and application. However, the sample size in this study is small and the follow-up period is short. Further large sample prospective randomized controlled studies and long-term follow-up are needed.

Author contributions: Conceptualization, XL and RZ; methodology, XL; software, RZ; validation, JY, XL and RZ; formal analysis, RZ; investigation, JY; resources, XL; data curation, XL; writing—original draft preparation, JY, XL; writing—review and editing, XL; visualization, XL; supervision, JY and RZ; project administration, XL; funding acquisition, XL. All authors have read and agreed to the published version of the manuscript.

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