Focused ultrasound for high-risk human papillomavirus infection-related low-grade cervical lesions: a prospective cohort study

Wenping Wanga,b, Yuqin Yaoa, Yuyuan Liua, Jiaojiao Ren, Liming Chen, Zhibiao Wanga and Honggui Zhoua

aDepartment of Gynecology, Affiliated Hospital of North Sichuan Medical College, Sichuan, China; bState Key Laboratory of Ultrasound in Medicine and Engineering, College of Biomedical Engineering, Chongqing Medical University, Chongqing, China

ABSTRACT

Objectives: To assess the efficacy and safety of focused ultrasound (FU) for high-risk human papillomavirus (HR-HPV) infection-related cervical low-grade squamous intraepithelial lesions (LSIL).

Methods: Of 185 patients who met the inclusion criteria for this prospective study from October 2020 to November 2021, 95 received FU and 90 were followed up only. At the six-month follow-up, the HR-HPV clearance and LSIL regression rates of the groups were compared and factors affecting HR-HPV clearance were analyzed. The safety and side effects of FU were evaluated.

Results: No significant difference was found in the baseline clinical data between the two groups (p > 0.05). At the six-month follow-up, the HR-HPV clearance rates were 75.6% in the FU group and 25.6% in the observation group (p = 0.000). The LSIL regression rates were 89.5% in the FU group and 56.4% in the observation group (p = 0.000). Multivariate logistic regression analysis showed that the HR-HPV clearance rate in the FU group was 9.03 times higher than that in the observation group (95% confidence interval [CI], 3.75–21.73, p = 0.000), and the clearance rate of single-type HR-HPV infections was 5.28 times higher than that of multi-type infections (95% CI, 1.83–15.23, p = 0.002). The mean intraoperative bleeding was 1.8 ± 0.6 (1–3) mL; the mean intraoperative pain score was 2.6 ± 1.0 (1–6).

Conclusions: For patients with HR-HPV infection-related histological LSIL, FU can eliminate HR-HPV infection and cause lesions to regress in a short time, with few adverse effects and good tolerance.

Introduction

Worldwide, cervical cancer is the fourth most common female malignant tumor, and in low- and middle-income countries, cervical cancer ranks second most common cancer among women [1]. Persistent high-risk human papillomavirus (HR-HPV) infection is closely associated with the occurrence and development of cervical precancerous lesions and cervical cancer [2]. Cervical low-grade squamous intraepithelial lesion (LSIL) is the histological manifestation of HPV infection [3]. Although LSIL has a high rate of spontaneous regression, women with HR-HPV infection have a longer duration of LSIL, lower rate of regression, and higher rate of progression than HR-HPV-negative women [4,5]. Moreover, positive HPV-DNA tests, abnormal cytology results, and colposcopy during follow-up result in different levels of psychological distress and financial burden to patients [6–8]. Therefore, in economically underdeveloped areas, some women diagnosed with HR-HPV infection and histological LSIL at the first visit urgently require treatment rather than observation.

At present, ablation and excision are the most commonly used modalities for the treatment of cervical intraepithelial neoplasia (CIN); however, both have their advantages and disadvantages. In the past 10 years, focused ultrasound (FU) has also been used in treating cervical lesions in China and has attracted much attention because of its unique treatment mode (thermal destruction of the target tissue at depth without damaging surrounding or overlying tissues [9]), good curative effect, and fewer side effects [10–12]. Our previous retrospective cohort study confirmed the efficacy of FU for HR-HPV infection-related low-grade cervical lesions and preliminarily analyzed the factors that affect HR-HPV clearance [13]. Owing to the limitations of retrospective studies, some bias is inevitable. Meanwhile, the baseline data of patients are incomplete, and relevant factors that affect HR-HPV clearance or persistence, such as the patient’s socioeconomic status, educational level, contraceptive methods, and past medical history [14], cannot be fully analyzed. Moreover, retrospective studies cannot be used to evaluate the safety and adverse effects of FU. Therefore, we conducted this prospective cohort study to assess the efficacy and safety of FU for HR-HPV infection-related LSIL and analyze the relevant factors that affect HR-HPV clearance.
Materials and methods

Study design

This prospective cohort study was approved by the ethics committees of Chongqing Medical University and the Affiliated Hospital of North Sichuan Medical College (Reference: 2020ER124-1) and was registered in the Chinese Clinical Trial Registry (ChiCTR2000040162). From October 2020 to November 2021, patients who visited the gynecology clinic of the Affiliated Hospital of North Sichuan Medical College, Nanchong City, Southwest China, were enrolled in this study if they met the following inclusion criteria: (1) women aged 18–55 years with a sexual history; had a confirmed HR-HPV infection by HPV-DNA test, and had negative cytological results for intraepithelial lesion or malignancy (NILM), or atypical squamous cells of undetermined significance (ASC-US), or LSIL; (2) had the entire squamocolumnar junction and all lesions fully visualized under colposcopy, and underwent cervical biopsy that revealed histological LSIL; (3) were non-pregnant and non-lactating; and (4) received no antiviral treatment 3 months before enrollment. The exclusion criteria were as follows: (1) cytological results were atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion (ASC-H), high-grade squamous intraepithelial lesion (HSIL), atypical glandular cells (AGC), or squamous cell carcinoma (SCC); or the histological results were HSIL or above; (2) underwent physical or surgical treatment of the cervix in the past year; (3) had acute inflammation of the reproductive tract, sexually transmitted diseases (STD), autoimmune diseases, and immunodeficiency diseases; (4) had severe cardiac, hepatic, renal, and coagulation dysfunctions; and (5) could not be followed up. All enrolled patients were assigned to the FU and observation groups in a ratio of 1:1 according to their wishes and signed a written informed consent form.

Based on the sample size calculation method for the superiority test, we estimated that 128 patients (given the sample allocation ratio of 1:1) are needed for this study, with a type I error, power, and loss rate of 5, 80 and 20%, respectively.

HPV testing and liquid-based cytology

HPV testing was performed using a High-Risk Human Papillomavirus Genotyping Real-Time PCR Kit (Shanghai ZJ Bio-Tech Co., Ltd. China) according to the manufacturer’s instructions. This kit tests for 15 HR-HPV genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68 and 82). LBC was performed using a ThinPrep 2000 processor (Hologic Inc., USA), and cytologists made cytological diagnoses and reports according to the 2014 Bethesda system [15].

Colposcopy and cervical biopsy

Colposcopy was performed in patients with HR-HPV infection and/or abnormal cytology results (≥ASCUS) by a professional gynecologist in a colposcopy clinic. Targeted biopsy under colposcopy was performed according to the 2019 American Society of Colposcopy and Cervical Pathology guidelines [16], and endocervical curettage was performed if necessary.

Histological diagnosis was provided by two experienced pathologists using a two-tier terminology as recommended by the Lower Anogenital Squamous Terminology Standardization Project for HPV-Associated Lesions guidelines [17].

Focused ultrasound treatment

Patients in the FU group were treated with an ultrasound therapeutic device (Model-CZF, Chongqing Haifu Medical Technology Co., Ltd. China). A trained and qualified physician performed the procedure. The patients were placed in the lithotomy position, and a sterile speculum was used to expose the cervix. The vaginal wall and cervical surface were sterilized, and the treatment probe was placed in close contact with the cervical surface using an ultrasonic coupling agent as the medium. Circular scanning was performed at a speed of 5–10 mm/s with the external cervical os as the center. Scanning can be strengthened in the cervical transformation zone and lesions. The operation lasted until the treatment area shrank and appeared slightly whitish, and the external cervical os was slightly depressed inward (Figure 1).

At the end of the procedure, the cervix was disinfected again, and the patient was kept under observation for approximately 20 min. The operation time, intraoperative blood loss, intraoperative pain score (visual analog scale [VAS] score), and other discomfort were recorded.

Follow-up

Patients were advised to avoid sexual intercourse and vaginal douches for 2 months after FU treatment. To avoid HPV reinfection, we advised that patients who enrolled in the study use condoms during sexual intercourse during follow-up. Follow-up visits were performed at 1 week, 3 months and 6 months after FU treatment. At the first follow-up, the characteristics and volume of the vaginal fluid, cervical healing, and pain scores (VAS) were recorded. At the second follow-up, vaginal discharge, cervical healing, and cervical appearance were recorded. At the third follow-up, the patient underwent HPV testing, cytology, colposcopy, and cervical biopsy under colposcopy, if necessary, and the results were recorded, as well as the patient’s vaginal discharge and cervical morphology.

Patients in the observation group were followed up irregularly through telephone interviews. Six months after enrollment, the patients were asked to return to the hospital for HPV testing, cytology, colposcopy, and cervical biopsy under colposcopy, if necessary, and the results were recorded, as well as the patient’s vaginal discharge and cervical morphology.

Definitions

The primary outcome was therapeutic efficacy, including HR-HPV clearance and LSIL regression rates. The secondary outcomes were therapeutic safety and adverse effects, including operative time, intraoperative bleeding, intraoperative pain score, postoperative vaginal discharge, postoperative pain
score, and cervical healing. HR-HPV clearance was defined as negative for all HR-HPV subtypes. The outcome of the LSIL lesions was comprehensively judged according to the results of HPV testing, cytology, colposcopy, and histology. We classified HR-HPV infections into four types [13,18]: single type infection with HPV16 or 18 (type I), co-infection with HPV16/18 (type II), single type infection without HPV16 or 18 (type III), and co-infection without HPV16/18 (type IV). The four types were combined and analyzed according to the presence or absence of HPV16/18 infection, types I and II were combined into HPV16/18 infection, and types III and IV were combined into non-HPV16/18 infection; according to whether they were single-type or multiple-type infections, types I and III were combined into single-type HR-HPV infection, and types II and IV were combined into multi-type HR-HPV infection.

Statistical analysis

SPSS version 22.0 (IBM Corp., Armonk, NY, USA) was used for statistical analysis. Continuous variables with a normal distribution are described as mean ± standard deviation, and the independent-sample t-test was used for analysis. Continuous variables with a skewed distribution are presented as the median (range), and nonparametric tests (Wilcoxon signed-rank test or Mann–Whitney U-test) were used for analysis. Categorical variables are expressed as frequency (percentage), and Pearson’s chi-square test or Fisher’s exact test was used for analysis. Binary logistic regression was used to calculate the odds ratios (ORs) with 95% confidence intervals (CIs) to evaluate the association between the variables and HR-HPV clearance. All tests were two-sided, with p < 0.05 considered significant.

Results

In total, 185 patients were enrolled in the study, with 95 and 90 patients in the FU and observation groups, respectively. At the 6-month follow-up, nine patients in the FU group and 12 in the observation group were lost, with a total loss rate of 11.4% (21/185) (Figure 2).

Demographic characteristics

As shown in Table 1, no significant differences were found in age, age at first sexual intercourse (sexarche), number of sexual partners, gravidity, parity, delivery mode, menarche age, or number of menopausal patients between the two groups (p > 0.05). A significant difference was found in the contraceptive methods between the two groups (p = 0.026).

Socioeconomic status

Educational categories were classified into three levels according to the Chinese school system: low (primary school or below), intermediate (junior or senior high school), and high (college or undergraduate and above). We divided monthly household income into three levels according to local conditions: low (<5,000 yuan), medium (5,000–10,000 yuan), and high (>10,000 yuan). Marital status was defined as unmarried, married, or divorced. The socioeconomic characteristics of the patients in the two groups are listed in Table 2, which shows no significant difference between the two groups (p > 0.05).

Medical history characteristics

We mainly focused on the past history of cervical lesions and the family history of gynecological malignant tumors. Only two patients in the study cohort were occasional smokers, and there were no heavy drinkers; therefore, tobacco and alcohol use were not included in the analysis. As shown in Table 3, no significant difference was noted in the past or family history between the two groups (p > 0.05).

HPV test results at enrollment

The HR-HPV subtype with the highest infection rate in the study cohort was HPV52 (34.8%), followed by HPV16 (23.8%), and HPV51 (11.0%). The distribution of HR-HPV infection types in the two groups was as follows: In the FU group, the highest proportion was type III (52.3%), followed by types I (23.3%), IV (14.0%), and II (10.5%). In the observation group, the highest proportion was type III (57.7%), followed by
types I (21.8%), IV (14.1%), and II (6.4%). No significant difference was found in the distribution of HR-HPV infection types between the two groups ($p = 0.781$).

**Follow-up outcome**

HR-HPV outcomes at the 6-month follow-up: Among the 86 patients in the FU group, 65 were cleared of HR-HPV, with a clearance rate of 75.6%, whereas 20 of the 78 patients in the observation group were cleared of HR-HPV, with a clearance rate of 25.6%. A significant difference was found in the HR-HPV clearance rate between the two groups ($p = 0.000$).

LSIL lesion outcomes at the 6-month follow-up: In the FU group, lesions regressed in 77 (89.5%) patients and persisted in 9 (10.5%) patients, whereas in the observation group, lesions regressed in 44 (56.4%) patients, 33 (42.3%) patients had persistent lesions, and 1 (1.3%) patient had lesion...
progression (cervical biopsy confirmed histological HSIL), and a significant difference was found in the LSIL regression rate between the two groups (p = 0.000).

**Logistic regression analysis**

We used univariate analysis to compare the clinical data (29 variables including demographic characteristics, socioeconomic status, medical history characteristics, type of HPV infection, vaginal discharge, etc.) between the HR-HPV-cleared group and the HR-HPV-non-cleared group to find relevant variables for regression analysis. These significant variables in the univariate analysis and covariates considered clinically influential were then analyzed using binary logistic regression to identify significant variables affecting HR-HPV clearance.

The results of binary logistic regression analysis are presented in Table 4. Multivariate logistic regression analysis showed that the intervention methods and HPV infection types had significant effects on the clearance rate of HR-HPV infection (p < 0.05). The HR-HPV clearance rate in the FU group was 9.03 (95% CI, 3.75–21.73) times higher than that in the observation group, and the clearance rate of single-type HR-HPV infection was 5.28 (95% CI, 1.83–15.23) times higher than that of multi-type HR-HPV infection. In addition, the comparison of contraceptive methods between the HR-HPV-cleared and non-cleared groups is presented in Table 5, which shows no significant difference between the two groups (p = 0.928).

**Subgroup analysis of FU group**

In the FU group, 82 (86.3%) patients were treated for 3–5 min, 7 (7.4%) were treated for <3 min, 5 (5.3%) were treated for 6–8 min, and only 1 (1.1%) was treated for 9–11 min. The average amount of intraoperative bleeding was 1.8 ± 0.6 (1–3) mL, and the average intraoperative pain score was 2.6 ± 1.0 (1–6) points. One (1.1%) patient experienced dizziness, 1 (1.1%) experienced nausea during the operation, and the other 93 (97.9%) experienced no other discomfort during the operation.

At the 1-week follow-up, 87 (91.6%) patients had small amounts of vaginal fluid (<1/2 of the menstrual volume), and none of the patients had heavy vaginal fluid (≥menstrual volume). Ninety (94.7%) patients had good cervical healing (the cervical treatment area contracted without bleeding), and none of the patients had poor cervical healing (active bleeding or purulent secretion attachment in the cervical treatment area). Moreover, 93 (97.9%) patients had a pain score of 0 and 2 (2.1%) patients had a pain score of 1. One patient was lost at the 3-month follow-up, and the remaining 94 (100.0%) patients had normal cervical morphology.
previous studies. Li et al. [11] reported that FU was used to treat patients with recurrent cervicitis and CIN1 with HR-HPV infections. At the 6-month follow-up, the regression rate of lesions was 75% (15/20), and the regression rate of lesions was 80% (16/20). Fu et al. [10] applied FU to treat patients with CIN1, including 19 patients with HR-HPV infection, 5 with low-risk human papillomavirus (LR-HPV) infection, 4 with mixed infection, and 2 who were HPV negative. At the 3-month follow-up, the HPV negative rate was 85.71% (24/28), and the CIN1 lesion regression rate was 83.33% (25/30). Several studies have confirmed that LR-HPV infection is easier to clear than HR-HPV infection [19,20], which may explain the higher HPV-negative rate reported by Fu et al.

As regards FU efficacy, our results showed that FU was not inferior to other modalities. Starks et al. [21] treated 291 HR-HPV-infected Mexican women with cryotherapy, and the HR-HPV-negative rate was 68% at the 6-month follow-up. Banerjee et al. [22] conducted a randomized controlled trial to compare the efficacy and safety of cryotherapy and thermal ablation in the treatment of CIN. The mean follow-up duration was 11 months. The results showed that the regression rates of CIN1 in the cryotherapy and thermal ablation groups were 74.1 and 81.0%, respectively. Moreover, our results showed that the FU treatment time was short (average time of 3–5 min), the intraoperative blood loss was minimal (1.8 ± 0.6 ml), and patients experienced only mild pain (pain score 2.6 ± 1.0), indicating that FU therapy was safe and well tolerated. The most

Discussion

In this prospective study, we evaluated the efficacy and safety of FU in the treatment of HR-HPV infection-related histological LSIL. We compared the clinical baseline data of the patients in the FU and control groups. The results showed no significant difference in the clinical baseline characteristics between the two groups, except for contraceptive methods. However, we did not find a significant correlation between contraceptive methods and HR-HPV clearance in the univariate analysis ($p = 0.928$). The comparison of baseline data indicated that although we adopted a cohort design according to clinical practice, there were relatively strict inclusion and exclusion criteria and trial structure that ensured the homogeneity of research subjects and the balance between groups to minimize bias in the research results.

Our primary outcomes were HR-HPV clearance and regression of LSIL lesions. Our findings are consistent with those of previous studies. Li et al. [11] reported that FU was used to treat patients with recurrent cervicitis and CIN1 with HR-HPV infections. At the 6-month follow-up, the negative rate of HR-HPV was 75% (15/20), and the regression rate of lesions was 80% (16/20). Fu et al. [10] applied FU to treat patients
common adverse effect was a small amount of vaginal fluid after treatment. Most patients showed good cervical healing after treatment, and no other serious adverse effects occurred. Our results are consistent with those of another study. Chen et al. [12] found that the efficacy of FU in the treatment of symptomatic cervical ectopy was equivalent to that of laser therapy, but the incidence of side effects was significantly lower than that of laser therapy. These findings suggest that FU represents a promising novel and noninvasive therapy for cervical lesions.

We also analyzed factors that affect HR-HPV clearance using binary logistic regression. Based on the nature of this prospective study, we comprehensively collected baseline characteristics of the patients. Multivariate logistic regression analysis revealed that only intervention methods and HR-HPV infection types had a significant effect on the clearance rate of HR-HPV infection. This result is consistent with that of our retrospective study and other studies [13]. Hu et al. [23] studied the factors related to HPV persistence or clearance after 5-aminolevulinic acid-based photodynamic therapy in patients with genital warts and found that single-type HPV infection was easier to clear. Cang et al. [24] also studied the efficacy of photodynamic therapy for HR-HPV-positive patients and found that the clearance rate of single-type HPV infection was higher than that of multi-type HPV infection; HPV16/18 infection was associated with a significantly higher clearance rate. However, we did not observe a significant correlation between HPV16/18 infection and HR-HPV clearance. These studies have suggested that surveillance should be strengthened in patients with multi-type HR-HPV infection. In both our retrospective and prospective studies of low-grade cervical lesions, HPV52 was the most common infection subtype, and single-type HR-HPV infection was more common than multi-type HR-HPV infection. These results are consistent with those in the existing literature [25–28].

This study has a few limitations. First, it was a single-center study and lacked a direct comparison between FU and other treatment modalities. Second, the effect of FU therapy on the fertility of patients requires a longer follow-up. Therefore, we plan to conduct a randomized controlled trial to compare the advantages and disadvantages of FU with other treatment modalities. In addition, 18 nulliparous women and women with fertility intention in this study will be the subjects of a long-term follow-up.

In conclusion, we conducted a prospective cohort study to explore the efficacy and safety of FU in the treatment of HR-HPV infection-related histological LSIL and analyzed the factors associated with HR-HPV clearance. The results showed that FU therapy can eliminate HR-HPV infection and cause LSIL lesions to regress in a short period, with few adverse effects, providing a better choice for patients who urgently require treatment.

**Author contributions**

Honggui Zhou and Zhibiao Wang conceived and designed the study. Wenping Wang, Yuqin Yao, Yujuan Liu, Jiaojiao Ren and Liming Chen performed the clinical and experimental work related to the study. Wenping Wang performed the data analyses and wrote the initial draft. Honggui Zhou reviewed and edited the manuscript. All authors read and approved the final manuscript.

**Disclosure statement**

No potential conflict of interest was reported by the author(s).

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**ORCID**

Wenping Wang  http://orcid.org/0000-0002-0775-5890

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