Original Article

Reproductive and Developmental Medicine®

Focused ultrasound ablation for women with cervical lesions: protocol of a randomized controlled trial

Li Yang[1,2,3,4], Zhao-Xin Wang[2,3,4], Yuan-Hang Zhu[2,3,4], Yang Bai[2,3,4], Yu-Ligh Liou[5], Qian Li[2,3,4], Zhong-Na Shi[2,3,4], Qiu-Ling Shi[1,6,*], Chen-Chen Ren[2,3,4,*]

Abstract

Objective: Persistent high-risk human papillomavirus (HPV) infection causes precancerous cervical lesions with subsequent progression to cervical cancer. HPV infection is very common in women of childbearing age, particularly with early onset of sexual activity. Treatments such as cold knife conization are usually used for women of childbearing age with cervical intraepithelial neoplasia 2+, which may cause delayed fertility, cervical insufficiency, and other adverse pregnancy outcomes during subsequent pregnancies.

Methods: Focused ultrasound (FUS) is a non-ionizing, non-invasive treatment method used to treat gynecological conditions such as cervicitis and vulvovaginal disease.

Results: A protocol has been developed to evaluate the value of FUS in the treatment of women of childbearing age with cervical lesions with fertility preservation.

Conclusion: This study aimed to evaluate the therapeutic effect of FUS treatment and fertility protection in women of childbearing age with cervical lesions who require family planning.

Trial registration number: Chinese Clinical Trial Registry (ChiCTR2300076739).

Keywords: Focused ultrasound ablation, Fertility, Cervical lesions, Protocol

Introduction

Cervical cancer is the fourth most common gynecologic cancer and commonly develops secondary to infection with high-risk human papillomavirus (HPV), with over half a million women diagnosed annually[1,2]. HPV infections are usually cleared in approximately 90% of those infected within 2 years, and persistent infection with high-risk genotypes can lead to cervical dysplasia, also known as cervical intraepithelial neoplasia (CIN)[3,4]. Patients diagnosed with CIN 1 have a 1% probability of developing invasive carcinoma[5]. Untreated CIN 1 lesions may progress to CIN 2/3 within 2–3 years of HR-HPV infection[6], and approximately 5% of patients with CIN 2/3 progress to invasive cervical cancer[7]. Timely treatment of CIN can prevent its progression to cervical cancer[8].

Currently, there are three main treatments for high-grade CIN: ablative treatment methods (thermal ablation and cryotherapy) or laser excision, loop electrosurgical excision procedure (LEEP; including large loop excision of the transformation zone [TZ] or cone biopsy with loop excision), and cold knife conization (CKC)[9]. Excisional treatment modalities, including LEEP and CKC, are feasible, effective, and have good post-operative outcomes. However, they are at risk of pregnancy-related complications such as cervical insufficiency, premature fetal membrane rupture, and preterm labor, and may add to the psychological burden on women of childbearing age[9,10]. Classical ablative treatment measures, such as cryotherapy, have fewer pregnancy-related complications. Therefore, for the histological diagnosis of CIN 2–3, the World Health Organization (WHO) guidelines recommend ablative or excisional treatment[11]. However, large post-operative vaginal discharges are disturbing and require a steady supply of liquid nitrogen or carbon dioxide[12]. This indicates that treatments with fewer side effects are needed for CIN 2–3.

With the launch of the three-child policy in 2021, fertility protection in China has become increasingly important[13,14]. Genital HPV infection is common and has a peak prevalence

Li Yang and Zhao-Xin Wang contributed equally as the co-first authors.

[1]State Key Laboratory of Ultrasound in Medicine and Engineering, College of Biomedical Engineering, Chongqing Medical University, Chongqing 400016, China; [2]Department of Gynecology, The Third Affiliated Hospital of Zhengzhou University, Zhengzhou 450052, China; [3]National Clinical Research Center for Obstetrics and Gynecology, Henan Branch, Zhengzhou 450052, China; [4]Zhengzhou Key Laboratory of Cervical Disease, Zhengzhou 450052, China; [5]Clinical Precision Medicine Research Center, The First Affiliated Hospital of Guangdong Pharmaceutical University, Guangzhou 510062, China; [6]School of Public Health, Chongqing Medical University, Chongqing 400016, China

**Corresponding to: Qiu-Ling Shi, Chongqing Medical University, No.74 Linjiang Road, Yuzhong District, Chongqing 400016, China. E-mail: qshi@cqmu.edu.cn; Chen-Chen Ren, The Third Affiliated Hospital of Zhengzhou University, No. 7, Front Kangfu Street, Zhengzhou, Henan 450052, China. E-mail: renchenchen1106@126.com*

Reproductive and Developmental Medicine (2026) 10:1

***Received:** 29 November 2023 **Accepted:** 1 April 2025*

http://dx.doi.org/10.1097/RD9.0000000000000132

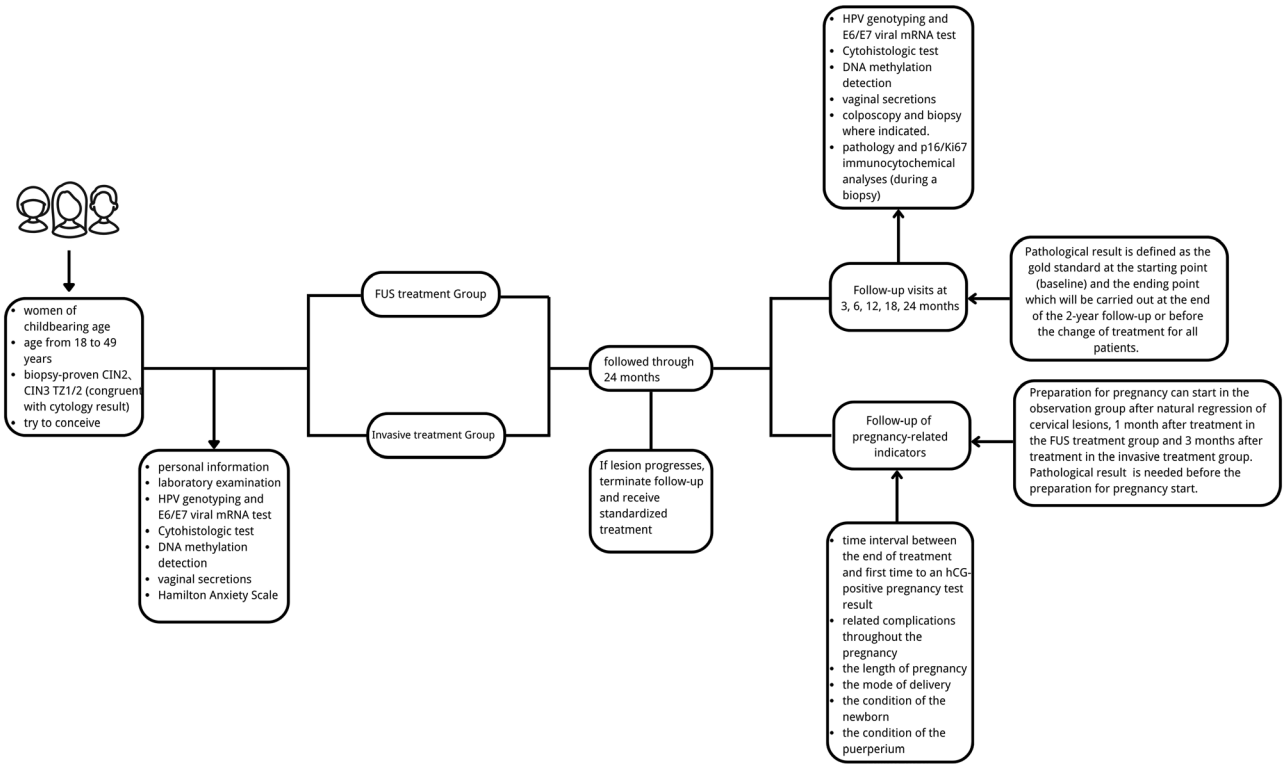

Fig. 1. Flow diagram of the study follow-up. FUS: focused ultrasound; hCG: human chorionic gonadotropin; HPV: human papillomavirus.

between the ages of 18 and 30[15]. The infection may clear spontaneously, and CIN 2 may regress to CIN 1 or normal without destructive treatment in most young women of childbearing age who are infected with HPV[16,17]. Most studies of CIN 2 regression have focused on waiting for regression strategies. However, the choice between waiting 1 and 2 years for CIN 2 to return to normal, taking the risk of preparing for pregnancy, or undergoing treatment is difficult for women of childbearing age who want to have children[18].

Focused ultrasound (FUS) is a combination of modern engineering physics with medical biology. The ultrasound has strong penetrating, directional, focusable characteristics, and can be emitted outside the body through the soft tissue and focus on the lesion. The local tissue temperature can instantly rise to more than 60°C, which will lead to irreversible cell death and coagulation necrosis, effectively removing the lesion without damaging the surrounding normal tissue. FUS is also widely used to treat gynecological conditions, such as cervicitis and vulvovaginal disease[19]. In 2009, Li *et al.*[20] first reported that FUS may be a safe, effective, and feasible treatment for HPV-infected cervicitis. Fu *et al.*[21] applied FUS to treat CIN 1 with an overall efficiency of 90% and an HPV clearance rate of 85.71%. These reported treatment outcomes are highly satisfactory; in 2021, Zeng *et al.* reported that FUS is an effective and safe therapy for treating CIN, which could improve the local immune milieu of the cervix to some extent. The authors analyzed the results of 154 patients and the efficacy rate at 3–6 months was 96.8%. The recurrence rate at 6–12 months was 2.0%. The HPV eradication rate of HPV was 72.4% at 3–6 months and 81.0% at 6–12 months. No severe adverse reactions or complications were observed[22].

Based on this concept, we aimed to establish a prospective cohort study to evaluate the efficacy and fertility preservation value of FUS for the treatment of cervical lesions in women over a follow-up of 2 years. We will include women of childbearing age (18–49 years old) with cervical lesions (CIN 2 and CIN 3 with TZ 1 and 2) who are planning to become pregnant as our study population. We will follow-up at 0, 3, 6, 12, 18, and 24 months after treatment, including routine tests (HPV testing, cytology, DNA methylation test, and colposcopy). These factors are potential predictors of disease progression. In this study, we aimed to evaluate whether FUS treatment has a good therapeutic effect and fertility protection for women of childbearing age with cervical lesions who require family planning.

Materials and methods

Study design and setting

Protocol and setting

This randomized controlled trial with a 2-year follow-up was conducted at The Third Affiliated Hospital of Zhengzhou University, China. This study was approved by the Institutional Review Board of The Third Affiliated Hospital of Zhengzhou University (2023-001). All patients will read and sign a written informed consent form before enrollment. All procedures involving human participants performed in this study are in accordance with the ethical standards of the institutional and National Research Committee and the 1964 *Declaration of Helsinki* and its later amendments or comparable ethical standards.

The data used in this study will be collected from patients who meet the following criteria: (1) age >18 years with histological CIN 2 and CIN 3 with TZ 1 and 2; and (2) family planning is required. The exclusion criteria are as follows: pregnancy or lactation, cervical conization, LEEP and CKC, vaginal lavage within 24 hours, and clinically observed acute or subacute inflammation of the cervix or vagina (Fig. 1).

Date collection schedule

Procedure	Visit 1 Baseline	Visit 2 Month 3	Visit 3 Month 6	Visit 4 Month 12	Visit 5 Month 18	Visit 6 Month 24	Visit 7 *	Visit 8 *
Eligibility criteria	●							
Research participants' consent	●							
Personal information	●							
Psychological scales	●							
Laboratory examination	●							
HPV genotyping and E6/E7 viral mRNA test	●	●	●	●	●	●	●	●
Liquid–based cytology test	●	●	●	●	●	●	●	●
DNA methylation detection	●	●	●	●	●	●	●	●
Colposcopy	●	●	●	●	●	●	●	●
Cervical biopsy*	●	○	○	○	○	●	●	●
Pathology and p16/Ki67 immunocytochemical analyses	●	○	○	○	○	●	●	●
Vaginal secretions	●	●	●	●	●	●	●	●

Fig. 2. Data collection schedule. Solid black dots indicate examinations performed during the follow-up visit. Hollow dots indicate examinations performed at the follow-up visit, as appropriate. Relevant tests for the evaluation of cervical lesions such as the TCT or HPV test are suspended during pregnancy. Relevant tests, including cervical biopsy, are performed before starting the preparation for pregnancy (Visit 7*) and separately after delivery (Visit 8*). HPV: human papillomavirus; TCT: ThinPrep cytologic test.

Treatment scheme and endpoint setting

Patients will be randomly divided into two groups, each with a different treatment method. The treatment methods included (1) FUS treatment and (2) invasive treatment. The baseline and follow-up visits are planned before the start of treatment and at 3, 6, 12, 18, and 24 months after treatment. The FUS treatment group will include patients who underwent FUS to destroy abnormal tissues. The invasive treatment group, namely resection, will include patients who underwent LEEP or CKC surgery to remove abnormal tissues. If progression of the lesion is found during follow-up, it will be terminated and standardized treatment will begin.

A change in treatment refers to the conversion of the current treatment plan (FUS or invasive treatment) to a different treatment plan or observation before or after completing the specified treatment and is defined as the period from the date of the first treatment regimen to the date of switching to another treatment regimen during the study period. All test results and the final histopathological result of colposcopy-guided biopsy must be performed as the final result of the previous treatment or at the end of the 2-year follow-up period.

Participants may begin preparing for pregnancy if their colposcopy (pathology), TCT, and HPV test results are negative after treatment. Natural pregnancy or artificial reproduction will be performed according to the patient's wishes, follow-up is close to being terminated, and all retests were evaluated 3–6 months after birth.

Data collection

The data collection protocol will be implemented according to the checklist shown in Fig. 2. All data will be obtained by the physician responsible for data collection. The participants will undergo a full physical examination and basic information collection. A psychological scale assessment will be completed through face-to-face interviews. All test results (HPV test,

thin-layer liquid cytology test, colposcopy, and pathology) will be obtained from recent medical records. Follow-up visits will take place at 3, 6, 12, 18, and 24 months after treatment.

Measurements

Baseline

The baseline data collected will be as follows: (1) personal information from medical records and health interviews; (2) laboratory examination (Fig. 2); (3) treatment scheme (treatment method and date); and (4) relevant psychological scales (such as fear and anxiety).

Personal information includes age, occupation, menstrual history (menarche/menopause), marital history, educational level, annual income, and race. Life and habit histories include sex (number of sex partners, frequency, and contraceptive methods), smoking, alcohol consumption, and hygiene habits. Birth history includes the times of gravidity, births, abortion, and the degree of expectation for the next birth. Gynecological examinations will be performed to assess the vulva, vagina, cervix, vaginal cleanliness, and venereal diseases.

Outcome measures

The primary outcome measures include pregnancy-related indicators. The secondary outcome measures include inspection indicators.

Pregnancy-related indicators

Pregnancy preparation will start 1 month after treatment in the FUS group and 3 months after treatment in the invasive treatment group. Colposcopy and cervical biopsy are required for all participants before they can start preparing for pregnancy. Follow-up of pregnancy-related indicators is also required, recording the time from treatment to the first positive human chorionic gonadotropin (hCG) test, any related complications throughout pregnancy, length of pregnancy, mode of delivery, and condition of the newborn and puerperium.

Inspection indicators

All eligible patients will undergo gynecological examinations. Clinical characteristics and laboratory examinations during all non-menstrual periods will be performed and assessed at baseline and 3, 6, 12, 18, and 24 months after treatment. Pathological results are defined as the gold standard at the starting point (baseline) and end point, which will be carried out at the end of the 2-year follow-up or before the change in treatment for all patients. All tests will also be performed at the start (baseline) and end points. HPV DNA test, HPV E6/E7 mRNA test, $PAX1^m$, and $JAM3^m$ detection, colposcopy, liquid-based cytology, vaginal microecological assessment, pathology, and p16/Ki67 immunocytochemical analyses (during biopsy) will be performed at baseline and post-treatment follow-up.

Quality control

At the time of patient enrollment, all pathological sections will be reviewed by a pathologist at our hospital. Diagnosis will be confirmed by routine pathology by two senior pathologists at each subsequent follow-up visit. All medical procedures will be performed by certified and well-trained doctors.

Data management and analysis

All participants will be assigned a PID code at the time of enrollment, which will be used in samples and documents during the 2-year study period. All data will be collected and managed using a firewall-protected electronic data management platform at Chongqing Medical University. Data on the platform can only be accessed by authorized researchers using private accounts and passwords. Any changes will be automatically recorded in the platform log and saved as separate files for data monitoring. For the data export process, de-identification of patient health information will be performed in accordance with the Health Insurance Portability and Accountability Act (HIPAA) rule. An independent security monitoring committee will be established at the start of the study and will monitor security throughout the study period. The data will be double-checked for incorrect or missing values.

Data analysis will mainly focus on pregnancy-related and inspection indicators. Pregnancy-related indicators, pregnancy outcomes, complications, and neonatal outcomes are the primary evaluation indices. Pathological results are the main evaluation indices for the inspection indicators. These data are qualitative, and each item will be assigned a value during the analysis. Data will be analyzed using SAS V.9.1.3 (SAS Institute Inc., Cary, NC, USA).

Therapeutic method

FUS treatment group

FUS treatment will be performed using an Ultrasound Therapeutic Device (Model CZF, Chongqing Haifu Medical Technology, Chongqing, China) with a therapeutic power of 3.5–4.5 W, working frequency of 9.8 MHz, and impulse of 1000 Hz. Therapeutic procedures will be performed by certified doctors trained in FUS treatment technology. Patients will be requested to remain in the lithotomy position. The cervix will be sterilized and fully exposed to the speculum. An ultrasonic coupling gel will be applied to the surface of the cervix before treatment. The treatment probe will be placed in close contact with the cervix and moved around continuously with the cervix as the center and against the skin over the diseased area with 2 mm more healthy tissue at a speed of 5–10 mm/s using a uniform linear or circular irradiation mode. The treatment will be continued until the lesion presents as a depressed area and the external cervical aperture is moderately introverted.

Invasive treatment group

The lesions around and under the cervical TZ will be removed under colposcopic guidance. Depending on the lesion range, loop electrodes of different diameters will be used. The resection range will include 3 mm of the normal tissue surrounding the TZ. Excision will be performed using 3.8 MHz high-frequency waves in a clockwise direction. All patients will receive a single treatment session. Three types of cervical conization require excision of the entire TZ and part of the cervical canal above the squamocolumnar junction (SCJ). Type I resection is usually used for the type 1 TZ, with a resection depth of 7–10 mm. Type II resection is used for the type 2 TZ, with a resection depth of 10–15 mm. The type of cervical conization will be selected based on colposcopic findings and cervical ultrasound status. All specimens will be analyzed by gynecologic pathologists in our pathology department.

Detection and diagnosis methods

Cytohistologic test

The cervical brush will be inserted into the cervix and swirled clockwise five to six times. Exfoliated cells from the cervical orifice and canal will be collected and preserved in PreservCyt solution (Hologic, Bedford, MA, USA). The cytopathological results will be classified based on the 2014 Bethesda System[18]. The reports will be interpreted as negative for intraepithelial lesions or malignancy (NILM), atypical squamous cells of undetermined significance (ASCUS), low-grade squamous intraepithelial lesions (LSIL), high-grade squamous intraepithelial lesions (HSIL), squamous cell carcinoma (SCC), and adenocarcinoma (ADC). The diagnostic result of NILM is considered normal, and positive results of ASCUS, LSIL, HSIL, SCC, and ADC are considered abnormal.

HPV genotyping and E6/E7 viral mRNA test

The Cobas HPV test (Roche, Basel, Switzerland) will be used with the same specimens collected from the PreservCyt solution. HPV16, 18 and other 12 types (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) will be detected using the Cobas HPV test with a polymerase chain reaction system following the manufacturer's protocol. E6/E7 viral mRNA will be detected using the Aptima HPV Assay Test (Hologic, Bedford, MA, USA) with the Panther system following the manufacturer's instructions.

PAX1 and JAM3 methylation ($PAX1^m$ and $JAM3^m$) detection

Methylation detection will be performed in a certified DNA laboratory, and the operators and staff will be blinded to the clinical information, including cytology, HPV genotyping, colposcopy, and pathological results. *PAX1* and *JAM3* methylation will be detected using the same specimens collected in the PreservCyt solution. Genomic DNA (gDNA) will be extracted from the exfoliated cervical samples using a JH-DNA Isolation and Purification Kit (OriginPoly Bio-Tec, Beijing, China) following the manufacturer's instructions. DNA concentration will be quantified using a NanoDrop 2000c spectrophotometer (Thermo Fisher Scientific, DE, USA). Briefly, 200–1000 ng of gDNA will be subjected to bisulfite conversion using a JH-DNA Methylation-Lightning MagPrep (OriginPoly Bio-Tec). Subsequently, the levels of $PAX1^m$ and $JAM3^m$ will be determined using the DNA Methylation Detection Kit for Cervical Cancer (Real-time PCR) with glyceraldehyde-3- phosphate dehydrogenase (GAPDH) as an internal control (OriginPoly Bio-Tec) on an ABI 7500 Real-time PCR System platform (Life Technology, Foster City, CA, USA) following the manufacturer's instructions. The hypermethylation level of the PAX1 gene will be determined based on the difference between the two Cp values (ΔCpP = Cp*PAX1* – CpGAPDH and ΔCpJ = Cp*JAM3* – CpGAPDH). A positive result of Ciscer methylation test ($Ciscer^m$) is defined as ΔCpP ≤6.6 or ΔCtJ ≤10.

Colposcopy and histology

Colposcopic examination (3mL LED; Leisegang, Berlin, Germany) will be performed by a qualified colposcopy physician. A 5% acetic acid and 5% Lugol's iodine solution will be applied to visualize the cervical lesions. Cervical TZ type will be determined by the physician according to the classification of TZ underlined by the International Federation for Cervical Pathology and Colposcopy (IFCPC)[19]. Cervical biopsies will be obtained from the suspicious lesion areas or random biopsies will be performed in the four quadrants of the cervix if no suspected lesion is found. Endocervical curettage (ECC) will be performed on the cervix with the TZ 3[20]. Colposcopy-directed biopsies will be performed to obtain histological results, according to standard procedures in China. Biopsy specimens will be histologically classified as normal, CIN 1, CIN 2, CIN 3, cervical carcinoma *in situ* (CIS), SCC, or adenocarcinoma (ADC), according to the international criteria. The pathological investigations will be performed by two expert pathologists.

Immunohistochemistry

Formalin-fixed paraffin-embedded (FFPE) tissue samples from primary tumors will be obtained for further immunohistochemical analysis. In addition, hematoxylin and eosin (HE) staining will be performed for each sample for morphological control using a standard protocol. After omitting the first three 10 µm sections of the FFPE-block, consecutive 4 µm sections will be prepared, transferred onto microscope slides, and dried in an incubator at 37°C overnight. Upon deparaffinization, heat-induced epitope retrieval will be performed in 10 mM citrate buffer (pH 6.0) and unspecific protein binding sites will be blocked by incubation in 200 mL phosphate-buffered saline (PBS) and 6 g bovine serum albumin (BSA) at pH 7.2 for 30 minutes at room temperature. Immunohistochemical staining targeting p16 and Ki67 will be performed using a modified manufacturer's protocol.

Vaginal secretions

A speculum without lubricant will be used. Secretions from the mucosa of the vaginal canal will be collected using a sterile pipette or swab. The swab will be placed back into the culture sleeve or viral transport medium. Swabs will be collected separately for each test. The Nugent scoring method examines the morphology of four dominant bacteria in vaginal secretion smears after Gram staining and counts *Lactobacillus*, *Gardnerella vaginalis*/*Prevosa vaginalis*, and *Camphobacter flextobacter*. The total value of each bacterial group will be calculated as the sum of their scores using a semi-quantitative evaluation method. The total score ranges from 0 to 3 as negative, 4–6 as intermediately suspected, and 7–10 as positive. In addition, a 16S rRNA gene assay or vaginal secretion real-time PCR assay will be used to detect the vaginal microbiota.

Psychological scales

General symptoms of anxiety and depression will be assessed using the clinician-rated Hamilton Anxiety Rating Scale and the Hamilton Rating Scale for Depression, in accordance with the Structured Interview Guide for the Hamilton Anxiety (HAMA) and Depression (HAMD) Rating Scale.

Discussion

To the best of our knowledge, there is no published real-world data regarding patients' treatment patterns after being diagnosed with CIN 2–3. This study focused on the characteristics of clinical outcomes in different treatment groups and the relationship between decision-making and follow-up indicators. Hence, this study was able to evaluate whether the patient's decision-making process was suitable for patients with CIN 2–3. This will also allow us to identify an effective method to assess the prognosis

of patients, such as HPV methylation testing. Ultrasound is a mechanical wave with good tissue penetration, positioning, and energy deposition properties. FUS therapy is a new technology for the non-invasive treatment of lesions in the skin and mucosal tissue without surgical incision, disruption, or puncture. It can focus the ultrasound emitted outside the body through the epidermis or mucosal epithelium in the superficial dermis or submucosa of the lesioned tissue to produce a high-energy area, which will cause the lesioned tissue in the target area to absorb energy and rapidly warm up within a short period. It can also produce biochemical reactions, eventually causing the lesioned tissue to be damaged and denatured, promoting tissue reconstruction and improvement of microcirculation. At the same time, the cavitation effect produced by ultrasound causes the cell membrane to lose continuity or change its permeability, thus achieving the purpose of treatment.

The effectiveness of FUS ablation therapy in the treatment of cervical lesions was demonstrated. Zeng *et al.* studied the effectiveness of FUS treatment and found cure rates of 96.8% for LSIL and 96.6% for HSIL after FUS ablation. Singh *et al.*[23] compared FUS with other therapies and reported cure rates of 95.2%, 78%, and 66% for CIN 1, CIN 2, and CIN 3 lesions treated with cryotherapy, and 96.6%, 89.18%, and 75% for CIN 1, CIN 2, and CIN 3 lesions treated with LEEP, respectively. The overall effectiveness of FUS was similar to that of cryotherapy and the previously reported LEEP. Pinder *et al.*[12] performed a meta-analysis and reported a recurrence rate of 5.3% at 12 months after cryotherapy or LEEP, compared with 1.4% after CKC. Zeng *et al.* showed an overall recurrence rate of 2.0% for FUS at 6–12 months follow-up, which is within the acceptable range. The adverse effects of FUS were mild and tolerable and no serious complications were observed. These results demonstrated that FUS is effective and safe for the treatment of CIN.

In addition to achieving satisfactory results, FUS has several advantages in clinical practice. First, it is a minimally invasive and environmentally friendly treatment that does not involve ionizing radiation or fumes. Second, the treatment requires only a power source and ultrasound coupling agent. In addition, the 5 mm diameter of the treatment probe facilitates movement to accommodate the different shapes of the lesion. Furthermore, the treatment can be administered on an outpatient basis and does not require special anesthesia or post-operative care. Third, FUS is a non-invasive treatment that not only reduces the patient's psychological burden and anxiety compared to traditional invasive treatments such as LEEP or CKC but also avoids the complications and subsequent effects on pregnancy associated with traditional treatments by using relevant psychological scales during enrollment. It allows women of childbearing age to start preparing for pregnancy as soon as possible after treatment, reducing the waiting time for fertility caused by treatment, and preventing infertility over time. Therefore, FUS is worthy of a prospective study on fertility protection.

In the second edition of the WHO Guidelines for Screening and Treatment of Cervical Precancerous Lesions for the Prevention of Cervical Cancer, DNA methylation testing was identified as one of the three possible future cervical cancer detection methods[11]. In China, hypermethylation of the paired box gene 1 (PAX1) and junctional adhesion molecule 3 (JAM3) (PAX1m and JAM3m) is widely used for the detection of CIN 3 and was approved by the National Medical Products Administration (NMPA) to obtain a class III medical device registration certificate (no. 20233400253), resulting in fewer colposcopic referrals than cytology[24–27]. Negative baseline methylation tests in untreated women with CIN 2/3 and strong evidence of clinical regression have been reported[28]. Methylation, combined with cytology or HPV genotyping, can be used to support wait-and-see policies for women with CIN 2/3[28]. These authors not only offer methylation gene testing as an initial management approach for cervical lesions (cancer) but may also use methylation of these genes as possible biomarkers for the development or regression prospects of cervical lesions. Owing to the excellent accuracy and predictive value of HPV DNA methylation testing, it can effectively prevent missed or undetected lesion progression in enrolled patients. Therefore, in this prospective randomized cohort study, we included methylation testing as a follow-up screening tool.

When we selected the inclusion criteria for this study, we investigated the natural history of untreated high-grade diseases (CIN 2, 3). The estimated spontaneous regression rate of CIN 3 is 32%–47%, with 12%–40% progressing to invasive cancer if left untreated[29–34]. We therefore will not enroll patients with CIN 3 and TZ 3 considering the higher risk of progression to invasive cancer if left untreated. However, it appears that approximately half of the patients with CIN 2 undergo regression if left untreated[35,36]. A meta-analysis of 36 studies (including randomized trials and observational studies) of 3160 patients with CIN 2 at month 24 found that lesions regressed in 50% of the patients, persisted in 32%, and progressed to CIN 3+ in 18%[35]. Based on the earlier evidence, we will select patients with CIN 2 and CIN 3 (TZ1 and TZ2) as one of the inclusion criteria.

In recent years, fertility in the Chinese population has continued to decline, and the fertility demand for patients of childbearing age with CIN lesions has increased. Therefore, it is important to identify effective treatment methods for CIN to protect the fertility of such patients. In this study, we will not only follow-up on the usual tests required after treatment of cervical lesions but will also focus on the pregnancy status of the patients to be enrolled. CIN treatment is associated with cervical stenosis, second-trimester pregnancy loss, and preterm birth (PTB). These risks are higher with excisional procedures than with ablative procedures and increase with increasing weight and volume of the tissue removed. We aimed to evaluate the role of FUS ablation therapy in fertility preservation in women of childbearing age by comparing the pregnancy status and outcomes of the three groups of patients. Overall, we can explore treatment patterns and their association with follow-up indicators, and unravel the changing trajectory of potential factors that affect decision-making. Most importantly, new real-world insights will be provided on the role of tailored disease management in the prevention and treatment of high-grade cervical lesions. These results imply that standardized management could help reduce the prevalence of cervical cancer and protect fertility in women. These data may help inform future clinical trial designs, highlight the need for better adherence to treatment guidelines, and inform clinical decision-making.

Conclusion

This study is the first prospective cohort study to compare the effectiveness of these two treatment methods of cervical lesion treatment: FUS ablation and invasive treatment (CKC or LEEP). The study will also closely monitor the pregnancy status of patients with fertility needs and aims to confirm through follow-up studies that there is no significant difference in the outcome of cervical lesion treatment with FUS ablation compared

to the conventional invasive treatment group, that there is no negative impact on pregnancy in women of childbearing age, that there is no additional unnecessary waiting time, that pregnancy preparation can begin 1 month after treatment, and that there are fewer complications during pregnancy. We expect that this study confirms the value of ultrasound ablation for fertility protection in women of childbearing age with cervical lesions and that the development of this study protocol will provide a theoretical basis for future prospective, large-scale, multicenter studies.

Acknowledgments

None.

Author contributions

The corresponding author was the guarantor for the overall content and attests that all listed authors meet the authorship criteria and that no others meeting the criteria have been omitted. L.Y., Z.X.W., Y.H.Z., Y.B., Y.L.L., Q.L., Q.L.S., and C.C.R. conceived and designed the study. L.Y., Z.X.W., Y.H.Z., Y.B., Y.L.L., and Q.L. collected and generated the study data. All the authors contributed to the development of this manuscript, had full access to the data, and provided their final approval before submission.

Funding(s)

This study was supported by the Open Project of the State Key Laboratory of Ultrasound Medical Engineering (2023KFKT003).

Conflicts of interest

All authors declare no conflicts of interest.

Ethics approval

The present study was approved by the Third Affiliated Hospital of Zhengzhou University (2023-001).

Informed consent statement

Owing to the rigor of the methodology, it is inappropriate or impractical for patients or the general public to participate in the design, conduct, report, or dissemination plans of our study.

Data availability statement

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

References

[1] Arbyn M, Weiderpass E, Bruni L, *et al*. Estimates of incidence and mortality of cervical cancer in 2018: a worldwide analysis. Lancet Glob Health 2019;8(2):e191–e203. doi:10.1016/S2214-109X(19)30482-6.
[2] Mougin C, Dalstein V, Prétet JL, *et al*. Epidemiology of cervical papillomavirus infections. Recent knowledge. Presse Med 2001;30(20):1017–1023.
[3] Moscicki AB, Schiffman M, Kjaer S, *et al*. Chapter 5: Updating the natural history of HPV and anogenital cancer. Vaccine 2006;24(Suppl 3):S42–S51. doi:10.1016/j.vaccine.2006.06.018.
[4] Castellanos MR, Nehru VM, Pirog EC, *et al*. Fluorescence microscopy of H&E stained cervical biopsies to assist the diagnosis and grading of CIN. Pathol Res Pract 2018;214(5):605–611. doi:10.1016/j.prp.2018.03.021.
[5] Pinto AP, Crum CP. Natural history of cervical neoplasia: defining progression and its consequence. Clin Obstet Gynecol 2000;43(2):352–362. doi:10.1097/00003081-200006000-00015.
[6] Winer RL, Kiviat NB, Hughes JP, *et al*. Development and duration of human papillomavirus lesions, after initial infection. J Infect Dis 2005;191(5):731–738. doi:10.1086/427557.
[7] Passmore JAS, Milner M, Denny L, *et al*. Comparison of cervical and blood T-cell responses to human papillomavirus-16 in women with human papillomavirus-associated cervical intraepithelial neoplasia. Immunology 2006;119(4):507–514. doi:10.1111/j.1365-2567.2006.02465.x.
[8] Santesso N, Mustafa RA, Schünemann HJ, *et al*. World Health Organization Guidelines for treatment of cervical intraepithelial neoplasia 2-3 and screen-and-treat strategies to prevent cervical cancer. Int J Gynaecol Obstet 2016;132(3):252–258. doi:10.1016/j.ijgo.2015.07.038.
[9] Santesso N, Mustafa RA, Wiercioch W, *et al*. Systematic reviews and meta-analyses of benefits and harms of cryotherapy, LEEP, and cold knife conization to treat cervical intraepithelial neoplasia. Int J Gynaecol Obstet 2016;132(3):266–271. doi:10.1016/j.ijgo.2015.07.026.
[10] Mathevet P, Chemali E, Roy M, *et al*. Long-term outcome of a randomized study comparing three techniques of conization: cold knife, laser, and LEEP. Eur J Obstet Gynecol Reprod Biol 2003;106(2):214–218. doi:10.1016/s0301-2115(02)00245-2.
[11] World Health Organization. WHO guideline for screening and treatment of cervical pre-cancer lesions for cervical cancer prevention, second edition. [2021-07-7]. Available from: https://www.who.int/publications-detail-redirect/9789240030824. [Accessed October 22, 2022].
[12] Pinder L, Parham G, Basu P, *et al*. Thermal ablation versus cryotherapy or loop excision to treat women positive for cervical precancer on visual inspection with acetic acid test: pilot phase of a randomised controlled trial. Obstet Gynecol Surv 2020;75:291–292. doi:10.1097/01.ogx.0000661548.12771.3d.
[13] Hesketh T, Zhou X, Wang Y. The end of the one-child policy: lasting implications for China. JAMA 2015;314(24):2619–2620. doi:10.1001/jama.2015.16279.
[14] Tatum M. China's three-child policy. Lancet 2021;397(10291):2238. doi:10.1016/S0140-6736(21)01295-2.
[15] Stanley M. Pathology and epidemiology of HPV infection in females. Gynecol Oncol 2010;117(2 Suppl):S5–10. doi:10.1016/j.ygyno.2010.01.024.
[16] Zhang J, Lu CX. Spontaneous regression of cervical intraepithelial neoplasia 2: a meta-analysis. Gynecol Obstet Invest 2019;84(6):562–567. doi:10.1159/000497286.
[17] Silver MI, Gage JC, Schiffman M, *et al*. Clinical outcomes after conservative management of cervical intraepithelial neoplasia grade 2 (CIN2) in women ages 21-39 years. Cancer Prev Res (Phila) 2018;11(3):165–170. doi:10.1158/1940-6207.CAPR-17-0293.
[18] Attali E, Yogev Y. The impact of advanced maternal age on pregnancy outcome. Best Pract Res Clin Obstet Gynaecol 2021;70:2–9. doi:10.1016/j.bpobgyn.2020.06.006.
[19] Zhang L, Zhang W, Orsi F, *et al*. Ultrasound-guided high intensity focused ultrasound for the treatment of gynaecological diseases: a review of safety and efficacy. Int J Hyperthermia 2015;31(3):280–284. doi:10.3109/02656736.2014.996790.
[20] Li CZ, Wang ZB, Yang X, *et al*. Feasibility of focused ultrasound therapy for recurrent cervicitis with high-risk human papillomavirus infection. Ultrasound Obstet Gynecol 2009;34(5):590–594. doi:10.1002/uog.7357.
[21] Fu Z, Fan Y, Wu C, *et al*. Clinical efficacy and mechanism for focused ultrasound (FUS) in the management of cervical intraepithelial neoplasia 1 (CIN1). Int J Hyperthermia 2020;37(1):339–345. doi:10.1080/02656736.2020.1749316.
[22] Zeng H, Liu M, Xiao L, *et al*. Effectiveness and immune responses of focused ultrasound ablation for cervical intraepithelial neoplasia. Int J Hyperthermia 2022;39(1):539–546. doi:10.1080/02656736.2022.2052365.
[23] Singh A, Arthur B, Agarwal V. LEEP verses cryotherapy in CIN. J Obstet Gynaecol India 2011;61(4):431–435. doi:10.1007/s13224-011-0048-1.
[24] Fang C, Wang SY, Liou YL, *et al*. The promising role of PAX1 (aliases: HUP48, OFC2) gene methylation in cancer screening. Mol Genet Genomic Med 2019;7(3):e506. doi:10.1002/mgg3.506.
[25] Kong L, Wang L, Wang Z, *et al*. DNA methylation for cervical cancer screening: a training set in China. Clin Epigenetics 2020;12(1):91. doi:10.1186/s13148-020-00885-7.
[26] Liou YL, Zhang TL, Yan T, *et al*. Combined clinical and genetic testing algorithm for cervical cancer diagnosis. Clin Epigenetics 2016;8:66. doi:10.1186/s13148-016-0232-3.

[27] Kremer WW, Steenbergen R, Heideman D, *et al*. The use of host cell DNA methylation analysis in the detection and management of women with advanced cervical intraepithelial neoplasia: a review. BJOG 2021;128(3):504–514. doi:10.1111/1471-0528.16395.
[28] Kremer WW, Dick S, Heideman DAM, *et al*. Clinical regression of high-grade cervical intraepithelial neoplasia is associated with absence of FAM19A4/miR124-2 DNA methylation (CONCERVE Study). J Clin Oncol 2022;40(26):3037–3046. doi:10.1200/JCO.21.02433.
[29] Peto J, Gilham C, Fletcher O, *et al*. The cervical cancer epidemic that screening has prevented in the UK. Lancet 2004;364(9430):249–256. doi:10.1016/S0140-6736(04)16674-9.
[30] McCredie MRE, Sharples KJ, Paul C, *et al*. Natural history of cervical neoplasia and risk of invasive cancer in women with cervical intraepithelial neoplasia 3: a retrospective cohort study. Lancet Oncol 2008;9(5):425–434. doi:10.1016/S1470-2045(08)70103-7.
[31] Gustafsson L, Adami HO. Natural history of cervical neoplasia: consistent results obtained by an identification technique. Br J Cancer 1989;60(1):132–141. doi:10.1038/bjc.1989.236.
[32] McIndoe WA, McLean MR, Jones RW, *et al*. The invasive potential of carcinoma in situ of the cervix. Obstet Gynecol 1984;64(4):451–458.
[33] American College of Obstetricians and Gynecologists. ACOG Practice Bulletin number 66, September 2005. Management of abnormal cervical cytology and histology. Obstet Gynecol 2005;106(3):645–664. doi:10.1097/00006250-200509000-00051.
[34] Chan JK, Monk BJ, Brewer C, *et al*. infection and number of lifetime sexual partners are strong predictors for "natural" regression of CIN 2 and 3. Br J Cancer 2003;89(6):1062–1066. doi:10.1038/sj.bjc.6601196.
[35] Tainio K, Athanasiou A, Tikkinen KAO, *et al*. Clinical course of untreated cervical intraepithelial neoplasia grade 2 under active surveillance: systematic review and meta-analysis. BMJ 2018;360:k499. doi:10.1136/bmj.k499.
[36] Sykes PH, Simcock BJ, Innes CR, *et al*. Predicting regression of cervical intraepithelial neoplasia grade 2 in women under 25 years. Am J Obstet Gynecol 2022;226(2):222.e1–222.e13. doi:10.1016/j.ajog.2021.09.009.

Edited by: Yong-Qing Zhu

How to cite this article: Yang L, Wang ZX, Zhu YH, Bai Y, Liou YL, Li Q, Shi ZN, Shi QL, Ren CC. Focused ultrasound ablation for women with cervical lesions: protocol of a randomized controlled trial. Reprod Dev Med 2026;10(1):40–47. doi: 10.1097/RD9.0000000000000132