Effectiveness and immune responses of focused ultrasound ablation for cervical intraepithelial neoplasia

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ABSTRACT

Purpose: To investigate the safety, efficacy, and the immune responses of focused ultrasound in cervical intraepithelial neoplasia (CIN).

Methods: Patients with biopsy-confirmed CIN were recruited for focused ultrasound treatment and asked to return during 3–6 and 6–12 months post-treatment to receive cervical cytology, high-risk human papilloma virus (HPV) detection, and colposcopy. The effective rate was evaluated within 3–6 months, whereas the recurrence rate was evaluated within 6–12 months. Cervicovaginal lavage and cervical tissue were sampled before and 3–6 months after treatment. The expression of interferon gamma (IFN-γ), endoplasmic reticulum aminopeptidase 1 (ERAP1), human leukocyte antigen 1 (HLA-I), cluster of differentiation 4 (CD4), and cluster of differentiation 8 (CD8) in the cervical tissue were observed by immunohistochemistry. Immunoglobulin A (IgA) and interleukin 10 (IL-10) levels in the cervicovaginal lavage were detected by enzyme-linked immunosorbent assay. Comparisons were made in immune analyte levels before and after treatment.

Results: We analyzed the results of 154 patients. The effective rate at 3–6 months was 96.8%. The recurrence rate at 6–12 months was 2.0%. The eradication rate of HPV was 72.4% at 3–6 months and 81.0% at 6–12 months. No serious adverse reactions and complications were observed. After treatment, a higher expression of ERAP1 was observed (p < 0.05). Significant down-regulation of IgA and IL-10 were detected (each p < 0.05). However, the expression of CD4, CD8, HLA-I, as well as the release of IFN-γ, did not reach statistical significance (each p > 0.05).

Conclusions: Focused ultrasound is an effective and safe therapy for treating CIN, which could improve the local immune milieu of the cervix to some extent.

Introduction

Cervical cancer is the fourth most common malignancy among women worldwide and one of the leading causes of cancer-related death among women in developing countries [1]. Human papillomaviruses (HPV) are found in over 99% of the invasive cervical cancer cases [2], and has been proved to be the main risk factor of cervical cancer and its precursor [3]. Studies show that HPV infections are usually cleared within 2 years in approximately 90% of infected individuals [4], whereas persistent infection with high-risk genotypes can lead to cervical dysplasia, also known as cervical intraepithelial neoplasia (CIN) [5]. A retrospective study of 188,095 cases reported 5.3% of low dysplasia (CIN 1), 0.18% of moderated dysplasia (CIN2), and 0.05% of high dysplasia (CIN3) [6]. Patients diagnosed with CIN 1 carry a 1% probability of developing invasive cancer [7]. CIN 1 lesions that do not regress may develop into CIN 2/3 within 2–3 years following infection [8], with approximately 5% of patients diagnosed with CIN 2/3 progressing to invasive cervical cancer [7]. The timely treatment of CIN can prevent the disease from progressing to cervical cancer.

Current therapeutics targeting CIN include ablation and resection therapies. High-grade CIN is commonly treated by conization procedures such as cold knife conization (CKC), loop electrosurgical excision procedure (LEEP) or laser excision. These excisional therapies are feasible, tolerable, and have favorable postoperative outcomes. However, they carry the risk for pregnancy-associated complications such as cervical insufficiency, preterm rupture of the membranes, and preterm labor [9]. Classic ablation therapeutic interventions, such as cryotherapy have low pregnancy-associated complications. However, the large amount of postoperative vaginal discharge is disturbing, and a stable supply of liquid nitrogen or liquid carbon dioxide is required [10].

Focused ultrasound (FUS) is a non-ionizing and noninvasive therapeutic modality and is primarily used to treat gynecology disorders, such as cervicitis and vulvar diseases [11]. Li has reported for the first time in 2009 that FUS could be a
safe, effective, and feasible therapy for patients with HPV-infected cervicitis [12]. FUS was then applied to treat CIN1 by Li’s group and showed a total effective rate of FUS for CIN1 of 90% and the rate of HPV clearance of 85.71% [13]. These reported therapeutic effects are very satisfactory; however, more clinical data are needed to promote this technology.

In this study, we aimed to explore the safety, efficacy, and the immune responses of FUS ablation in CIN, including CIN1, CIN2, and CIN2-3, and to provide more data for the effectiveness of focused ultrasound and its biological effect in CIN.

Materials and methods

Study population

This prospective study was approved by the institutional ethics committee of the Second Affiliated Hospital of Chongqing Medical University (approval No. 2019-38). The study population included women diagnosed with squamous intraepithelial lesion, and then called CIN, between 1 July 2019 and 30 September 2020 at the Second Affiliated Hospital of Chongqing Medical University. Inclusion criteria were as follows: patients aged from 18 to 60 years, those with biopsy-proven CIN (congruent with cytology result), those with negative findings on endocervical curettage, and those with satisfactory colposcopy examination (visualization of the squamocolumnar junction and the entire lesion). Exclusion criteria were as follows: suspicion or evidence of invasive lesions on cytology, biopsy, or colposcopic examination, positive pregnancy test or lactating women, previous history of ablation therapy or conization, current pelvic inflammatory disease, acute cervicitis, or other gynecological infection. Patients who agreed to sample cervicovaginal lavage and cervical tissue before and after treatment were included to study the changes of cervical immune indexes. Informed consent was obtained from all patients prior to their participation.

Sample collection

Cervicovaginal lavage was collected before and 3–6 months after treatment. The cervix was exposed with a speculum. The upper one-third segment of the vagina and the exocervix were lavaged with 4 ml sterile normal saline, and then the lavage fluid was pipetted from the vaginal posterior fornix using a sterile burette and preserved in liquid nitrogen for processing. The cervical tissue was obtained by biopsy under the guidance of a colposcope before and 3–6 months after treatment.

The process of focused ultrasound ablation

Treatment was performed under the guidance of a colposcope. The 3% acetic acid was applied to the cervix to demarcate the lesion. The CZF-300 ultrasonic therapeutic apparatus was manufactured by Chongqing HIFU Medical Technology Co, Ltd (Chongqing, China). The frequency was 9.65 MHz, the power was 7.7–8.6 W. Ultrasonic coupling agent was used to make the therapeutic probe better attached to the surface of the cervix. Circular and radial scanning with a speed of 2–5 mm/s were the therapeutic techniques commonly used, and the treatment area was 3–5 mm beyond the edge of the lesion. The total treatment duration of each patient was approximately 180–300 s. Circumstances were allowed to discontinue the operation when the treatment area convergence with the color of white or white with red in it, along with cervical sag inward were exhibited.

Evaluation

Once treated, patients were asked to return 3–6 months and 6–12 months after treatment. At each follow-up visit, patients had a complete physical and pelvic examination, conventional cytology, HPV DNA testing, and a colposcopic exam, which colposcopically directs biopsies if suspicious lesions are present. Common adverse events were lower abdomen cramping during treatment, and watery or occasionally blood-tinged discharge for up to 2–3 weeks. The possible complications were fever or shaking chills requiring oral or intravenous antibiotics, severe lower abdominal pain requiring pain medication, foul-smelling or pus-like discharge, excessive bleeding with clots requiring a hospital visit, and cervical stenosis requiring dilation.

The primary outcome was the therapeutic effect. The total effective rate including complete remission and improvement was evaluated at 3–6 months. Patients with negative cytology and colposcopy, or normal histology were considered as being in complete remission regardless of the HPV test results. Patients with better cytology and colposcopy, or better histology were considered to have improved. Persistent was defined as the diagnosis of CIN within 6 months after treatment. Progression was defined as worse cytology or colposcopy, or worse histology within 6 months after the treatment. Recurrence was defined as the diagnosis of CIN in ≥ 6 months after treatment in patients who had at least one negative cytology and colposcopy after treatment [14].

The secondary outcome was the HPV eradication rate within 3–6 months and 6–12 months. Among 154 patients included in our study, 116 patients had a positive HPV status, 28 were negative, and 10 were unknown. We analyzed the HPV eradication rate of 116 positive cases before treatment.

Cervical tissues for immunohistochemistry (IHC)

The expression of interferon gamma (IFN-γ), endoplasmic reticulum aminopeptidase 1 (ERAP1), human leucocyte antigen I (HLA-I), Cluster of differentiation 4 (CD4), and Cluster of differentiation 8 (CD8) in the cervical tissue were observed by IHC. Operations were performed according to the instructions of the manufacturer. Paraffin-embedded cervical tissues were sectioned into 5 μm slices and blocked with 1% H2O2, and incubated with trypsin at 37 °C for 30 min for antigen retrieval. Sections were incubated with primary antibodies...
overnight at 4°C, including CD4 (1:400, anti-rabbit, ab133616, Abcam, USA), CD8 (1:100, anti-rabbit, ab93278, Abcam, USA), HLA-I (1:100, anti-mouse, ab70328, Abcam, USA), ERAP1 (1:500, anti-rabbit, ab124669, Abcam, USA), and IFN-γ (1:100, anti-rabbit, YT2279, Immunoway, China). Sections were visualized with a 3,3′-diaminobenzidine substrate kit and counterstained with hematoxylin. The expression of CD4, CD8, IFN-γ, ERAP1, and HLA1 were observed under a microscope. Immunoreactive cells were stained brown yellow. Region of interests (ROIs) were defined as the cervical epithelium and stroma immediately beneath the epithelium. Three visual fields (magnification: 200×) were randomly chosen as ROIs and imaged using a CoolSnap digital camera. The integrated optical density (IOD) and area of these immunohistochemical figures were semi-quantitatively measured by Image-Pro Plus 6.0 (Media Cybernetics, USA). The mean density was calculated by IOD/area.

Cervicovaginal lavage for enzyme-linked immunosorbent assay

Immunoglobulin A (IgA) and interleukin 10 (IL-10) levels in the cervicovaginal lavage were detected by enzyme-linked immunosorbent assay (ELISA). The assay for IgA and IL-10 (Neobioscience, CHN) was used according to the manufacturer’s instructions. The absorbance was measured at 450 nm with an Epoch Microplate reader (BioTek, VT, USA), and cytokine levels were calculated according to the standard curve.

Statistical analysis

All data were processed by Graphpad prism 8.0. The distribution of patient baseline characteristics, measures of efficacy, and safety were presented as the adoption rate (%) and medians (lower and upper quartiles) as appropriate. Fisher exact test was used to test differences in the ratio of the response rate. T-test was performed to compare the differences of local immune data before and after treatment and statistical significance was set at $p < 0.05$.

Results

Participants’ characteristics

A total of 161 patients were enrolled in our study, seven of whom were lost to follow-up. Finally, 154 patients were included in this study. The median follow-up was 4 (3, 4) months for the 3–6 months period, and 9 (8, 11) months for the 6–12 months period. Ten patients agreed to sample cervicovaginal lavage and cervical tissue before and after treatment. Figure 1 displays a consort diagram that illustrates the progress of all participants through the trial. Characteristics of the patients are shown in Table 1.

The outcome of the FUS treatment

The total effective rate for primarily 3–6 months was 96.8% (149/154). The total persistence rate was 3.2% (5/154), and no patient suffered disease progression in 3–6 months after...
H. ZENG ET AL.

The total recurrence rate was 2.0% ((3/149) at 6–12 months. The response to FUS is shown in detail in Table 2. Colposcopic image characteristics of the cervix before and 3 months after treatment are shown in Figure 2.

Among the 154 cases, pathology was 125 low-grade CIN and 29 high-grade CIN. In low-grade CIN, the effective rate was 96.8% (121/125), compared with 96.6% (28/29) in high-grade CIN (p = 1.000). The persistence rates were 3.2% (4/125) for low-grade CIN and 3.4% (1/29) for high-grade CIN (p = 1.000). The recurrence rates were 2.5% (3/121) for low-grade CIN and 0% (0/28) for high-grade CIN (p = 1.000; Table 2).

In the HPV positive group, the effective rate was 96.55% (112/116), compared with 96.42% (27/28) for the HPV negative group (p = 1.000). The persistence rates were 3.45% (4/116) for HPV positive and 3.57% (1/28) for HPV negative group (p = 1.000). The recurrence rates were 0.89% (1/112) for HPV positive patients and 7.41% (2/27) for HPV negative patients (p = 0.096; Table 2).

Eradication of HPV was achieved in 72.4% (84/116) of FUS patients in the analyzed population at 3–6 months, and increased to 81.0% at 6–12 months. The HPV eradication rates were 70.5% (62/88) for low-grade CIN and 78.6% (22/28) for high-grade CIN at 3–6 months and increased to 79.5% (70/88) for low-grade CIN and 85.7% (24/28) for high-grade CIN at 6–12 months. No significant difference was found in HPV eradication between low-grade CIN and high-grade CIN (p = 1.000; Table 3).

Lower abdominal discomfort was commonly seen in 88.31% (136/154) of the cases during the treatment, and the symptom could alleviate rapidly after treatment without medical intervention. All the cases suffered a watery and occasionally blood-tinged discharge, which lasted for approximately 2–3 weeks. Vaginal bleeding requiring a hospital visit occurred in 3.2% (5/154) of the cases after treatment during the decrustation period and was solved using the gauze compression method. No patients suffered an infection requiring oral or intravenous antibiotics. We did not observe the cervical stenosis requiring dilation after treatment. No severe complications were observed.

**The immune response after FUS treatment**

All 10 patients were HPV positive before treatment, including seven with low-grade CIN and three with high-grade CIN. The cytology and colposcopy results were all negative.

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### Table 1. Demographic clinical characteristics of the included patients.

| Characteristic                        | Patients participated in follow-up (n = 154) | Patients agreed to cervical sampling (n = 10) |
|--------------------------------------|---------------------------------------------|---------------------------------------------|
| Age (years)                          |                                             |                                             |
| Median (lower quartile, upper quartile) | 33 (28, 36)                                | 30.5 (26, 32)                              |
| Range                                | 20–57                                      | 24–33                                      |
| BMI (kg/m²)                          |                                             |                                             |
| Median (lower quartile, upper quartile) | 20.9 (19.1, 22.5)                          | 20 (19.4, 20.4)                           |
| Range                                | 16.2–30.5                                  | 18.8–22.3                                  |
| Sexual partners, n (%)               |                                             |                                             |
| <1                                   | 80 (51.9)                                  | 4 (40.0)                                   |
| ≥2                                   | 74 (48.1)                                  | 6 (60.0)                                   |
| Parity, n (%)                        |                                             |                                             |
| ≤1                                   | 125 (81.2)                                 | 5 (50.0)                                   |
| ≥2                                   | 29 (18.8)                                  | 5 (50.0)                                   |
| Contraception, n (%)                 |                                             |                                             |
| Condom                               | 105 (68.2)                                 | 7 (70.0)                                   |
| Intrauterine device                  | 17 (11.0)                                  | 2 (20.0)                                   |
| Othersa                              | 4 (2.6)                                    | 0                                          |
| None                                 | 28 (18.2)                                  | 1 (10.0)                                   |
| HPV status, n (%)                    |                                             |                                             |
| Positive                             | 116 (70.0)                                 | 10 (100.0)                                 |
| Negative                             | 28 (18.2)                                  | 0                                          |
| Unknown                              | 10 (8.2)                                   | 0                                          |

Others include contraceptives, subdermal arm implant and tubal ligation.

### Table 2. Efficacy of CIN treated by focused ultrasound.

| Outcome                          | CIN (n = 154) | HPV status (n = 154) |
|----------------------------------|---------------|---------------------|
|                                  | Low-grade CIN | High-grade CIN      | Positive | Negative | Unknown |
|                                  | (n = 125)     | (n = 29)            | (n = 116) | (n = 28) | (n = 10) |
| 3–6 months follow up             |               |                     |          |          |         |
| Total effective rateb            | 121/125 (96.8%) | 28/29 (96.6%)     | 112/116 (96.55%) | 27/28 (96.42%) | 10/10 (100%) | 1.000    |
| Complete remissionb              | 119/125 (95.2%) | 26/29 (89.7%)      | 109/116 (93.97%) | 26/28 (92.86%) | 10/10 (100%) | 145/154 (94.2%) |
| Improvementc                     | 2/125 (1.6%)  | 2/29 (6.9%)        | 3/116 (2.59%)   | 1/28 (3.57%)   | 0/10 (0.00%) | 4/154 (2.6%) |
| Persistenced                     | 4/125 (3.2%)  | 1/29 (3.4%)        | 4/116 (3.45%)   | 1/28 (3.57%)   | 0/10 (0.00%) | 5/154 (3.2%) |
| 6–12 months follow up            |               |                     |          |          |         |
| Recurrencea                      | 3/121 (2.5%)  | 0/28 (0%)          | 112/116 (96.55%)| 27/28 (96.42%) | 10/10 (100%) | 3/149 (2.0%) |

Abbreviations: CIN, cervical intraepithelial neoplasia. aEffective rate equals to complete remission rate plus improvement rate. bNo evidence of disease at 3–6 months. cDisease consistently existed within 6 months after treatment. dDisease reappeared 6–12 months. The difference of efficacy between low-grade CIN and high grade CIN, the HPV positive and negative CIN were compared. Fisher exact test, p < 0.05 was considered significant.
3–6 months after treatment. The HPV results showed six negative and four positive, similar as before. In 6–12 months of follow-up, the cytology and colposcopy remained normal. The HPV results showed nine negative and only one positive.

Changes in immune indexes are shown in Figure 3, and the statistical results revealed that the ERAP1 expression was significantly increased after the FUS treatment (0.00357 vs. 0.00592, \( p = 0.0398 \)). In contrast, no significance was found in the infiltration of the CD4\(^+\) T cell (0.0103 vs. 0.0112, \( p = 0.701 \)), CD8\(^+\) T cell (0.0189 vs. 0.0109, \( p = 0.105 \)), the expression of HLA-I (0.0278 vs. 0.0252, \( p = 0.599 \)), and the release of IFN-\( \gamma \) (0.0307 vs. 0.0316, \( p = 0.898 \)) before and after treatment. FUS formulation significantly down-regulated IgA (6400 vs. 962.9, \( p = 0.0032 \)) and IL-10 (0.512 vs. 0.411, \( p = 0.0355 \)) levels in cervicovaginal lavage samples compared to the unprocessed status.

### Discussion

FUS is a new method for the treatment of CIN. Different from other ablation therapies, FUS has good penetrability and a different treatment mode (from inside to outside). The focus of the ultrasound was located 3–6 mm deep into the cervical tissue. By focusing the ultrasound beam to the target, FUS induces heat, mechanical and cavitation effects that denature the targeted lesions at depth or superficiality, facilitating necrosis and allowing them to be replaced by surrounding normal tissue.

Here, we studied the therapeutic effect of FUS. We found a cure rate of 96.8% for LSIL and 96.6% for HSIL after FUS ablation. Comparing FUS to other therapies, Singh Abha et al. reported a cure rate of 95.2% for CIN1, 78% for CIN 2, and 66% for CIN 3 lesions after cryotherapy, and 96.6% for CIN1, 89.18% for CIN 2, and 75% for CIN 3 lesions after LEEP [15]. The total effective rate of FUS was similar to that of cryotherapy and LEEP previously reported. As for the recurrence, Nancy Santesso et al. performed a meta-analysis and reported that the recurrence rate was 5.3% in 12 months after cryotherapy and LEEP previously reported. As for the recurrence, Nancy Santesso et al. performed a meta-analysis and reported that the recurrence rate was 5.3% in 12 months after cryotherapy and LEEP previously reported. As for the recurrence, Nancy Santesso et al. performed a meta-analysis and reported that the recurrence rate was 5.3% in 12 months after cryotherapy and LEEP previously reported. As for the recurrence, Nancy Santesso et al. performed a meta-analysis and reported that the recurrence rate was 5.3% in 12 months after cryotherapy and LEEP previously reported. As for the recurrence, Nancy Santesso et al. performed a meta-analysis and reported that the recurrence rate was 5.3% in 12 months after cryotherapy and LEEP previously reported. As for the recurrence, Nancy Santesso et al. performed a meta-analysis and reported that the recurrence rate was 5.3% in 12 months after cryotherapy and LEEP previously reported. As for the recurrence, Nancy Santesso et al. performed a meta-analysis and reported that the recurrence rate was 5.3% in 12 months after cryotherapy and LEEP previously reported. As for the recurrence, Nancy Santesso et al. performed a meta-analysis and reported that the recurrence rate was 5.3% in 12 months after cryotherapy and LEEP previously reported. As for the recurrence, Nancy Santesso et al. performed a meta-analysis and reported that the recurrence rate was 5.3% in 12 months after cryotherapy and LEEP previously reported.
smoke. Second, only an electrical supply and coupling agent for ultrasound are required for treatment. Furthermore, the therapeutic probe diameter of 5 mm is convenient for movement to adapt to the different shapes of lesions. Moreover, treatment can be applied in the outpatient clinic without requiring special anesthesia or post-surgical care. Based on these advantages, FUS is worthy of clinical promotion.

During the follow up, we found a high eradication of HPV. The mechanism behind this phenomenon has not been fully understood. Previous study in the cryotherapy for CIN has showed that significant upregulation of IFN-γ and downregulation of IL-10 in 6 months after cryotherapy [16]. An exploratory study which detected 16 cytokines in cervical secretions before and 6 months after LEEP suggested few changes in the cervical microenvironment [17], suggesting that comparing the ablation to the excision procedure for CIN treatment, the necrosis of the lesion in situ may induce some immune effect in the cervical microenvironment. In this study, the local immune milieu of the cervix was explored before and 3–6 months after FUS treatment. Since HPV infections remain localized in the epithelium, a systemic immune response is rare, and levels of circulating immune markers in response to HPV infection are not easily established [18], the systemic immune response was not detected in this study.

Adaptive immunity plays an important role in HPV infectious diseases. To initiate an adaptive immune response, antigen-presenting cells need HPV antigens, followed by activation, and present them to immune effector cells [19]. HLA class I antigen-presenting system is responsible for the presentation of HPV antigens to the immune system. ERAP1, which trims peptides to generate ligands for HLA-I loading, is a crucial regulator of the peptide repertoire and affects formation of stable HLA-I at the cell surface [20,21]. IFN-γ can upmodulate the expression of major histocompatibility complex I (MHC-I) and promote the efficacy of CD8+ CTL [22]. Cytotoxic CD8+ T cell is the principal effector cell in HPV infectious diseases. CD4+ helper T cells also participate in the anti-virus immunity and play an important role in the activation of CD8+ cytotoxic T cells. However, HPV has several mechanisms for avoiding the immune system. Downregulating the expression of the interferon and upregulating IL-10 could produce a local immunosuppressive environment, which, along with alters infectious cell surface antigens and forms an immunosuppressive network that inhibits the immune response [23].

Our data showed the significant upregulation of ERAP1 in local CIN tissue after FUS therapy, which may imply a positive impact on the presentation of individual HLA class I antigens [19,20]. However, the expression level of HLA-I and IFN-γ were not significantly increased. We noticed a slight increase in CD4+ T cells infiltration and a decrease in CD8+ T cells, although not statistically significant. The mechanism behind this phenomenon remains unelucidated. We propose that the eradication of CIN by FUS is mainly due to the thermal and non-thermal effects of ultrasound irradiation, and the necrosis of the lesion in situ by thermal effect may induce a non-thermal effect, such as the upregulation of the ERAP1 expression and decrease in the secretion of IL-10, which relieve the local immunosuppressive environment with a decreased likelihood for HPV infection and benefit for HPV eradication [23,24].

Figure 3. Variations in immunological indexes before and 3–6 months after treatment. A. Immunohistochemical staining was done on cervical sections obtained (original magnification 200×). B. The mean density of immunological indexes. C. The level of IgA and IL-10 in the cervicovaginal lavage was detected by ELISA. *p < 0.05.
We did not identify statistically significant differences in the expression of CD4⁺ T cell, CD8⁺ T cell, HLA-1, and IFN-γ before and 3–6 months after FUS treatment, which may be related to the limited time point for detection. Previous study has shown that a significant increase in the population of CD4 lymphocytes and the ratio of CD4/CD8 in the peripheral blood were detected 7–10 days after high-intensity FUS treatment for solid tumor [25]. IFN-γ secretion by T lymphocytes is transient, reaching its peak at approximately 24 h after stimulation and then returning to baseline expression levels [26]. Besides 3–6 months after FUS treatment, the immunological indexes should also be detected in 7–10 days after FUS treatment in the further studies for a more comprehensive understanding of the immunomodulatory effect of FUS treatment in CIN.

As the main product of humoral immune response, which occurs later than the cellular immune response, the secretion of IgA often elicits a lesser effect to eliminate the virus and neoplastic cells compared with cellular immunity [27]. Dillner et al. [28] found that the IgA ELISA absorbance values of the CIN group were significantly higher than those of the normal group, which suggested that IgA antibodies may be a useful marker for CIN. A following study also showed that both the frequency and magnitude of cervical IgA with HPV 16 infection was significantly elevated in women with CIN 2/3 compared with women with CIN 1 (p = 0.0045) [29], suggesting that the IgA level was positively associated with the grade of the cervical lesion. Our result demonstrated that FUS application significantly decreased IgA in cervical secretions. Combined with the conclusions of prior studies, it could be inferred that a decreased IgA might be a potential biomarker of validity of FUS in CIN [28,29].

This study has several limitations. First, as for the setting of the follow-up period, the initial follow-up time reported in many studies was 3–6 months [30,31]. The sixth month after treatment was a crucial time point to evaluate the persistency (<6 months) and recurrence (at least 6 months) [14]; therefore, we set the second follow-up time to 6–12 months. However, the time period is rather long, and it is better for the future studies to set a shorter time period. Second, the subgroup analysis was made according to the grade of CIN and HPV status before treatment. The effective rate seemed similar between the groups without statistically significant differences. However, we only included 29 patients in the high-grade CIN group, which were much fewer than those in the low-grade CIN group. The negative HPV cases were also less than the positive. Since the samples were different, it could reduce the power of the test to some extent and further studies with more HSIL cases are needed to evaluate the difference. Third, the local immunological indexes for FUS treatment should be a dynamic change process, and more time points should be chosen by future studies to detect the changes in immunological indexes.

Conclusion

This study is the first to analyze the efficacy and changes of immunological indicators of the FUS in CIN. Our data showed that FUS is a safe and effective method for the treatment of cervical intraepithelial lesions with a high HPV eradication rate, and it could relieve the local immunosuppressive environment by the upregulation of the ERAP1 expression and decrease the secretion of IL-10, which may decrease the likelihood for HPV infection and benefit for HPV eradication. Moreover, further studies of a large sample and multiple time nodes are still needed.

Disclosure statement

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

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