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ORIGINAL RESEARCH

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Clinical study of Modified Ermiao Granules activating AIM2 inflammasome-mediated pyroptosis to clear HR-HPV and ameliorate LSIL

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Abstract

This study aimed to examine the efficacy and safety of Modified Ermiao Granules in clearing high-risk human papillomavirus (HR-HPV) and reversing low-grade squamous intraepithelial lesions (LSIL), as well as to examine cervical tissues before and after drug treatment for absent in melanoma (AIM2) inflammasome-mediated pyroptosis. A total of 60 patients with persistent HR-HPV infection and cervical histopathology of the LSIL were randomly divided into control group and observation groups (n = 30 each). Observation group participants received Modified Ermiao Granules orally, whereas the control group received recombinant human interferon α2b vaginal effervescent tablets. After treatment, the observation group had a significantly lower HPV DNA loads than the control group ($p < 0.05$). Compared with the control group, the observation group had a significantly higher HR-HPV clearance rate ($\chi^2 = 4.267$, $p = 0.039$). Accordingly, the LSIL reversal rates were 33.33% and 60.00% in the control and observation groups, respectively ($\chi^2 = 4.286$, $p = 0.038$). Both treatments reduced clinical symptoms in patients with HR-HPV with LSIL, but the observation group showed greater improvement ($p < 0.05$). Control and observation groups expressed AIM2 protein positively at 36.67% and 66.37%, respectively ($\chi^2 = 5.406$, $p < 0.05$). Caspase-1, gasdermin D (GSDMD), and interleukin (IL)-1β levels in vaginal douche were significantly higher in the observation group than in the control group ($p < 0.05$). There was a significant negative correlation between HPV DNA load and AIM2 expression in cervical tissues ($rho = -0.493$, $p < 0.05$), with caspase-1 ($r = -0.439$, $p < 0.05$), GSDMD ($r = -0.508$, $p < 0.05$) and IL-1β levels ($r = -0.347$, $p = 0.007$) in vaginal douche. In conclusion, the Modified Ermiao Granules effectively cleared persistent HR-HPV infections and reversed LSIL, which may be associated with its activating effect on AIM2 inflammasome-mediated pyroptosis.

Keywords

Modified Ermiao Granules; HPV; Pyroptosis; Inflammasome; AIM2

1. Introduction

Persistent high-risk human papillomavirus (HR-HPV) infection is an established risk factor for the development of squamous intraepithelial lesions (SIL) of the cervix and cervical cancer [1]. Despite the long progression from HPV infection to SIL and cervical cancer, early reversibility is possible [1]. Preventing cervical cancer onset and progression requires an early diagnosis and intervention to block lesions at the precancerous stage [2].

HPV can be treated with medication, local physical therapy, surgery, immunotherapy, *etc.*, but overtreatment is still controversial [3]. Traditional Chinese medicine (TCM) preparations have gained a great deal of attention in recent years for their effectiveness in treating HR-HPV infections and blocking cervical lesions [4]. Based on Ermiao Pill, our team developed "Modified Ermiao Granules" to clear heat and dry dampness [5]. It was licensed as a Chinese medicine preparation by the national invention patent in 2013 (Patent No.: ZL201310037166.6).

Immune abnormalities and inflammation are key components of cervical pathology [2]. Against invasion from foreign pathogenic microorganisms, the innate immune system is the first line of defense. Originally identified as genes associated with malignant phenotype reversal in melanoma cells, Absent in Melanoma (AIM2) is a crucial component of innate immunity, before being classified as a DNA sensor [6]. Among the AIM2 structural domains, the Hin structural domain binds directly to dsDNA; the pyrin domain (PYD) structural domain interacts with the PYD structural domain of the adaptor protein apoptosis associated speck-like protein containing a caspase recruitment domain (ASC). As well, ASC's caspase

recruitment domain (CARD) can interact with pro-Caspase-1's CARD structural domain to create an inflammasome complex. By phosphorylating and linear ubiquitinating ASC, the AIM2 inflammasome activates pyroptosis, a cell death mediated in part by the caspase substrate gasdermin D (GSDMD) [7]. AIM2 inflammasome are necessary for the host's immune response to viruses. All four types of viruses are recognized by AIM2 after their DNA enters the cytoplasm, including the vaccinia virus, mouse cytomegalovirus (MCMV), HPV and hepatitis B virus (HBV) [8]. Activating AIM2 inflammasomes may be a new strategy for preventing and treating HPV infections [9].

This study evaluated the efficacy and safety of Modified Ermiao Granules in clearing HR-HPV and reversing low-grade squamous intraepithelial lesions (LSIL), and analyzed the changes in AIM2 inflammasome-mediated pyroptosis in cervical tissues before and after administration. Modified Ermiao Granules are expected to provide a clinically effective treatment for HR-HPV persistent infection combined with LSIL, and provide information for future studies exploring the mechanism of action.

2. Material and methods

2.1 Patients

This study was an assessor-participant-data analyst blinded, randomized, sham-controlled trial. Patients with low-grade squamous intraepithelial lesions (LSIL) with persistent HR-HPV infection who were treated at Jiangsu Provincial Hospital of Traditional Chinese Medicine from March 2021 to December 2021 were selected. Positive results from the first HR-HPV screening test remained positive after repeated screening after a time interval of more than 12 months, indicating persistent HR-HPV infection.

Inclusion criteria: (1) Age 30–55 years old, regular menstrual cycle, history of sexual intercourse. (2) Positive HR-HPV type test results (HPV16, HPV18, HPV31, HPV33, HPV35, HPV39, HPV45, HPV51, HPV52, HPV53, HPV56, HPV58, HPV59, HPV66, HPV68). (3) LSIL confirmed by cytology according to the Bethesda classification system. (4) No involvement in other clinical studies. Exclusion criteria: (1) Preparing for pregnancy or breastfeeding. (2) Severe organ diseases, hematologic diseases, malignant tumors, immune diseases, or abnormalities of liver and kidney functions that affect the clinical use of medication. (3) Patients with psychiatric disorders who could not cooperate. (4) Allergic to medications or foods. (5) Patients with combined cervical polyps, previous cervical trauma, or a history of physical therapy for cervical lesions. Withdrawal criteria: (1) poor adherence to or withdrawal requests mid-study. (2) Adverse events during treatment follow-up made continuing the study inappropriate. (3) Invalid data generated after randomization. (4) Incomplete clinical or pathological information.

This study involved 78 women, 11 of whom were withdrawn due to poor compliance, 6 were excluded due to incomplete pathologic findings after treatment, and 1 was unable to continue in the study due to a car accident during follow-up. Finally, 60 subjects who met the requirements were enrolled in this study and randomly divided into a control group and an observation group (n = 30 each) at a ratio of 1:1 according to the randomization sequence. An independent biostatistician generated the randomization sequence using Microsoft Excel prior to study initiation. Individual randomization codes were sealed in sequentially numbered opaque envelopes and opened after participants completed baseline assessments by attending physician.

2.2 Treatment

Control group participants were given recombinant human interferon α2b vaginal effervescent tablets (#S20120019; Kawin, Beijing, China). Medication was started on the 3rd day after menstruation, 1 capsule every other day, 9 times per month, and discontinued during menstruation. Modified Ermiao Granules were administered orally to the observation group. Premenstrual period, 1 dose in the morning, and 1 dose in the evening, 14 days per month, and discontinued during menstruation. The drug composition included 10 g each of Phellodendri Cortex (Huang Bo), Rhizoma Atractylodis, Atractylodes macrocephala Koidz., Paris polyphylla Smith var. yunnanensis, Hedyotis diffusa Willd. (Rubiaceae), Radix Isatidis (Isatis indigotica Fortune), and Smilax glabra Roxb., and 30 g of Coix lacryma-jobi L. In both groups, patients were continuously treated for 3 months without having sexual contact.

2.3 Cervical specimens

Each eligible participant underwent a gynecological examination. Using a cytobrush, we exfoliated cervical cells from the ecto- or endo-cervical canals. For HPV genotyping, specimens were stored in preservation solution in 2 mL vials at −20 °C. Cytology samples were stored at 4 °C in 20 mL vials using ThinPrep® PreservCyt® (Hologic, Waltham, MA, USA). Samples were transferred to the laboratory and cytological site for HR-HPV genotyping and cytology.

2.4 Observation indicators

Biological indicators: (1) HR-HPV DNA load and HPV clearance before (T0) and after (T1) treatment. (2) LSIL regression after treatment. (3) AIM2 expression in cervical biopsies from LSIL patients was analyzed by immunohistochemistry before (T0) and after (T1) treatment. (4) Caspase-1, GSDMD and interleukin (IL)-1β levels in vaginal douche were measured by enzyme-linked immunosorbent assay before (T0) and after (T1) treatment. Clinical indicators: (1) TCM evidence score before (T0) and after (T1) treatment. Safety indicators: Adverse reactions to drugs, including dizziness and palpitations, nausea and vomiting, skin allergies, abdominal pain, and diarrhea.

2.5 HPV genotyping

The linear array (LA) method was used for typing 37 HPV types (HPV6, HPV11, HPV16, HPV18, HPV26, HPV31, HPV33, HPV35, HPV39, HPV40, HPV42, HPV45, HPV51, HPV52, HPV53, HPV54, HPV55, HPV56, HPV58, HPV59, HPV61, HPV62, HPV64, HPV66, HPV67, HPV68, HPV69,

HPV70, HPV71, HPV72, HPV73, HPV81, HPV82, HPV83, HPV84, HPV1S39 and HPV CP6108). With the LA method, DNA sequence of the HPV L1 region, which is about 450 bp long, are amplified by polymerase Chain Reaction (PCR) using PGMY09/11 primers and reverse hybridization. Subsequently, we used β-globin gene as an internal reference [10] to ensure the validity of the sample and the reliability of the assay [11]. LA test results determined HPV types and single or multiple infections. HR-HPV infection was defined when any of the 13 HR-HPVs (HPV16, HPV18, HPV31, HPV33, HPV35, HPV39, HPV45, HPV51, HPV52, HPV56, HPV58, HPV59 and HPV68) were positive among the above types. Whenever two or more HR-HPV types above are positive, it is defined as HR-HPV multiple infection.

2.6 High-risk hybrid capture 2 (HR HC2)

A full-length viral RNA probe is used by the HC2 to bind to the viral target DNA, forming a RNA-DNA complex, that is captured by an antibody encapsulated in a microtiter plate. HPV DNA in the specimen is detected by signal amplification and chemiluminescent signal generation. HR HC2 is capable of detecting 13 HR-HPV types simultaneously, but cannot distinguish between different HPV types. Therefore, in this study, we only assessed HPV DNA content in the samples based on the semi-quantitative HC2 results instead of determining the negativity and type of the samples. To evaluate the results, the ratio of relative light unit (RLU) to cut off (CO), *i.e.*, signal intensity (RLU/CO) was used. RLU/CO reflects HPV DNA content, the greater its ratio, the higher the HPV DNA content in the sample. Hence, 1.0 RLU/CO is equivalent to 1.0 pg/mL of viral load in the sample [12]. HPV viral load in specimens was expressed in pg/mL.

2.7 Liquid-based cytology test

An exfoliated cell specimen from cervix was collected using a broom-like brush. In accordance with the Bethesda classification system, specimens were prepared and stained for diagnosis. In liquid-based cytology testing, regression of LSIL is defined as a negative result for malignant intraepithelial lesions.

2.8 Chinese medicine symptoms score

Based on the relevant standards of the Diagnostic Efficacy Criteria for Chinese Medicine Diseases complied by the State Administration of Traditional Chinese Medicine of China and clinical features of the TCM evidence scoring scale [13], the scoring was done. Seven indicators totaling 0–15 are included in the scale. Higher scores indicate a more severe condition.

2.9 Immunohistochemical analysis

We baked, dewaxed and hydrated paraffin sections of cervical tissue (4 mm) before antigenically repairing them using citric acid antigen repair solution (pH = 6.0) for 2 min. Sections were rinsed with phosphate buffer solution (PBS) and incubated at room temperature with 3% hydrogen peroxide for 10 min. After rinsing with PBS, an anti-AIM2 monoclonal antibody (1:50, ab93015, Abcam, Fremont, CA, USA) was added dropwise to the sections and incubated at room temperature for 1 h. Next, a ready-to-use MaxVision reagent was used to incubate the sections at room temperature for 15 min. After diaminobenzidine (DAB) color development, the sections were counterstained with hematoxylin. A PBS rinse was performed to return the blue color. Gradient ethanol was used for dehydration, and xylene clear, neutral gum for clearing. Two experienced pathologists determined the results. Cells stained yellowish, tan, or brown staining in the nucleus or cytoplasm for AIM2 protein were considered positive. Staining intensity and percentage of positive cells were calculated to score the results: (1) staining intensity: 0 for no staining, 1 for light yellow, 2 for tan, and 3 for brown; (2) percentage of positive cells: 0 for $\leq$5%, 1 for 6%–25%, 2 for 26%–75%, and 3 for $>$75%. Staining intensity + percentage of positive cells = total score. A total score of 0 was recorded as (−), and a score of 1 to 9 was recorded as (+). AIM2-positive expression rate (%) = positive cases/total cases $\times$ 100%.

2.10 Enzyme-linked immunosorbent assay (ELISA)

During colposcopy, patients received 5 mL of 0.9% Sodium Chloride (NaCl) solution through a 10 mL sterile disposable syringe. Following the rinsing of the posterior vaginal fornix and cervix, the solution was recovered in a sterile centrifugal tube. The supernatant was collected after centrifugation at 2000 r/min for 10 min. Caspase-1, GSDMD and IL-1β levels in the vaginal lavage were measured by ELISA. Human caspase-1 ELISA kit (#ab241860), human GSDMD ELISA kit (#ab272463), and human IL-1β ELISA kit (#ab214025) from Abcam (Fremont, CA, USA). Reference ranges of ELISA results for caspase-1, GSDMD and IL-1β were as follows: caspase-1, 15.6 pg/mL–1000 pg/mL; GSDMD, 0.78 ng/mL–50 ng/mL; and IL-1β, 14.06 pg/mL–900 pg/mL, respectively.

2.11 Statistical analysis

Using Chi-square test or Fisher's exact tests, proportions of nominal variables were compared between treatment and control groups. Continuous variables were presented as mean and standard deviation or median and range. Continuous variables were compared using the *t* test. An analysis of pre- and post-treatment data was conducted using a paired *t* test. Statistical analyses were performed using IBM SPSS Statistics (Version 16.0. Armonk, NY, USA: IBM Corp), with p values < 0.05 indicating statistically significant.

3. Results

3.1 General information

This study included 60 LSIL patients with persistent HR-HPV infection. average age was (40.43 $\pm$ 6.49) years old, ranging from 30 to 54 years old. TCM evidence score ranged from 6 to 15, with a mean of (10.92 $\pm$ 2.34). This study population harbored all 13 common HR-HPV subtypes, including HPV type 16 in 19 cases (31.67%), HPV type 18 in 12 cases (20.00%), and HPV type 31 in 4 cases (13.33%). Among them, 34 cases (56.67%) were infected with only one subtype, while 26 cases

(43.33%) were mixed with two or more subtypes. Neither the control group nor the observation group had statistically significant differences in age, Chinese medicine symptoms score, HPV subtype distribution, and infection types ($p > 0.05$, Table 1).

3.2 HPV DNA load and LSIL reversal

Before treatment (T0), the control group and the observation group didn't differ significantly in HPV DNA load ($p > 0.05$). Both groups experienced significant decreases in HPV DNA load after three months of treatment (T1) ($p < 0.05$), with the observation group significantly lower than the control group ($p < 0.05$, Fig. 1). After treatment (T1), 11 patients (36.67%) in the control group turned negative from positive HR-HPV, whereas19 patients (63.33%) in the observation group turned negative HR-HPV. HR-HPV clearance rate was significantly higher in the observation group than in the control group ($p < 0.05$, Table 2). Additionally, LSIL reversal rates for the control and observation groups were respectively 33.33% (10/30) and 60.00% (18/30) ($p < 0.05$, Table 2).

3.3 Chinese medicine symptom score

Patients with HR-HPV with LSIL could be treated with either treatment modality, but the observation group improved significantly more, as evidenced by the significantly lower Chinese medicine symptoms score in the observation group compared to the control group after treatment ($p < 0.05$, Fig. 2).

3.4 AIM2 expression in cervical tissue

In immunohistochemical analysis, AIM2 appeared throughout the basal cell layer as continuous, strong nuclear or nuclear plus cytoplasmic brownish-yellow staining extending upwards (Fig. 3). Before treatment (T0), both control and observation groups expressed AIM2 protein positively at 20.00% (6/30) and 23.33% (7/30) respectively ($\chi^2 = 0.098$, $p > 0.05$). The positive expression rate of AIM2 protein in control and observation groups after two courses of treatment (T1) was 36.67% (11/30) and 66.37% (20/30) respectively ($\chi^2 = 5.406$, $p < 0.05$).

3.5 Levels of caspase-1, GSDMD and IL-1β in vaginal douches

Before treatment, vaginal douche fluid from both groups did not differ significantly in caspase-1, GSDMD and IL-1β levels ($p < 0.05$, Fig. 4). After treatment, caspase-1, GSDMD and IL-1β levels in vaginal douche fluid in the observation group were significantly higher than in the control group ($p < 0.05$, Fig. 4).

3.6 Correlation analysis

After treatment, Spearman correlation analysis revealed a significant negative correlation between HPV DNA load and AIM2 expression in cervical tissues ($p < 0.05$, Table 3). Pearson correlation analysis showed HPV DNA load was significantly negatively correlated with caspase-1, GSDMD and IL-1β levels in vaginal douche ($p < 0.05$, Table 3).

3.7 Security analysis

Neither group experienced any obvious adverse reactions during the medication process. In the blood routine indexes and liver and kidney functions, no obvious abnormalities were observed following treatment.

4. Discussion

Cervical cancer develops from persistent HR-HPV infection [14]. Typically, cervical precancerous lesions appear over the course of more than 10 years from HR-HPV infection to invasive cervical cancer [15]. In most cases, cervical precancerous lesions are long-lasting and reversible. Cervical cancer incidence can be significantly reduced if patients are treated during cervical pre-cancer. Verhoef *et al*. [16] reported that 30% to 50% of HPV-infected patients developed mild lesions on the cervical epithelium, but most reverted to normal within 3 to 4 months after viral clearance. Paaso *et al*. [17] also pointed out that HR-HPV infection is closely related to cervical lesions grade. A cure rate of over 95% can be achieved if active intervention treatment is performed at the SIL stage.

Overtreatment should be avoided in patients with persistent HR-HPV infection combined with SIL [18]. However, appropriate interventions should also be made to eliminate persistent HR-HPV infection and etiologically block SIL. Prophylactic vaccines against HPV infection have begun to be used in clinics, but they are only effective against certain subtypes. Age and application scope limitations are present, while therapeutic vaccines are still under development [19]. Though effective in blocking cervical lesions progression, physical and surgical treatments have the disadvantages of residual lesions, recurrence, complications, and inability to remove HPV completely. Interferon and povidone suppositories are medications with long treatment periods and inaccurate efficacy. TCM has advantages over Western medicine when it comes to regulating immunity, anti-inflammation, and anti-virus [20]. Chinese medicine also has a unique multi-target effect. Therefore, clinical research has concentrated on developing TCM therapies that eliminate HPV infection effectively.

With LSIL, we found remarkable clinical efficacy for patients with persistent HR-HPV infection. Modified Ermiao Granules not only effectively cleared persistent HR-HPV infection and reversed LSIL, but also improved TCM symptoms and greatly relieved clinical symptoms. Chinese herbs have been shown to have multi-target effects on HPV infection-related cervical pre-cancer. The aqueous extract from Cortex Phellodendri is shown to have immunomodulatory effects with broad-spectrum antiviral activity in modern pharmacological studies [21]. A volatile oil constitutes the main pharmacological component of Rhizoma Atractylodis, and its active ingredients have strong antibacterial, antiviral, antitumor, hepatoprotective, inhibitory inflammatory response, diuretic, central inhibitory, and antiulcer effects [22]. HeLa cell proliferation is significantly inhibited by Atractylenolide I, the active ingredient of Atractylodes macrocephala Koidz [23]. Immune checkpoint blockade therapy responsiveness can be enhanced by activating tumor antigen presentation [24]. Coix lacryma-jobi L., Paris polyphylla Smith var. yunnanensis,

TABLE 1. General information.

Parameters	Control group (n = 30)	Observation group (n = 30)	$t/Z/\chi^2$	p
Age (years, mean ± standard deviation)	41.10 ± 7.21	39.77 ± 5.84	0.787	0.435
Chinese medicine symptoms score (mean ± standard deviation)	10.97 ± 2.37	10.87 ± 2.39	0.163	0.871
HPV DNA load (pg/mL, median and range)	33.17 (22.83, 54.65)	41.81 (28.97, 57.58)	1.005	0.315
HR-HPV subtype (n, %)	-	-	0.093	0.993
HPV16	9 (30.00%)	10 (33.33%)	-	-
HPV18	6 (20.00%)	6 (20.00%)	-	-
HPV31	2 (6.67%)	2 (6.67%)	-	-
Others	13 (43.33%)	12 (40.00%)	-	-
HR-HPV infection type	-	-	0.271	0.602
Mono-infection	18 (60.00%)	16 (53.33%)	-	-
Multi-infectious	12 (40.00%)	14 (46.67%)	-	-

HR-HPV: high-risk human papillomavirus.

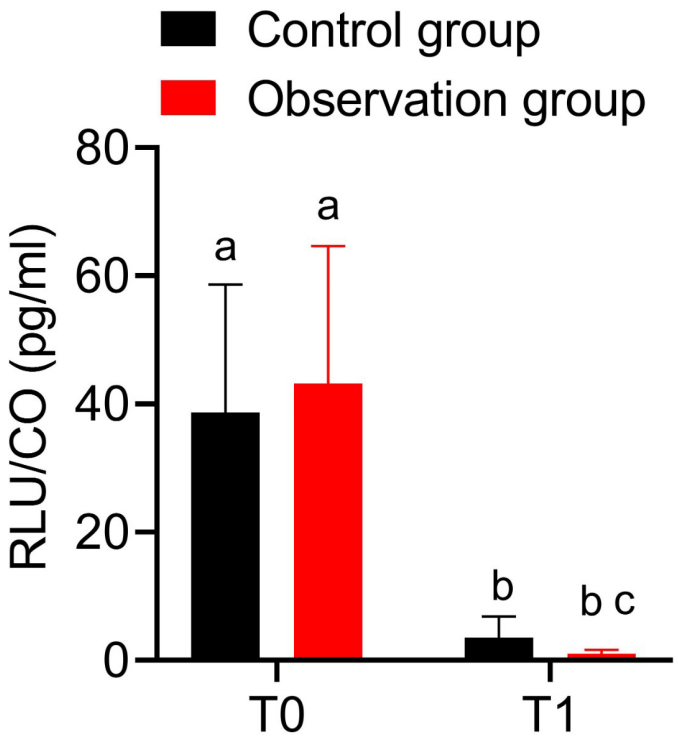

FIGURE 1. Comparison of HPV DNA load before (T0) and after treatment (T1) between control and observation groups. a, b and c: The same superscripted letter in T0 treatment indicate no significant differences based on (*t* test) ($p > 0.05$). Differing superscripted letters in T1 treatment indicate significant differences according to (*t* test) ($p < 0.05$). RLU: relative light unit; CO: cut off.

TABLE 2. Comparison of cervical HR-HPV clearance and LSIL reversal rate between both groups after treatment (n, %).

Parameters	Control group (n = 30)	Observation group (n = 30)	χ^2	p
HR-HPV cleared rate	11 (36.67%)	19 (63.33%)	4.267	0.039
LSIL reversal rate	10 (33.33%)	18 (60.00%)	4.286	0.038

HR-HPV: high-risk human papillomavirus; LSIL: low-grade squamous intraepithelial lesions.

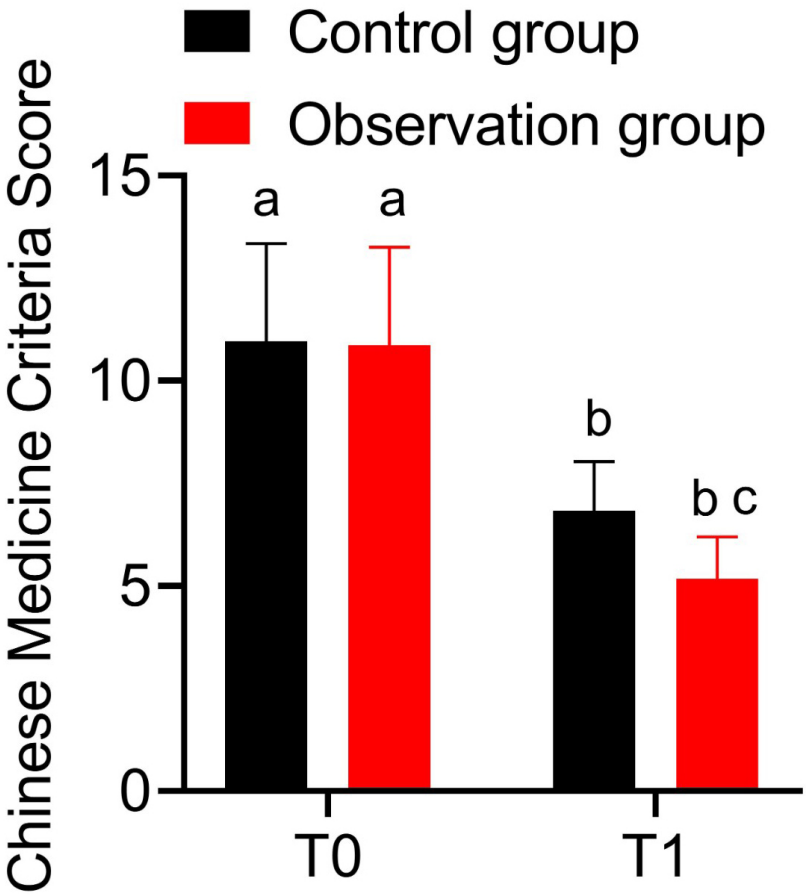

FIGURE 2. Comparison of changes in Chinese medicine symptoms score before (T0) and after (T1) treatment between both groups. a, b and c: The same superscripted letter in T0 treatment indicate no significant differences based on (*t* test) ($p > 0.05$). Differing superscripted letters in T1 treatment indicate significant differences according to (*t* test) ($p < 0.05$).

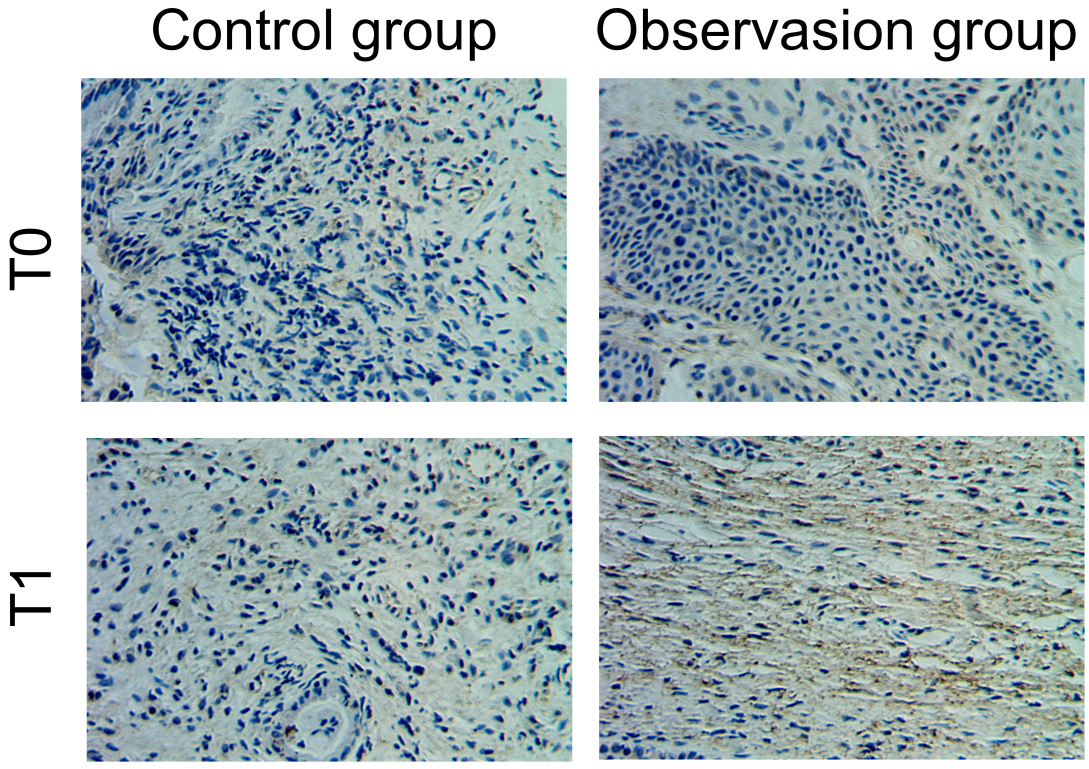

FIGURE 3. Immunohistochemical analysis to detect AIM2 protein expression in cervical tissues (×200).

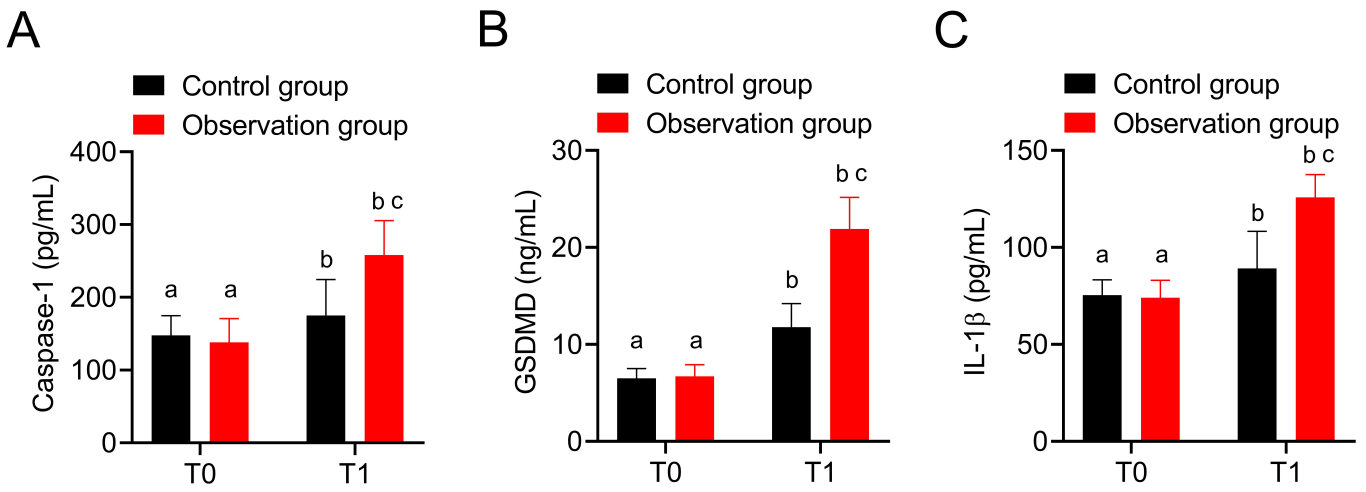

FIGURE 4. Comparison of Caspase-1, GSDMD and IL-1β levels in vaginal douche before (T0) and after (T1) treatment in both groups. (A) Caspase-1 level; (B) GSDMD level; (C) IL-1β level. a, b and c: The same superscripted letter in T0 treatment indicate no significant differences based on (*t* test) ($p > 0.05$). Differing superscripted letters in T1 treatment indicate significant differences according to (*t* test) ($p < 0.05$). GSDMD: gasdermin D; IL: interleukin.

TABLE 3. Correlation analysis.

Parameters	HPV DNA load	
	rho/r	*p*
AIM2	−0.493	<0.001
caspase-1	−0.439	<0.001
GSDMD	−0.508	<0.001
IL-1β	−0.347	0.007

AIM2: absent in melanoma; GSDMD: gasdermin D; IL: interleukin.

Hedyotis diffusa Willd. (Rubiaceae), Radix Isatidis (Isatis indigotica Fortune) and Smilax glabra Roxb. have antitumor, antibacterial and anti-inflammatory properties [25]. This study demonstrates the advantages of TCM in preventing and treating HPV infection through the use of Modified Ermiao Granules, as having detoxifying and anti-inflammatory effects.

Pattern recognition receptors (PRRs), apoptosis associated speck-like protein (ASC), and caspase-1 precursor compose the inflammasome, found in cells' cytoplasm. Inflammasome activation induces the production of multiple cytokines and plays an important role in intrinsic immunity. AIM2 inflammasome was first proposed in 2002, consisting of AIM2, ASC and pro-caspase-1 [26]. As part of the body's anti-infection immune response, AIM2 recognizes dsDNA from pathogens invading the organism and assembling into the inflammasome, activating caspase-1, causing pyroptosis as well as the maturating and releasing IL-1β and IL-18 [27]. AIM2 inflammasome was activated by Francisella tularensis, Listeria monocytogenes, Streptococcus pneumoniae, hepatitis B virus, and murine cytomegalovirus infection *in vitro* cellular. AIM2 inflammasome activation, caspase-1 cleavage, and IL-1β release were detected in HPV-infected skin [28]. Inhibition of AIM2 gene transcription was observed in HPV-infected cervical cancer cells to contribute to cancer cell evasion of antiviral immune responses [9]. AIM2 inflammasome-mediated pyroptosis in enterovirus A71-infected neuronal cells limits viral replication [29]. Furthermore, IL-1β activates other immune genes expression, promotes lymphocytes recruitment to the primary infection site, and controls HPV invasion of the pathogen as a pleiotropy inflammatory factor [30]. This study revealed that Modified Ermiao Granules significantly increased AIM2 expressions in cervical tissues of patients with persistent HR-HPV infection with LSIL and induced caspase-1 activation and IL-1β release. Meanwhile, AIM2, Caspase-1, GSDMD and IL-1β were all significantly negatively correlated with HPV DNA load in HR-HPV LSIL patients. From an immunoinflammatory perspective, it is hypothesized that Modified Ermiao Granules eliminate HR-HPV and block cervical lesions progression by activating AIM2-mediated pyroptosis.

Patients in this study did not experience any adverse effects, suggesting Modified Ermiao Granules are safe for treating persistent HR-HPV infection with LSIL. There are, however, many limitations to this study. The efficacy of different HPV subtypes was not compared. A better management and treatment of groups after clarifying HPV subtypes could be provided by further clinical studies. Due to the short observation period in this study, the results may be biased, since the outcome of cervical lesions could change as time passes. There is still a need for follow-up to expand the sample size and conduct long follow-up statistics. It is still necessary to examine further how HPV infection and AIM2-mediated pyroptosis may be regulated.

5. Conclusions

In summary, Modified Ermiao Granules effectively cleared persistent HR-HPV infections and reversed LSIL, which may be associated with its activating effect on AIM2 inflammasome-mediated pyroptosis. Modified Ermiao Granules need to be validated in animal or cellular models to modulate the AIM2 inflammasome. New insights from this study may help develop and apply HR-HPV-targeting drugs.

ABBREVIATIONS

HPV, Human papillomavirus; HR-HPV, high-risk HPV; LSIL, Low-grade squamous intraepithelial lesion; AIM2, Absent in melanoma.

AVAILABILITY OF DATA AND MATERIALS

The authors declare that all data supporting the findings of this study are available within the paper and any raw data can be obtained from the corresponding author upon request.

AUTHOR CONTRIBUTIONS

JF and QLR—designed the study and carried them out; prepared the manuscript for publication and reviewed the draft of the manuscript. JF, QLR and YH—supervised the data collection, analyzed the data, interpreted the data. All authors have read and approved the manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Ethical approval was obtained from Medical Research Ethics Committee of Jiangsu Provincial Hospital of Traditional Chinese Medicine (Approval no. 2021NL-025-03). Written informed consent was obtained from a legally authorized representative(s) for anonymized patient information to be published in this article.

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Not applicable.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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