Original Research

Clinical observation and mechanism of the elimination of HPV16/18/58 subtype infection and the reversal of grade I cervical intraepithelial neoplasia in Han Chinese women treated with modified Ermiao granules

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Objective: To evaluate the efficacy and molecular mechanism of modified Ermiao granules in eliminating HPV16/18/58 subtype infection and reversing cervical intraepithelial neoplasia (CIN) I in Han Chinese women. *Materials and methods*: A total of 135 cases of CIN I with HPV16/18/58 infection from May 2016 to April 2019 were randomly and equally divided into three groups: control group, no treatment given; traditional Chinese medicine treatment group, treated with modified Ermiao granules; and interferon group, treated with recombinant human interferon α2b gel. Each group had 45 cases. The negative rate of HPV, CIN I reversal, the levels of CD3+, CD4+, CD8+, IgA, IgM, and IgG, the expression of TLR4, p16, and ki67, the amplification of *hTERC*, and the mRNA expression levels of P16 and Ki67 were compared. *Result*: Modified Ermiao granules and interferon could reduce the negative HPV rate, promote the CIN I course reversal, significantly decrease the TLR4, p16, and ki67 expression levels, and decrease the *hTERC* amplification. The difference among the three groups was statistically significant ($P < 0.0167$). The CD3+, CD4+, IgA, IgM, IgG, and CD8+ levels were significantly increased by modified Ermiao granules and interferon, and the P16 and Ki67 expression levels were significantly decreased ($P < 0.05$). Modified Ermiao granules had more obvious advantages in eliminating HPV, reversing CIN I, reducing TLR4 protein expression and *hTERC* amplification, and improving IgA, IgM, and IgG levels compared with those of the interferon group ($P < 0.0167$, $P < 0.05$). *Conclusion*: Modified Ermiao granules can improve the HPV clearance rate, reverse the CIN I course, inhibit cell proliferation, enhance cervical local immune function, and improve inflammatory stress level. Modified Ermiao granules has a good curative effect on HPV16/18/58 subtype infection and CIN I in Han Chinese women.

Keywords

Human papilloma virus; Modified Ermiao granules; Cervical intraepithelial neoplasia; Human telomerase RNA component

1. Introduction

Human papilloma virus (HPV), which prevents the high risk of persistent infection, is highly correlated with precancerous lesions and cervical cancer. Persistent HPV infection can significantly increase the proportion of cervical intraepithelial neoplasia (CIN), which is the most important pathogenic factor of cervical cancer.

Ermiao granule is a compound prescription comprised of Phellodendri Chinensis Cortex and Atractylodis Rhizoma in a specified ratio. Ermiao granule has been used for treating arthralgia syndrome in Chinese medicine since the Ming Dynasty (15th Century CE) because of its function of "clearing heat and removing dampness". In the traditional theory of Chinese medicine, the main pathogenic factors are wind, cold, heat, dampness, and dryness. Damp-heat, is a term of used in traditional Chinese medicine (TCM). Downward diffusion of damp-heat refers to the two pathogenic factors of dampness and heat, which together lead to pathological conditions of the lower body organs. In TCM the manifestations of the syndrome of downward diffusion of damp-heat include body weakness, anorexia, abdominal pain. Morbid leucorrhoea increases in quantity and is yellow, viscous, foul, and sometimes purulent. Rash and pruritus of the genitals may be present when damp-heat act on the female reproductive system.

Different from Western medicine in TCM the spleen also has the function of promoting food digestion and fluid metabolism. Malfunction of the spleen leads to fluid metabolism disorder and pathologic conditions (dampness). In TCM, strengthening the spleen function results in the elimination of dampness.

Nowadays, Ermiao granule is also being prescribed for treating HPV infection. It has been modified in order to improve its clinical effectiveness. Modified Ermiao granules are

derived from the experience of Professor Ren Qingling, who is a well-known gynecology expert of the Affiliated Hospital of Nanjing University of Chinese Medicine, the Jiangsu Provincial Hospital of Traditional Chinese Medicine. Modified Ermiao granule is a heat-clearing and moistening compound in (TCM used to treat HPV infection. The compound was given a national invention patent in 2013 (Patent no.: ZL201310037166.6). In this study, Han Chinese women infected with HPV16/18/58 accompanied with CIN 1 (CIN I) were treated with modified Ermiao granules to observe their efficacy in treating HPV infection and reversing CIN 1 and to understand the mechanism of action.

2. Materials and methods

2.1 General information

The study included 135 patients who had HPV16/18/58 single-type infection with CIN I, were 22–45 years old (mean age = 32.3 years, SD = 5.7 years), and were admitted to the gynecology clinic of Jiangsu Provincial Hospital of Traditional Chinese Medicine from May 2016 to April 2019. The course of the disease was 2–26 months with an average of 5.4 ± 1.4 months.

2.2 Diagnostic criteria

2.2.1 Diagnostic criteria of Western medicine

The diagnostic criteria of Western medicine for CIN I (Textbook of the 12th five-year plan of the Ministry of Health, Obstetrics and Gynecology, People's Medical Publishing House, 8th edition, 2014, edited by Xie Xing and Gou Wenli) are as follows:

(1) Possible symptoms: vaginal discharge is occasionally increased, has or has no odor, or has contact bleeding. Most patients have no specific symptoms. (2) Possible signs: the cervix is smooth; however, increased vaginal secretions, cervical erosion, or local erythema is present upon specialist examination. No obvious abnormality is detected in bilateral adnexa. (3) Auxiliary examination: Cervical cytology has a higher specificity than high-risk HPV detection. The Bethesda System for reporting cervical cytology is utilized. A cytology result of atypical squamous cells of undetermined significance (ASC-US) or worse is considered positive diagnosis. Reflex HPV-DNA testing is performed if a patient's cytology result is ASC-US or worse. Immediate colposcopy examination is conducted for HPV 16/18-positive patients. HPV-DNA and its typing test: second-generation gene hybridization capture (HC2) is used to detect HPV-DNA, and its results are combined with those of the cytological test. The HC2 quantitative result of RLU/CO 1.0 is a positive indication. If a positive result is obtained, HC2 can be combined with a cytological test, a kappe diversion hybridization typing test, and other screening methods for verification. Diagnosis of low-grade CIN: Patients with positive HC2 test results for HPV-DNA and cytological diagnosis of ASC-US or worse require colposcopy and histopathological examination. Colposcopy is one of the key steps in the three-step diagnosis (cytologic, HPV testing, colposcopy, and histopathology) in

making the diagnosis. Cervical biopsy histopathology is the gold standard for identifying lesions. In this study, patients who satisfied the colposcopic and histopathological diagnoses of CIN I were included.

2.2.2 Diagnostic criteria of TCM

In TCM pathology of the lower genital tract is classified as leucorrhea disease with the syndrome of downward diffusion of damp-heat. The diagnostic standard of is in accordance with the State Administration of Traditional Chinese Medicine, a 1994 reference; "Standard of Diagnosis of Disease and Curative Effect of Traditional Chinese Medicine" trial version (version 1, 2002); "Chinese Medicine: New Medicine Clinical Research Guiding Principle (10th edition)", a reference material used in teaching Chinese medicine in higher colleges and universities; and "The Gynecology of Traditional Chinese Medicine in the New Century (4th edition)" published by China Press of Traditional Chinese Medicine and edited by Tan Yong. (1) Main symptoms: morbid leucorrhoea increases in quantity and is yellow, viscous, foul, and sometimes purulent. Pruritus and heat rash of the genitals may be present. (2) Secondary symptoms: body weakness, sense of suppression in the chest, mouth pain, anorexia, abdominal pain, yellow and greasy coating on the tongue, and frequent urination with yellow urine are observed. (3) Tongue and pulse signs: Red tongue, yellow and greasy, presence of fur, and smooth pulse are recorded. A diagnosis is made when the main symptom and two or more secondary symptoms are present and combined with the tongue and pulse signs.

2.3 Inclusion criteria

The following criteria were used in the selection of participants in this study: patients who (1) met the diagnostic criteria of CIN I disease syndrome and leucorrhea disease with the syndrome of the downward diffusion of damp-heat; (2) are or had been sexually active, aged between 30 and 65 years; (3) were Han Chinese women having a regular menstrual cycle and avoiding pregnancy (e.g., using contraception) in the past year or were postmenopausal; (4) tested positive for HPV16/18/58 and pathological tissue result of CIN I; and (5) gave informed consent volunteering to participate in the study. The study was approved by the Ethical Review Board and the process of obtaining informed consent complied with relevant regulations. (6) Peripheral blood parameters, liver and kidney function, blood coagulation function, and cardiopulmonary function were normal, and no serious diseases of the liver, brain, kidney, and other vital organ were present. (7) Had no history of mental illness and were able to cooperate during treatment.

2.4 Exclusion criteria

The following were grounds for exclusion from this study: (1) cervical lesions diagnosed as CIN II/III or above by histopathological examination; (2) complicated with cervical polyps, previous history of cervical trauma, or history of physical therapy due to cervical lesions; (3) use of other Chi-

nese and Western drugs to treat CIN I disease after entering the study; (4) women who were not avoiding pregnancy (e.g., using contraception), were pregnant, or were breastfeeding; (5) women who had severe primary heart, liver, lung, kidney, blood, or serious diseases affecting their survival; (6) failure to give full informed consent due to mental or behavioral impairment; (7) suspected or had a history of alcohol or drug abuse; (8) presence of other conditions that could jeopardize adherence, such as frequent changes in working conditions that might easily lead to loss of follow-up; (9) allergic constitution, such as allergic history of two or more drugs and food, or allergic to the ingredients of a given drug; and (10) patients participating in clinical trials of other drugs.

2.5 Therapeutic method

(1) The placebo group (N = 45) without drug intervention received one course of treatment and physical examination every 3 months. (2) The modified Ermiao granule group (N = 45) ingested the TCM Ermiao granules dissolved in water (drug composition: 10 g of fried atractylotis glutamata, 10 g of cortex phellodendri, 30 g of coix seed, 10 g of chonglou, 10 g of ban lan gen, 30 g of *Panax* anemone, and 10 g of tu fu ling; Patent no.: ZL201310037166.6) for 14 days before menstruation. They stopped taking it upon the onset of the menstrual period. One course of treatment lasted 3 months, and the treatment continued for two courses. (3) The participants in the interferon group (N = 45) applied interferon gel after they cleaned their vulva before bedtime at night. A disposable vaginal applicator was used. Recombinant human interferon 2b gel (1 g; Mega pharmacy [HeFei] Co., Ltd.; approval number S20010054) was applied once every other day during the course of treatment. One course of treatment lasted 1 month, and was repeated for six consecutive courses. Treatment started 3 days after the end of menstrual bleeding and stopped with the next menstruation.

2.6 Observation indicators and methods

2.6.1 Cell specimen collection method

The transformation zone of the cervix was sampled with a brush and the sample submitted for liquid-based cytology.

2.6.2 HPV-DNA examination

Fresh cervical biopsy tissue was obtained using colposcopy before and after treatment and frozen at -80 °C in liquid nitrogen for examination. HC2 test/kappa diversion hybridization. HPV-DNA typing test was used to identify persistent HPV infection and calculate the HPV-negative conversion rate.

2.6.3 Cervical histopathology

Some of the cervical tissue obtained before and after treatment was fixed with formaldehyde, dehydrated, embedded in paraffin, and stained with hematoxylin and eosin (HE) to observe the progression of precancerous cells.

2.6.4 CD 3/4/8+ T-lymphocyte expression and IgA/M/G levels

The expression rates of CD3+, CD4+, and CD8+ in cervical T lymphocytes were detected through flow cytometry. The supernatant was extracted after tissue homogenization. The IgA, IgM, and IgG levels were determined with an enzyme-linked immunosorbent assay. The effects of the modified Ermiao granules on the cellular immunity of high-risk HPV-infected cervical tissues were compared.

2.6.5 Immunohistochemical staining for TLR4, P16, and Ki67 observation

Immunohistochemical staining was performed on cervical tissue to observe the expression of toll-like receptor 4 (TLR4), P16, and Ki67 in the cervical tissue of patients with CIN I after high-risk HPV infection. Immunohistochemical positivity criteria: the staining of TLR4 was in the cell membrane or cytoplasm, the staining of P16 was in the nucleus and/or cytoplasm, and the staining of Ki67 was in the nucleus. Interpretation results included the staining intensity and number of stained cells.

The specific method was as follows. (1) Staining intensity: Ten fields were randomly selected, and 1000 positive cells were counted for semiquantitative grading, including nonstaining (0 points), pale yellow (1 point), brown yellow (2 points), and deep brown yellow (3 points). (2) The number of stained cells (represented by the percentage of stained positive cells) was rated according to the following: ≤ 5% (0 point), 6%–24% (1 point), 25%–50% (2 points), and > 50% (3 points). Positive scores were obtained after multiplying the scores of the staining intensity and the number of stained cells, where 0 and 1 were negative (-), 2 to 3 were weakly positive (+), 4 to 6 were moderately positive (++), and 7 to 9 were strongly positive (+++).

2.6.6 hTERC amplification and mRNA expression of P16 and Ki67

Double-color interphase fluorescence in situ hybridization (FISH) was adopted to compare the effects of modified Ermiao granules and interferon treatment on high-risk HPV infection-related genes and detect the cervical cell telomere enzyme (human telomerase RNA component, *hTERC*) gene amplification. Reverse transcription-polymerase chain reaction (RT-PCR) technique was used to detect the mRNA expression levels of P16 and Ki67 in cervical tissues.

2.6.7 Establishment of hTERC amplification threshold

FISH was applied to detect the amplification of *hTERC* in 20 normal cervical isolated cells to establish the threshold value, which was calculated as follows: threshold value = mean + 3 × standard deviation, and the result was 4.98%. This value indicated that the number of abnormal cells/the total number of counting cells was ≥ 5, and *hTERC* was amplified. Each case was randomly counted for 100 interphase cells by observing under a microscope with an oil lens at 1000× magnification. The ratio of red and green signals in the nucleus of a single interphase was determined. A ratio of 2 : 2 indicated normal cells, whereas two red signals of > 2

and two green signals of ≥ 2 were positive cells. The number of positive cells $\geq$ threshold (9 in this experiment) was positive for *hTERC* amplification according to the recorded number of red and green signals.

2.7 Statistical analysis

Data were statistically analyzed with SPSS 22.0 software. Measurement data were examined with ANOVA. $P < 0.05$ indicated statistically significant differences. The rate values were compared for categorical data and evaluated with a χ^2 test. The test level was significant at $P = 0.05/3 = 0.0167$.

3. Results

3.1 HPV changes before and after treatment in the three groups of patients

After 6 months of treatment, 38 (84%) of 45 patients in the TCM treatment group, 27 (60%) of 45 cases in the interferon group, and 8 (18%) of 45 cases in the placebo group turned negative for high-risk HPV. As shown in Table 1, the HPV-negative conversion rate in the TCM treatment group was significantly higher ($P < 0.0167$) than that in the placebo group ($P = 0.000$) and the interferon group ($P = 0.010$). Therefore, modified Ermiao granules had a significant inhibitory effect on HPV.

3.2 Histological changes before and after treatment in the three groups

The cervical tissues obtained before and after treatment were observed through HE staining for the reversal of CIN. Table 1 shows that the 31 (69%) of 45 cases from the TCM treatment group, 19 (42%) of 45 cases from the interferon group, and 5 (11%) of 45 cases from the placebo group had CIN reversal. The CIN reversal rate of the TCM treatment group was significantly higher ($P < 0.0167$) than those of the placebo group ($P = 0.000$) and interferon group ($P = 0.011$). Therefore, the modified Ermiao granules had a significant inhibitory effect on reversing the course of CIN I.

Table 1. Comparison of the HPV-negative conversion rate and the CIN reversal rate between groups.

Groups	HPV negative conversion	CIN reversal
TCM treatment group (n = 45)	38*△	31*△
Interferon group (n = 45)	27*	19*
Placebo group (n = 45)	8	5

*indicates $P < 0.0167$ compared with the placebo group; $\triangle$ indicates $P < 0.0167$ compared with interferon group.

3.3 Protein expression of TLR4, P16, and Ki67 in the tissues of the three groups before and after treatment

No significant difference was observed in the protein expression levels of TLR4, P16, and Ki67 in the TCM treatment, interferon, and placebo groups before treatment ($P = 0.970$, $P = 0.913$, $P = 0.968$, respectively). The positive expression rate of TLR4 was significantly decreased in the TCM treatment group after treatment ($P = 0.001$), whereas no significant difference was found in the interferon and placebo groups before and after treatment ($P = 0.398$, $P = 0.832$). After treatment, the positive expression rate of TLR4 was significantly lower ($P < 0.0167$) in the TCM treatment group than in the interferon group ($P = 0.004$) and the placebo group ($P = 0.001$) as shown in Table 2.

The positive expression rates of P16 and Ki67 in the TCM treatment group significantly decreased ($P = 0.000$, $P = 0.006$). Similarly, their positive expression rates in the interferon group significantly decreased ($P = 0.000$, $P = 0.035$). After the treatment, the positive expression rates of P16 and Ki67 in each group were significantly lower than those in the placebo group ($P = 0.004$, $P = 0.006$). The positive expression rate of P16 in the interferon group was significantly lower ($P < 0.0167$) than that in the placebo group ($P = 0.004$). The positive expression rate of Ki67 was not significantly different from that in the placebo group ($P = 0.058$). The positive expression rates of p16 and ki67 in the TCM treatment group were not significantly different from those in the interferon group ($P = 0.598$, $P = 0.384$, $P < 0.0167$). Details are shown in Table 3.

3.4 CD3/4/8+ and IgA/M/G of T lymphocytes in the three groups before and after treatment

Before the treatment, the cell percentages of CD3+, CD4+, and CD8+ and the ratio of CD4+/CD8+ between the groups had no significant differences ($P = 0.892$, $P = 0.921$, $P = 0.933$, $P = 0.874$). The percentages of CD3+ and CD4+ cells and the ratio of CD4+/CD8+ in the TCM treatment group and the interferon group after the treatment were significantly higher than those before the treatment ($P = 0.013$, $P = 0.021$, $P = 0.008$). The percentage of CD8+ cells after the treatment significantly decreased compared with before treatment ($P = 0.027$). Significant differences were observed between the TCM treatment group and the interferon group compared with the placebo group ($P = 0.002$, $P = 0.003$). By contrast, no significant difference was found between the TCM treatment group and the interferon group ($P = 0.167$). Details are shown in Table 4.

In addition, no significant difference in the IgA, IgM, and IgG values of the cervical tissues among the groups before the treatment ($P = 0.428$, $P = 0.615$, $P = 0.910$). The IgA, IgM, and IgG values after the treatments with TCM ($P = 0.000$, P = 0.000, P = 0.000) and interferon ($P = 0.011$, $P = 0.009$, $P = 0.000$) were higher than those before the treatments. The amplitude of increase in the TCM treatment group was significantly higher than that in the interferon group ($P < 0.05$). Details are shown in Table 5.

3.5 hTERC amplification before and after treatment in the three groups

No significant difference was observed in the *hTERC* amplification among the three groups before treatment, and the groups were comparable ($P = 0.839$). After treatment, the *hTERC* amplification ratio of the TCM treatment group and the interferon group decreased significantly ($P = 0.000$, $P = 0.004$, $P < 0.05$). The *hTERC* amplification ratios of the TCM treatment group and the interferon group were significantly

Table 2. Comparison of the positive expression of TLR4 protein.

Groups	Before treatment	After treatment
	Number of TLR4 positive cases	Number of TLR4 positive cases
TCM treatment group (n = 45)	25	9*△
Interferon group (n = 45)	26	22
Placebo group (n = 45)	26	25

*indicates $P < 0.0167$ compared with the placebo group; $\triangle$ indicates $P < 0.0167$ compared with interferon group; ** indicates $P < 0.05$ compared with the treated group.

Table 3. Comparison of the positive expression of P16 and Ki67 proteins.

Groups	Before treatment		After treatment	
	P16	Ki67	P16	Ki67
TCM treatment group (n = 45)	26**	28**	8*	15*
Interferon group (n = 45)	27**	29**	10*	19
Placebo group (n = 45)	25	29	23	28

*indicates $P < 0.0167$ compared with the placebo group; **indicates $P < 0.05$ compared with the treated group.

lower than that of the placebo group ($P = 0.000$, $P = 0.008$). The *hTERC* amplification ratio of the TCM treatment group was significantly lower ($P < 0.0167$) than that of the interferon group ($P = 0.009$). These results indicated that the modified Ermiao granules could affect *hTERC* amplification (Table 6).

3.6 mRNA expression of P16 and Ki67 before and after treatment in the three groups

RT-PCR showed that the mRNA expression levels of P16 and Ki67 were widely expressed in the cervical tissues of patients with CIN I. No significant difference ($P < 0.05$) was observed in the positive mRNA expression rates of P16 and Ki67 in each group before treatment ($P = 0.923$, $P = 0.898$). After the treatment, the positive mRNA expression rates of P16 and Ki67 ($P = 0.000$, $P = 0.014$) in the TCM treatment and interferon groups ($P = 0.000$, $P = 0.001$) were significantly lower than that in the placebo group ($P < 0.0167$). In addition, the mRNA expression levels of P16 and Ki67 in each group were also significantly different. In Table 7, the mRNA expression levels of P16 and Ki67 in the TCM treatment group and the interferon group were significantly lower than that in the blank control group ($P < 0.05$).

4. Discussion

The incidence and mortality rate of cervical cancer in China account for one-third of the world's, and this value is closely related to the high infection rate of HPV in China. Globally, HPV16 and 18 have been detected in approximately 50% and 20% of cervical cancer cases , respectively [1]. In Southeast Asia, HPV58 is the third-most common HPV type and accounts for 11.5%–28% of all HPV infections among women in China and other Asian countries. CIN is a group of precancerous lesions closely related to invasive cervical carcinoma. It reflects the continuous process that leads to the development of cervical cancer [2, 3]. Patients with persistent

high-risk HPV infection with cervical intraepithelial lesions can present with diverse clinical features, including vaginal discharge, contact bleeding, and vulvar pruritus. In TCM it is thought that it can also cause soreness and weakness of the waist and knees, which could affect the quality of daily life and the physical and mental health of patients. The function of DNA damage repair factors affected by HPV is related to the progress of CIN [4]; therefore, timely and effectively blocking HPV infection and retarding the progression of CIN in its early stages are important for the prevention of cervical cancer.

According to TCM patients with a continuous infection with high-risk HPV and CIN I often have a large amount of a thick vaginal discharge with a peculiar smell. This, combined with the pulse and condition of tongue coating, and modern research on the mechanism by which HPV infection causes cervical lesions led us to propose that the key to the pathogenesis of this disease is damp heat diffusing downward. Therefore, "modified Ermiao granules" based on "ermiao powder", a famous prescription for clearing heat and drying dampness in Danxi's Mastery of Medicine, were proposed to be patented (Patent no.: ZL201310037166.6). The medicine is composed of *Phellodendron*, *Atractylodes macrocephala*, coix seed, *Paris polyphylla*, isatis root, *Hedyotis diffusa*, and *Smilax glabra*. The combined use of various traditional Chinese medicines can play a role in clearing heat, drying dampness, strengthening the spleen, and stopping the belt (i.e., decrease leucorrhea) from the two aspects of strengthening and dispelling evil. This approach can eliminate HPV infection and reverse CIN I.

The 3q26 region of the human chromosome contains *hTERC*; the distortion of the chromosome arm 3q is one of the most common chromosomal aberrations in cervical cancer, and this condition can be detected in approximately 70% of patients with cervical cancer [5–7]. Therefore, *hTERC* detection has clinical significance in screening cervical precancerous lesions, establishing the prognosis, and evaluating treatment effects. In this study, *hTERC* amplification decreased considerably after the administration of modified Ermiao granules; therefore, modified Ermiao granules could well reverse precancerous lesions caused by HPV.

P16 and Ki67 expression has been widely used to detect cell proliferation [8]. The excessive expression of P16 and Ki67 is associated with high cell proliferation activity and hyperplasia in precancerous or cancerous lesions. P16 and Ki67

Table 4. Differences in CD3/4/8+ among the three groups.

Groups	Before treatment				After treatment			
	CD3+	CD4+	CD8+	CD4+/CD8+	CD3+	CD4+	CD8+	CD4+/CD8+
TCM treatment group (n = 45)	51.37 ± 8.26*	37.16 ± 6.9*	30.97 ± 7.09*	1.36 ± 0.66*	53.75 ± 8.6$^\triangle$	39.57 ± 8.2$^\triangle$	28.12 ± 5.4$^\triangle$	1.71 ± 0.85$^\triangle$
Interferon group (n = 45)	51.21 ± 8.27*	37.34 ± 6.7*	30.53 ± 7.11*	1.35 ± 0.61*	53.38 ± 8.4$^\triangle$	39.24 ± 8.3$^\triangle$	28.23 ± 5.3$^\triangle$	1.69 ± 0.82$^\triangle$
Placebo group (n = 45)	51.44 ± 8.31	37.20 ± 7.18	31.04 ± 7.07	1.35 ± 0.72	50.75 ± 8.04	37.29 ± 7.16	30.92 ± 6.89	1.36 ± 0.68

$\triangle$ indicates $P < 0.0167$ compared with the placebo group; * indicates $P < 0.05$ compared with the treated group.

Table 5. Differences in IgA/M/G among the three groups.

Groups	Before treatment			After treatment		
	IgA	IgM	IgG	IgA	IgM	IgG
TCM treatment group (n = 45)	14.76 ± 4.23**	81.85 ± 9.36**	11.21 ± 3.04**	19.13 ± 4.88*$^\triangle$	91.52 ± 9.78*$^\triangle$	16.34 ± 4.26*$^\triangle$
Interferon group (n = 45)	14.82 ± 4.34**	82.21 ± 9.28**	11.29 ± 3.14**	16.32 ± 4.42*	85.62 ± 8.98*	14.34 ± 3.26*
Placebo group (n = 45)	14.55 ± 4.29	82.10 ± 9.33	11.24 ± 3.16	14.33 ± 3.75	81.52 ± 9.78	11.34 ± 3.01

*indicates $P < 0.0167$ compared with the placebo group; $\triangle$ indicates $P < 0.0167$ compared with the interferon group; ** indicates $P < 0.05$ compared with the treated group.

Table 6. Comparison of *hTERC* amplification between groups.

Groups	Before treatment	After treatment
	Number of *hTERC* amplification cases	Number of *hTERC* amplification cases
TCM treatment group (n = 45)	36**	11*$^\triangle$
Interferon group (n = 45)	36**	23*
Placebo group (n = 45)	34	35

*indicates $P < 0.0167$ compared with the placebo group; $\triangle$ indicates $P < 0.0167$ compared with the interferon group; **indicates $P < 0.05$ compared with the treated group.

Table 7. mRNA expression levels of P16 and Ki67 before and after treatment in the three groups.

Groups	Before treatment		After treatment	
	P16 mRNA	Ki67 mRNA	P16 mRNA	Ki67 mRNA
TCM treatment group (n = 45)	0.87 ± 0.18**	1.17 ± 0.11**	0.76 ± 0.22*	0.92 ± 0.06*
Interferon group (n = 45)	0.88 ± 0.17**	1.16 ± 0.09**	0.77 ± 0.21*	1.02 ± 0.10*
Placebo (n = 45)	0.89 ± 0.19	1.17 ± 0.13	0.86 ± 0.18	1.18 ± 0.12

*indicates $P < 0.0167$ compared with the placebo group; **indicates $P < 0.05$ compared with the treated group.

are widely used to detect and grade CIN and cervical cancer [9, 10]. In this study, the protein and mRNA expression levels of P16 and Ki67 decreased significantly after the administration of modified Ermiao granules, suggesting that modified Ermiao granules might reverse CIN by inhibiting cell proliferation.

The effect of HPV infection on the local cervical micorenvironment also exists in varying degrees, including immune function as an important aspect related to infection. Changes in local immunity reflect the host's response to infection-induced stress and could correlate with response to treatment and clinical outcome [11–13]. HPV infection, and the presence of inflammatory stress caused by infection can lead to changes in immune regulation [14]. T-lymphocyte subsets in cellular immunity and IgA, IgG, and IgM in humoral immunity maintain the body's high immune status. Therefore, we compared the levels of T lymphocytes CD3+, CD4+, and CD8+ and IgA, IgM, and IgG in our patients before and after the treatment. The differences in the results confirmed

that the T lymphocyte subgroups and IgA, IgM, and IgG are closely related to the treatment effect.

TLR4 is considered a receptor associated with immunity and inflammation. TLR4 and its mediated signaling pathways play an important role in the occurrence and development of cervical cancer, especially in inflammatory tumors. TLR4 activation may destroy MyD88 by activating the NF-kB signal to release a large number of inflammatory factors and inhibit the cervical local immune function by interacting with the host's immune system. Hence, the immune system cannot properly identify and attack HPV, inducing the formation of an inflammatory microenvironment and promoting the development of cervical lesions [15]. The present study found that the modified Ermiao granules can decrease high-risk HPV persistent infections with CIN I on cervical biopsy by reducing TLR4 and the expression of the downstream cytokine NF-kB P65. Prompt administration of modified Ermiao granules might prevent the release of inflammatory factors and improve inflammatory lesions by inhibit-

ing TLR4 signaling transduction pathways, which are similar to the function of TLR4 blockers. Our results suggest that modified Ermiao granules could enhance the local immunity of cervical tissues to treat HPV and reverse CIN. However, whether or not the modified Ermiao granules could treat high-risk HPV and reverse CIN I through its effect on the TLR4/NF-KB signaling pathway should continue to be studied.

In summary, modified Ermiao granules are based on the TCM treatment concept of "strengthening body resistance". We have shown that the modified Ermiao granules have a significant effect on patients with CIN I due to HPV infection through the effective elimination of the persistent high-risk HPV infection and reversing CIN I by clearing heat, inhibiting dampness, and invigorating the spleen. Its mechanism of action may be related to the inhibition of cell proliferation and the enhancement of local immune function. Further studies on the clinical impact of the modified Ermiao granules on HPV infection and CIN are warranted.

Author contributions

WWX and JT conceived and designed the experiments. WWX, JT, YMZ and QLR performed the experiments. YMZ and JT analyzed the data. WWX and JT wrote the paper.

Ethics approval and consent to participate

All subjects gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the ethical principles of NHFPC: Measures for Guidelines on Ethical Review of Biomedical Research Involving Human Subjects (2015), CFDA: Chinese Good Clinical Practice (2003), Provisions for Clinical Trials of Medical Devices (2015), WMA: Declaration of Helsinki and CIOMS: International Ethical Guidelines for Biomedical Research Involving Human Subjects,and the protocol was approved by the IRB of Affiliated Hospital of Nanjing University of Chinese Medicine (Jiangsu Province Hospital of Chinese Medicine) (approval number: 2015NL-108-02).

Conflict of interest

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

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