

## Original Article

# Effects of warm silver needle acupuncture combined with myofascial trigger point intervention on peripheral neutrophil autophagy and alleviation of chronic pelvic pain in women

Fubo Tian<sup>1\*</sup>, Yu Cao<sup>2\*</sup>, Yaojun Lu<sup>1</sup>, Zixuan Ren<sup>1</sup>, Shen Sun<sup>1</sup>, Jianhua Xia<sup>3</sup>

<sup>1</sup>Department of Anesthesiology, Shanghai Obstetrics and Gynecology Hospital, Fudan University, 128 Shenyang Road, Shanghai 200090, China; <sup>2</sup>Department of Urology, Ningxiang Hospital, Hunan University of Chinese Medicine, Ningxiang 410600, Hunan, China; <sup>3</sup>Department of Anesthesiology, Shanghai Pudong New Area People's Hospital, 490 Chuanhuan South Road, Shanghai 200090, China. \*Equal contributors.

Received September 29, 2023; Accepted December 28, 2024; Epub April 15, 2025; Published April 30, 2025

**Abstract:** Objective: To explore the single and combined therapeutic effects of warm silver needle acupuncture and myofascial trigger point (MTrP) intervention on chronic pelvic pain (CPP). Methods: CPP patients underwent treatment with warm silver needle acupuncture and/or MTrP intervention, and the therapeutic efficacy of each method was evaluated. Peripheral blood neutrophil autophagy and related plasma inflammatory factors, including IL-6, IL-8, IL-10, and monocyte chemoattractant protein-1 (MCP-1), were measured. Results: Our findings indicated that both warm silver needle acupuncture and MTrP intervention significantly relieved CPP-associated pain and substantially improved patients' physical and mental health. Notably, compared with healthy controls, increased neutrophil autophagy and elevated plasma inflammatory factor levels were observed in CPP patients. These changes were inhibited by both single and combined treatment modalities. The combined treatment exhibited particularly remarkable efficacy, conferring additional benefits in pain relief and overall well-being improvement for women with CPP. The levels of all inflammatory factors (IL-6, IL-8, IL-10, and MCP-1) in the combined intervention group were significantly lower than those in the single intervention groups (all  $P < 0.05$ ). Conclusion: Warm silver needle acupuncture and MTrP intervention effectively alleviate CPP by modulating neutrophil autophagy and inflammatory factors, and their combination yields enhanced therapeutic benefits for women with CPP.

**Keywords:** Chronic pelvic pain in women, warm acupuncture with silver needles, myofascial trigger points, neutrophil, autophagy

## Introduction

Chronic pelvic pain (CPP) is a prevalent and clinically refractory syndrome, with a global prevalence ranging from 6% to 27% among women aged 18-50 years [1]. The underlying mechanism of CPP is complex and its cure rate is low, exerting a significant toll on patients' physical and mental well-being as well as their quality of life [2, 3]. At present, in Western medicine, common treatment modalities include physical therapy, oral analgesics, laparoscopy surgery and psychotherapy. However, these approaches often fall short in terms of curative efficacy, with a high recurrence rate. In fact,

clinical evidence suggests that a single therapeutic method typically struggles to achieve satisfactory outcomes [1, 4, 5].

Pain generation and transmission occur via multiple pathways, involving nociceptors, afferent fibers, the dorsal horn of the spinal cord, spinal cord thalamic tract and other ascending tracts, cortical and limbic system control areas, descending control system and neurotransmitter release [6-8]. Dorsal root ganglia (DRG) are considered primary sensory afferent neurons and they are a crucial relay station for transmitting sensory impulses from the periphery to the central nervous system. DRG play a vital role in

conveying both somatic and visceral sensations [9]. DRG bridge the spinal cord with the body's internal and external environments. Specifically, DRG neurons receive peripheral primary sensory information and relay it to the spinal cord and other higher centers. As the principal type of sensory ganglia and the initial site of pain induction, they govern the sensory perception of most body parts, including internal organs [10].

Clinically, acupuncture at Zusanli (ST36) has been employed to treat pain, such as in cases of irritable bowel syndrome visceral pain, following the principle of "belly Zusanli stay". Physiological and anatomical investigations into the relationship between Zusanli and the intestines have revealed that the afferent nerves connecting Zusanli and the intestinal organs may converge in the lumbosacral DRG [11, 12]. Additional studies have demonstrated that Zusanli-based interventions, such as acupoint catgut embedding and acupuncture therapy, yield superior efficacy in managing CPP syndrome [13, 14]. It is hypothesized that acupuncture may achieve sensitivity inhibition and analgesic effects by activating the afferent neurons at Zusanli, which in turn regulate adjacent intestinal afferent neurons through the inhibitory signal pathway mediated by satellite glial cells.

Myofascial pelvic pain accounts for over 22% of CPP cases [15]. Myofascial trigger points (MTrPs), previously known as trigger points and also referred to as pain points or trigger spots, constitute the primary drivers of chronic myofascial inflammation [16]. MTrPs associated with visceral pain are widely dispersed, often located within the referred pain areas, although many are situated some distance away. Notably, brief and intense stimulation of MTrPs can frequently lead to prolonged pain relief [17]. Previous research has distinguished between potential MTrPs and activated MTrPs [18]. Activation of MTrPs typically manifests as spontaneous pain, where deep pressure can elicit local or referred pain at a distance, and acupuncture can trigger a local muscle twitching response. In contrast, potential MTrPs only produce pain upon firm pressure [19]. Visceral diseases can induce sensitization on the body surface, representing a pathological connection. Consequently, surface stimulation techniques like acupuncture can potentially influ-

ence various conditions, including visceral pain, through the integration of sensory convergence and incoming information. This may also be linked to the out-of-phase activation of primary neurons in the DRG.

Acute inflammatory responses mediated by peripheral neutrophils have been indicated to potentially prevent the progression of chronic pain. An active inflammatory response, particularly one regulated by neutrophils, is conducive to pain relief [20]. Neutrophil autophagy, as a conserved self-degradation process, plays a pivotal role in the phagocytosis of pathogens by neutrophils [21]. Under inflammatory conditions, increased peripheral blood neutrophil autophagy occurs concomitantly with elevated concentrations of inflammatory factors like IL-6, IL-8, IL-10, and monocyte chemoattractant protein-1 (MCP-1) [22]. Previous studies have demonstrated that elevated levels of IL-6, IL-8, IL-10, and MCP-1 are associated with CPP [23-25]. Growing evidence suggests that abnormal autophagy levels, along with the resultant dysfunction of the immune system, are linked to the pathogenesis and progression of endometriosis. Recent studies have revealed heightened expression of autophagy-related proteins in the ectopic tissues of patients with endometriosis [26]. Autophagy plays a role and may serve as a potential target for endometriosis, which commonly leads to CPP [27].

Traditional Chinese medicine (TCM) emphasizes holistic syndrome differentiation and Qi-blood regulation. In the present study, we explored a combined TCM approach. Specifically, we intended to regulate peripheral receptors by MTrP intervention and spinal dorsal horn nerve conduction mediated by silver needles for comprehensive treatment of CPP. Additionally, we sought to investigate whether peripheral blood neutrophil autophagy was implicated. As far as we know, no similar studies have been reported. Previous research has predominantly focused on either acupuncture or myofascial trigger points in isolation and has not utilized the combination of the two to treat CPP. Warm silver needle acupuncture combined with MTrP intervention holds promise as a candidate treatment for CPP by modulating the low (peripheral sensor), middle (spinal dorsal horn), and high (cerebral cortex) levels along the ascending nerve conduction pathway.

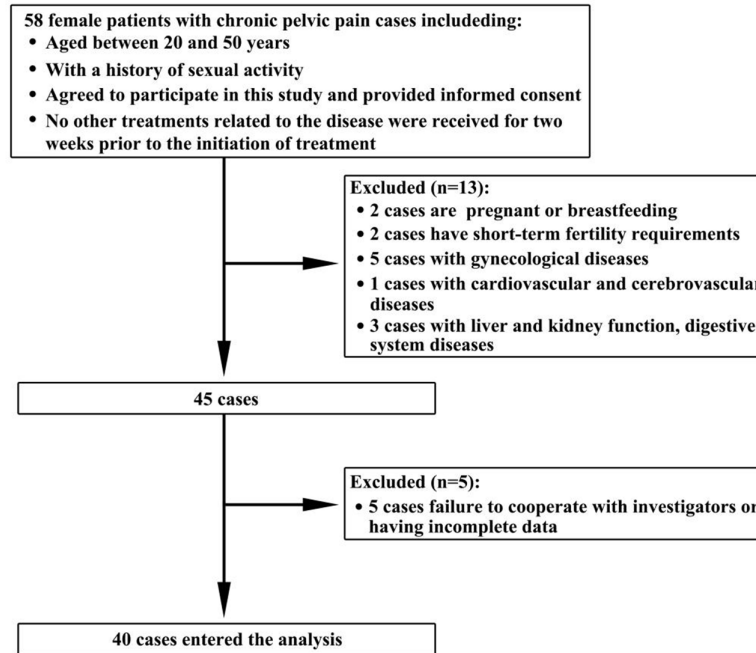


Figure 1. Flow diagram.

## Materials and methods

### Reagents and instruments

Human IL-6 (JL14113), IL-8 (JL19291), IL-10 (JL19246), MCP-1 (JL19334), serotonin (5-HT) (JL10725), and  $\beta$ -endorphin ( $\beta$ -EP) (JL11415) assay kits were procured from Shanghai Jianglai Biotechnology Co., Ltd. Silver needles (0.25×145 mm) were purchased from Shanghai Taicheng Technology Development Co., Ltd. Millimeter needles, namely disposable sterile acupuncture needles (0.45×75 mm, No. 2270463), were provided by Jiangsu Wujiang Jiachen Acupuncture Instrument Co., Ltd. The human peripheral blood neutrophil isolation solution kit (P9040) was purchased from Solarbio Technology Co., Ltd.

### Participants

This study was conducted in accordance with the Ethics Committee of Fudan University (Ethical approval in obstetrics and gynecology: 2022-144) and adopted a retrospective cohort study design. Forty patients with CPP were enrolled in the present study based on the diagnostic criteria of both Western medicine and TCM [28-30]. The Western diagnostic criteria

for CPP include a prior history of pelvic inflammatory disease, pelvic surgery, and endometriosis. CPP typically manifests with varying intensities, from mild to severe, and persists for over six months. Gynecological examinations usually yield negative results, and the pain severity does not correlate with physical findings. The TCM diagnostic criteria for CPP correspond to the key points of TCM viscera syndrome differentiation and qi-blood syndrome differentiation. Primary symptoms involve lower abdominal and lumbosacral pain, while secondary symptoms often exacerbate with fatigue, sexual intercourse, or occur before and after menstruation. Patients may also experience a sense of heaviness in the

anal region, accompanied by low-grade fever, increased menstruation, and leucorrhea, as well as symptoms of irritability or depression. Examination findings might reveal a dark red tongue, potentially showing petechiae or ecchymosis, with a white or yellow coating, and astringent pulse with a string-like quality. The inclusion criteria were as follows: 1) Women who fulfilled the above diagnostic criteria for CPP in both Chinese and Western medicine; 2) Female patients aged between 20 and 50 years with a history of sexual activity; 3) Patients who voluntarily consented to participate in this study and provided informed consent; 4) Those who had not received any other treatments related to the disease for two weeks prior to treatment initiation. Exclusion criteria comprised: 1) Female patients who were pregnant, breastfeeding, or had short-term fertility requirements; 2) Patients with organic lesions of the reproductive system or gynecological acute abdominal and other gynecological diseases; 3) Patients with cardiovascular and cerebrovascular diseases, liver and kidney function, digestive system and other serious diseases; 4) Those who failed to cooperate with investigators (Figure 1). All patients received treatment after the experiment was completed.

# Acupuncture and myofascial trigger point intervention in CPP

## *Groups and treatment*

Thirty CPP patients were randomly divided into three groups, with ten patients in each group.

(1) For patients in the warm silver needle acupuncture group, they were asked to lie prone and relax their limbs to widen the vertebral space first. Subsequently, acupuncture treatment was administered at points including both sides of the Sanjiao Shu point, Weishu point, Shenshu point, Danchang Shu point, Ciliao point, and Zhongliao point. The spinal nerve root outlet was located under portable ultrasonic positioning to ensure accurate injection. For the acupuncture procedure, after routine skin disinfection, the needles were held in both hands until a strong qi sensation was achieved. All silver needles were heated for acupuncture for approximately 20 minutes. After burning, the ash was removed, the needles were withdrawn, and a sterile cotton ball was used to press the needle eye for a while. Occasionally, if patients felt that the local skin could not endure the heat of moxibustion, local acupoints were blocked to prevent skin burns.

(2) In the MTrP group, patients were placed in the supine position with relaxed legs and a pillow under the popliteal fossa. Centering on the pain site, the fingertip pulp was used to identify and record the trigger points. After routine skin disinfection, the needle (disposable sterile acupuncture needle, 0.45×75 mm) was held in both hands, with the left hand used for positioning and the right hand quickly inserting into the trigger points until the local muscle bundle twitched, and then the needle was withdrawn [31-33]. Occasionally, bleeding was observed, and an alcohol cotton ball was used for compression.

(3) In the silver needle combined with MTrP group, patients lay prone with relaxed limbs, and the points were selected according to the above technical procedures. Treatment was administered twice a week for a course of five weeks, with a total of 10 sessions. Occasionally, if patients felt that the local skin could not endure the heat of moxibustion, a small cardboard was provided to block the local acupoints to prevent skin burns.

## *Observation of curative effect*

A single-blind method was utilized to blind the patients. Outpatient doctors were randomly

divided into the experimental group and the control group based on the treatment order. The efficacy evaluation was carried out by a non-treating physician. The observation period was 35 days after treatment. Patients were required to fill in the follow-up registration form in detail, recording their symptoms and signs, observing the long-term efficacy, and checking for any secondary adverse reactions or complications. The general indicators included age, marital status, disease course, present history, past history, menstrual history, and pregnancy history.

The curative effect was indicated by the following therapeutic indicators in accordance with the Guiding Principles for Clinical Research of New Chinese Medicine for the Treatment of Pelvic Inflammatory Disease.

Changes in clinical symptoms: Clinical symptoms were observed before and after treatment and one month after treatment, covering the location, nature, degree of pain, aggravating or mitigating factors, relationship with menstruation, accompanying symptoms, changes in pain characteristics, whether accompanied by dysmenorrhea and pain during intercourse, sexual life, and the impact on patients' quality of life.

Changes in physical signs: Abdominal physical and gynecological examinations were conducted before and after treatment and one month after treatment, and the syndrome and signs score were evaluated. The main syndrome items included lower abdomen or lower abdominal distension pain, dull pain, and depression distension, each scored 5 points. Lumbosacral pain and swelling pain were scored with 5 points. Abnormal leukorrhea was scored with 5 points. Secondary symptoms such as breast pain were scored with 3 points. Lumbar and abdominal pain during fatigue, after intercourse, and before and after menstruation were scored with 3 points. Epigastric fullness was scored with 3 points. Poor appetite was scored as 3 points. Irritability was scored as 3 points. Emotional depression or occasional sighing were scored as 3 points. Irregular menstruation was scored with 3 points. Abnormal tongue image was scored with 2 points. Abnormal pulse was scored as 2 points. Physical signs scoring included uterine mobility limitation or fixation, each scored 3 points. Uterine tenderness was scored as 3 points.

## Acupuncture and myofascial trigger point intervention in CPP

Tubal cord thickening was scored as 2 points. Tubal tenderness was scored as 5 points. Parauterine lamellar thickening was scored as 2 points. Lateral uterine tenderness was scored as 5 points. The syndrome score in this study was 40 points, the physical signs score was 20 points, and the total score was 60 points.

**Pain Degree VAS Scale:** Before and after treatment and one month after treatment, a 0-10 scale was used to represent different degrees of pain, where 0 indicated no pain and 10 represented the most severe pain. Patients circled a number that best reflected their pain level. A score of 1-3 indicated mild pain, 4-6 indicated moderate pain, and 7-10 indicated severe pain.

**Evaluation of depression and anxiety:** Hamilton Depression Scale (HAMD) (17 items) and Hamilton Anxiety Scale (HAMA) were used before and after treatment and one month after treatment. The reduction rate of HAMD and HAMA was calculated as: [(pre-treatment score - post-treatment score)/pre-treatment score<sup>3</sup> 100%].

**Quality of life (QOL) assessment:** The Chinese version of the Health status questionnaire (SF-36) was used before and after treatment and one month after treatment to score the patients' quality of life, including functional status, health satisfaction, and overall health evaluation. A total of 36 items were scored.

### *ELISA assay*

Plasma levels of IL-6, IL-8, IL-10, MCP-1, 5-HT, and  $\beta$ -EP were measured at each time point. For plasma 5-HT and  $\beta$ -EP determination: three mL of fasting venous blood was collected before and after treatment and quickly injected into a pre-chilled glass test tube containing 2500 IU aprotinin and 50  $\mu$ L of 1% heparin. The sample was then centrifuged at 3000 r/min for 15 min at 4°C. The plasma was aspirated and stored at -40°C until measurement. The plasma serotonin level was quantified using ELISA kits, with the operation strictly adhering to the manufacturer's instructions.

### *Peripheral blood neutrophil isolation and autophagy observation*

Neutrophils were purified from peripheral blood samples following the kit instructions. Briefly, 5

mL of peripheral blood from the aforementioned participants was layered with the lymphoprep density gradient solution provided in the kit in a centrifuge tube and centrifuged at 500 g for 20 min at room temperature. The neutrophils were collected and washed with red blood cell lysis buffer to remove red blood cells, followed by two washes with PBS. After centrifugation at 350 g for 5 min, the neutrophils were collected for autophagy observation.

A transmission electron microscope (TEM) (HITACHI, 7800, Japan) was used to observe autophagy. The neutrophils were sequentially fixed in 3% glutaraldehyde and 1% osmium tetroxide. After serial acetone dehydration and infiltration, the samples were centrifuged at 10,000 g, and ultrathin sections were prepared. The ultrathin sections were stained with 2.6% lead citrate solution and then examined under TEM to identify the typical autophagic double-membrane vacuoles in the cytoplasmic region.

### *Data statistical analysis*

All data were meticulously preserved and managed by dedicated personnel. Clinical observations were recorded on uniformly printed, paginated observation forms. Data statistics were coordinated with professional statisticians for comprehensive statistical analysis and description. All data were analyzed using SPSS 18.0 and GraphPad Prism 9. Measurement data were statistically presented as mean  $\pm$  standard deviation (mean  $\pm$  SD). The significance [of the difference between two samples was evaluated using the t-test. ANOVA followed by post hoc Bonferroni test was employed to analyze differences when comparing three or more groups. A *P* value less than 0.05 was considered statistically significant.

## **Results**

### *Comparison of general conditions*

In this study, 40 patients with CPP were screened for inclusion, among which 30 patients underwent different interventions. There were no statistically significant differences in mean age, BMI, disease duration, as well as peripheral blood eosinophils, leukocytes, and neutrophil counts among the three pre-treat-

## Acupuncture and myofascial trigger point intervention in CPP

**Table 1.** Comparison of general conditions

Characteristic	Control (n=10)	CPP+WA (n=10)	CPP+MTrPs (n=10)	CPP+WA+MTrPs (n=10)
Age (years)	34.9±6.61	35.2±5.77	35.3±5.31	35.1±5.24
Body mass index (kg/m <sup>2</sup> )	22.9±2.19	23.1±3.02	22.8±2.52	23.2±2.64
Duration of disease (months)	13.3±0.7	13.2±0.5	13.5±0.8	14.6±0.6
Treatment				
Surgery	No	No	No	No
Non-central analgesics	10 (100)	10 (100)	10 (100)	10 (100)
Traditional medicine	No	No	No	No
Eosinophil (%)	1.70±0.67	0.90±0.74	1.00±0.47	0.90±0.74
Leukocyte (10 <sup>9</sup> /L)	4.23±0.73	9.06±1.64	9.00±1.84	8.91±1.45
Neutrophil (%)	2.37±0.50	6.19±1.79	6.34±1.97	6.10±1.56

CPP+WA: chronic pelvic pain (CPP) patients treated with warm silver needle acupuncture. CPP+MTrPs: CPP patients treated with myofascial trigger points. CPP+WA+MTrPs: CPP patients treated with warm silver needle acupuncture combined with myofascial trigger points.

ment groups (all  $P > 0.05$ ), indicating comparability, as shown in **Table 1**. Notably, all CPP patients did not receive prior TCM treatment.

### *Comparison of clinical symptoms, signs, and pain scale (VAS)*

Pre-treatment scores for syndrome and physical signs did not differ significantly among the groups (both  $P > 0.05$ ) (**Figure 2A-C**). After treatment and follow-up, the total scores of the warm silver needle acupuncture combined with MTrP group were significantly lower than those of the warm silver needle acupuncture group or the MTrP group when treated alone (both  $P < 0.05$ ). No significant differences were detected between the two single-treatment groups (both  $P > 0.05$ ). VAS scores were comparable among the three groups before treatment ( $P > 0.05$ ), but were significantly reduced after treatment and at follow-up ( $P < 0.05$ ) (**Figure 2D**). The combination group exhibited lower VAS scores than either single-treatment group ( $P < 0.05$ ).

### *Depression and anxiety rating (HAMD, HAMA), quality of life (QOL) assessment and 5-HT and $\beta$ -EP measurement*

No significant differences were observed in the reduction of depression and anxiety between the warm silver needle acupuncture group and the MTrP intervention group (both  $P > 0.05$ , **Figure 3A**). However, the combination of warm silver needle acupuncture and MTrP intervention showed additive effects in reducing depres-

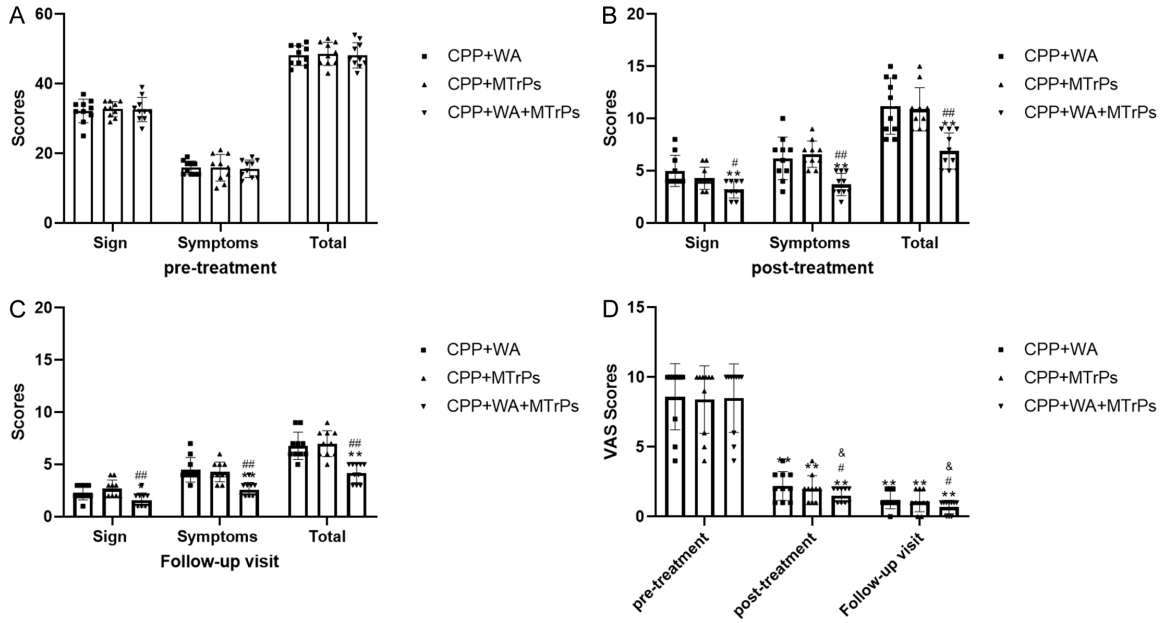
sion and anxiety (both  $P < 0.05$ ). Quality of life scores showed significant improvements across all groups after treatment ( $P < 0.05$ ) (**Figure 3B**). The total scores of patients in the three groups improved to varying degrees after treatment, with the combined treatment yielding superior results compared to the single treatments ( $P < 0.05$ ).

The levels of 5-HT and  $\beta$ -EP were measured at each time point to evaluate the therapeutic effect, as per previous research [34]. After treatment, the plasma 5-HT level decreased and the plasma  $\beta$ -EP level increased in all groups, and these differences were statistically significant compared with those before treatment (both  $P < 0.05$ ) (**Figure 3C, 3D**). Based on the 5-HT and  $\beta$ -EP assays, after treatment, the improvement in the combination group was more pronounced than that in the other two single-treatment groups ( $P < 0.05$ ).

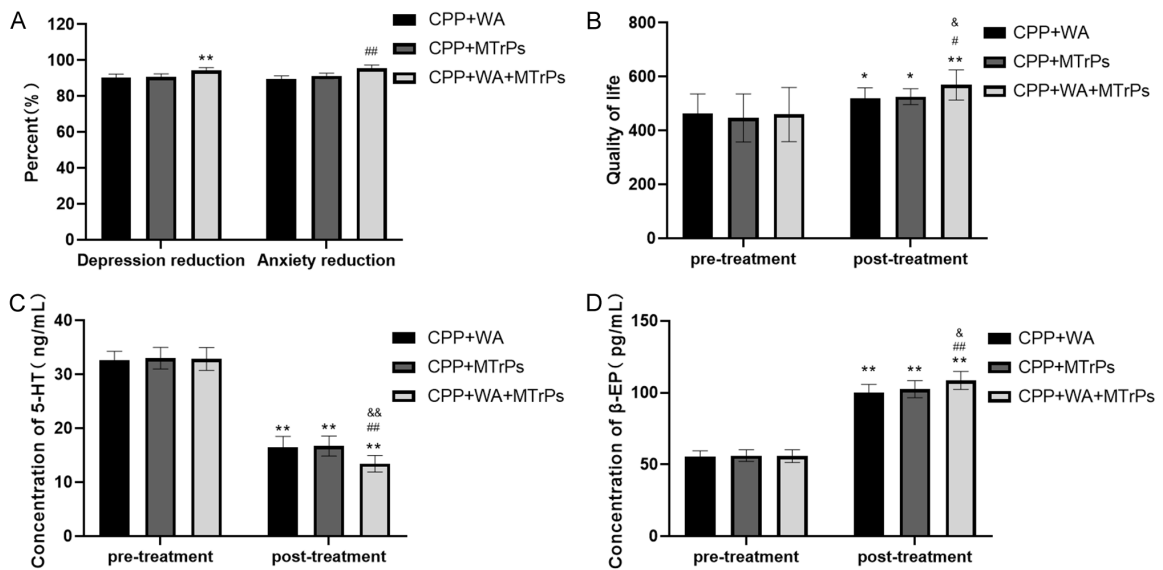
### *Impact of combination intervention on peripheral blood neutrophil autophagy in CPP*

To elucidate the mechanism underlying the treatment of CPP using warm silver needle acupuncture and MTrP intervention, peripheral blood neutrophil autophagy was investigated in this study. As shown in **Figure 4A**, the neutrophil count in CPP patients tended to decrease after treatment compared with the pre-treatment level, although the difference was not statistically significant. TEM was used to observe neutrophil autophagy in patients from each group. The results, presented in **Figure 4B, 4C**,

## Acupuncture and myofascial trigger point intervention in CPP



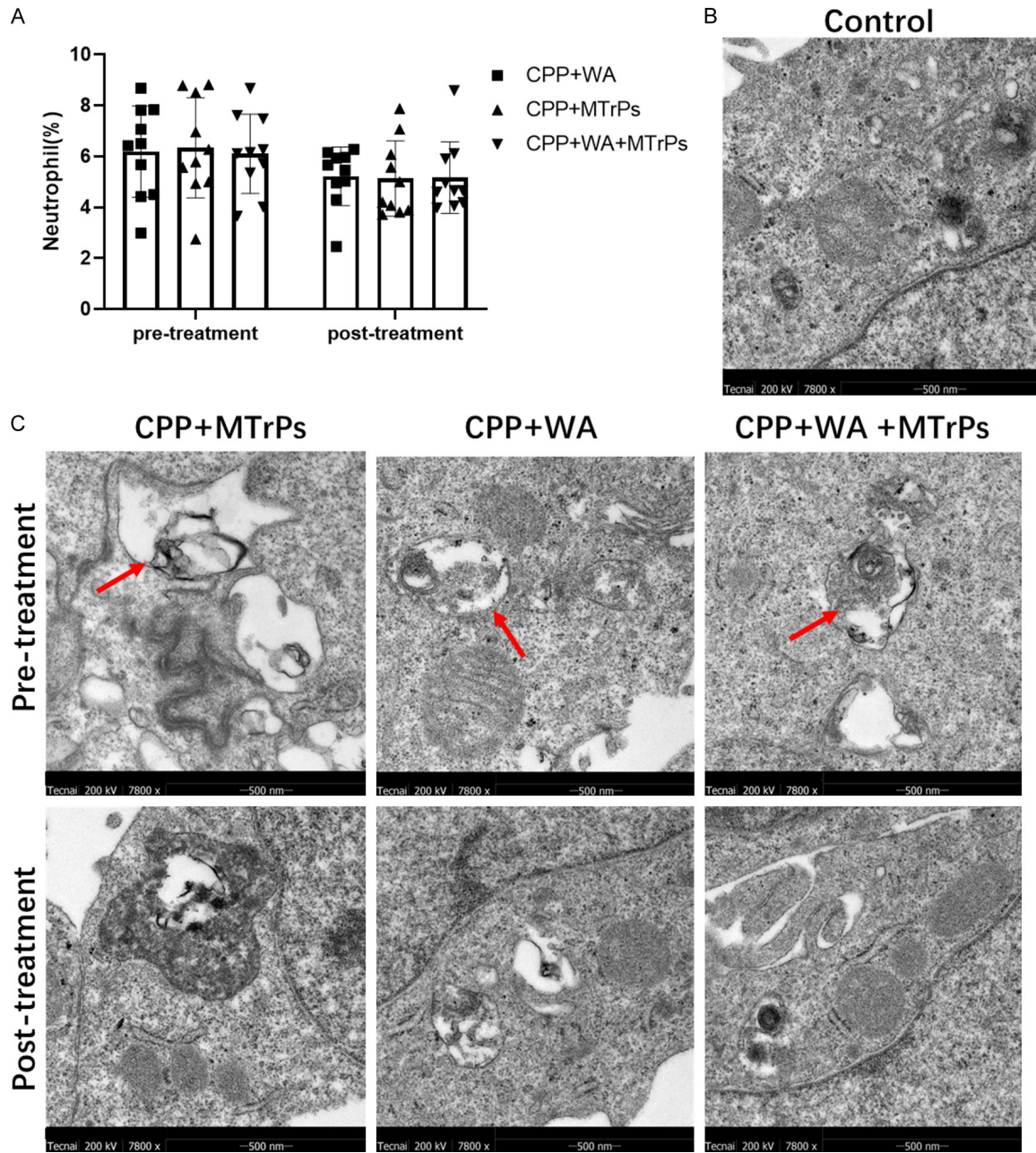
**Figure 2.** Comparison of clinical observations. CPP+WA, chronic pelvic pain (CPP) patients treated with warm silver acupuncture (n=10). CPP+MTrPs, CPP patients treated with myofascial trigger points (n=10). CPP+WA+MTrPs, CPP patients treated with warm silver acupuncture combined with myofascial trigger points (n=10). A. Clinical symptoms and signs of pre-treated patients in each group. B. Clinical symptoms and signs of post-treated patients in each group. C. Clinical symptoms and signs of follow-up visited patients in each group. \* $P < 0.05$ , \*\* $P < 0.01$ , the patients in each group compared with those in CPP+WA group. ## $P < 0.05$ , ### $P < 0.01$ , the patients in each group compared with those in the CPP+MTrPs group. D. Pain scale (VAS) of patients in each group. \* $P < 0.05$ , \*\* $P < 0.01$ , the patients in each group with corresponding pre-treatment. # $P < 0.05$ , the patients compared with those in the CPP+WA group. & $P < 0.05$ , the patients compared with those in the CPP+MTrPs group. CPP+WA: chronic pelvic pain (CPP) patients treated with warm silver needle acupuncture. CPP+MTrPs: CPP patients treated with myofascial trigger points. CPP+WA+MTrPs: CPP patients treated with warm silver needle acupuncture combined with myofascial trigger points.



**Figure 3.** Clinical efficacy evaluation. CPP+WA, chronic pelvic pain (CPP) patients treated with warm silver acupuncture (n=10). CPP+MTrPs, CPP patients treated with myofascial trigger points (n=10). CPP+WA+MTrPs, CPP patients treated with warm silver acupuncture combined with myofascial trigger points (n=10). A. Depression and Anxiety Rating of patients in each group. \*\* $P < 0.01$ , the patients in each group compared with those in CPP+WA group. ## $P < 0.01$ , the patients in each group compared with those in the CPP+MTrPs group. B. Life quality of patients in

## Acupuncture and myofascial trigger point intervention in CPP

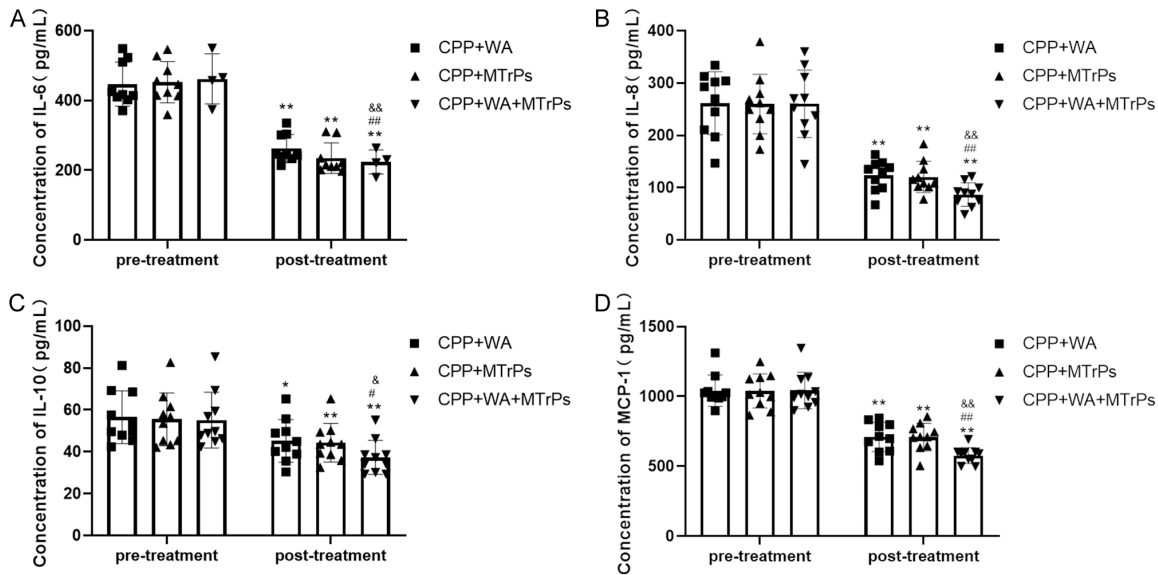
each group. C. The levels of 5-HT in patients of each group. D. The levels of  $\beta$ -EP in patients of each group.  $*P < 0.05$ ,  $**P < 0.01$ , the patients in each group with corresponding pre-treatment.  $\#P < 0.05$ ,  $\#\#\#P < 0.01$ , the patients compared with those in the CPP+WA group.  $\&P < 0.05$ ,  $\&\&P < 0.01$ , the patients compared with those in the CPP+MTrPs group. CPP+WA: chronic pelvic pain (CPP) patients treated with warm silver needle acupuncture. CPP+MTrPs: CPP patients treated with myofascial trigger points. CPP+WA+MTrPs: CPP patients treated with warm silver needle acupuncture combined with myofascial trigger points.



**Figure 4.** The effect of Warm acupuncture with silver needles and intervention in myofascial trigger point on neutrophil autophagy in chronic pelvic pain (CPP). CPP+WA, CPP patients treated with warm silver acupuncture (n=10). CPP+MTrPs, CPP patients treated with myofascial trigger points (n=10). CPP+WA+MTrPs, CPP patients treated with warm silver acupuncture combined with myofascial trigger points (n=10). A. The neutrophil (%) in the patients. B. Photograph of neutrophil from normal volunteer (Control). C. Photograph of neutrophil from CPP patients in each group. The red arrow indicates autophagosomes. CPP+WA: chronic pelvic pain (CPP) patients treated with warm silver needle acupuncture. CPP+MTrPs: CPP patients treated with myofascial trigger points. CPP+WA+MTrPs: CPP patients treated with warm silver needle acupuncture combined with myofascial trigger points.



## Acupuncture and myofascial trigger point intervention in CPP



**Figure 5.** The plasma levels of IL-6, IL-8, IL-10 and MCP-1 in chronic pelvic pain (CPP) patients. CPP+WA, CPP patients treated with warm silver acupuncture (n=10). CPP+MTrPs, CPP patients treated with myofascial trigger points (n=10). CPP+WA+MTrPs, CPP patients treated with warm silver acupuncture combined with myofascial trigger points (n=10). \* $P < 0.05$ , \*\* $P < 0.01$ , the patients in each group with corresponding pre-treatment. # $P < 0.05$ , ## $P < 0.01$ , the patients compared with those in the CPP+WA group. & $P < 0.05$ , && $P < 0.01$ , the patients compared with those in the CPP+MTrPs group. CPP+WA: chronic pelvic pain (CPP) patients treated with warm silver needle acupuncture. CPP+MTrPs: CPP patients treated with myofascial trigger points. CPP+WA+MTrPs: CPP patients treated with warm silver needle acupuncture combined with myofascial trigger points.

revealed that more vesicular autophagosomes with a double or multilayer structure were observed in neutrophils from CPP patients in each group before treatment than in the control group. After treatment, the CPP-mediated increase in autophagosomes was significantly reduced in all patients, particularly in those treated with the combination of silver needle acupuncture and MTrP.

### Plasma levels of IL-6, IL-8, IL-10 and MCP-1 in CPP patients treated with combination treatment

Plasma levels of IL-6, IL-8, IL-10, and MCP-1 were measured at each time point. After treatment, the plasma levels of IL-6, IL-8, IL-10, and MCP-1 decreased in all groups compared with the pre-treatment levels (all  $P < 0.05$ ) (Figure 5A-D). After treatment, the plasma levels of IL-6, IL-8, IL-10, and MCP-1 in the combination groups were lower than those in the patients treated with silver needle acupuncture or MTrP alone. The effect of the combination group was more obvious than that of the other two single-treatment groups on the plasma levels of IL-6, IL-8, IL-10, and MCP-1 in CPP patients (all  $P < 0.05$ ).

## Discussion

CPP is a prevalent disease among women, with an increasingly younger age of onset, posing a significant threat to women's physical and mental well-being [35-37]. Currently, due to the complexity of the disease, the clinical efficacy of treating CPP remains limited [5]. TCM, however, holds a unique edge in CPP management, characterized by fewer side effects, diverse treatment modalities, and well-established therapeutic effects [38, 39]. In this study, warm silver needle acupuncture, MTrP intervention, and their combination were employed in the treatment of CPP in women, and the underlying molecular mechanisms were explored.

TCM emphasizes a systematic holistic approach. In TCM theory, CPP is categorized as "abdominal pain", "mass", "female abdominal pain", "heat invading the blood chamber", "lower belt disease", and "dysmenorrhea" [40, 41]. As stated in "Female Section Jinglun", "For menstrual disorders and abdominal pain, it is not just about the pain; all stem from blood disharmony, and treatment should focus on regulating qi and blood" [42]. Female abdominal pain is further classified into patterns such as

“Qi-cold blood stasis”, “Qi stagnation and blood coagulation”, “liver channel blood deficiency”, “liver channel dampness-heat”, “liver-spleen deficiency”, and “liver-spleen deficiency-cold” [43]. The application of acupuncture and MTrP intervention in CPP patients aligns with the holistic principles of TCM.

In this study, a thicker and longer silver needle, compared to the ordinary filiform needle, was utilized for warm acupuncture in CPP treatment. This was based on prior research indicating that the therapeutic use of silver needles in pain disorders has yielded remarkable results [44, 45]. Studies have demonstrated that silver needles possess excellent thermal conductivity, enabling them to fully harness the medicinal properties of moxa sticks during acupuncture, with the body temperature reaching approximately 52°C [46]. The deeper and stronger stimulation provided by silver needles can elicit a potent response in local tissues, leading to more pronounced analgesic effects after warm acupuncture and moxibustion. Our findings corroborated previous studies, suggesting that silver needle warm acupuncture significantly alleviated CPP-induced pain and improved patients’ physical and mental health [44, 45].

Our study also revealed the efficacy of MTrP intervention in relieving CPP. This may be attributed to the fact that MTrPs are thought to be present in the pelvic floor muscles and/or lower abdominal wall, associated with myofascial pelvic pain, a common non-articular musculoskeletal disorder and one of the most prevalent gynecological conditions, implicated in an estimated 22% to 94% of cases of CPP globally [15]. Myofascial pelvic pain may coexist with endometriosis and dysmenorrhea [15, 47]. In women with endometriosis-associated CPP, myofascial dysfunction and hypersensitivity extend beyond the pelvis [48]. Active MTrPs are more frequently observed in women with primary dysmenorrhea, particularly during menstruation, with a prevalence of over 50% in the iliococcygeus muscle and 100% in the internal obturator muscle during the menstrual phase [49]. Given the pathological connection that visceral diseases can induce sensitization on the body surface, surface stimulation like acupuncture can effectively address visceral pain through the integration of sensory convergence and incoming information. In light of the role of MTrPs in CPP, acupuncture was used to deacti-

vate trigger points in our patients. Our results demonstrated that MTrP intervention could also mitigate pain and enhance the physical and mental health of CPP patients, yielding outcomes comparable to those of silver needle warm acupuncture.

In accordance with the systematic holistic theory of TCM and the diverse mechanisms of silver needle warm acupuncture and MTrPs, the clinical therapeutic effects of combining silver needle warm acupuncture with MTrPs were investigated in CPP patients in this study. The results demonstrated that the combined treatment of silver needle warm acupuncture and MTrPs exerted the most significant impacts on the symptoms of pain, depression and anxiety ratings, and quality of life in CPP patients, compared to single treatments using either silver needle warm acupuncture or MTrP intervention alone. These findings were corroborated by the levels of 5-HT and  $\beta$ -EP assays and pain-related therapeutic effect indicators [34, 50]. Our results suggested that silver needle warm acupuncture and MTrP intervention had additive effects in the treatment of CPP in women.

Subsequently, the underlying mechanisms of silver needle warm acupuncture and MTrP intervention in CPP were explored. Previous research has indicated that an active inflammatory response, especially one regulated by neutrophils, can aid in pain relief, while suppressing this active immune response may lead to prolonged pain [20]. Neutrophil autophagy represents one of the self-protection mechanisms under abnormal conditions, and peripheral neutrophil autophagy can enhance inflammatory factors such as IL-6, IL-8, IL-10, and MCP-1 [22] (1, 18). Moreover, elevated levels of IL-6, IL-8, IL-10, and MCP-1 have been observed in the peripheral blood of CPP patients [51, 52]. It has been proposed that CPP may be alleviated by reducing the levels of these inflammatory factors [52, 53]. Therefore, in this study, the effects of silver needle warm acupuncture and MTrP intervention on peripheral blood neutrophil autophagy and the levels of IL-6, IL-8, IL-10, and MCP-1 in CPP were examined. Results revealed that, compared with the control group, more prominent peripheral blood neutrophil vesicular autophagosomes were detected in CPP patients. After treatment with silver needle warm acupuncture, MTrP intervention, or their combination, a decrease in neutrophil

autophagy was observed in pre-treated CPP patients, with the combination of silver needle warm acupuncture and MTrP intervention significantly suppressing CPP-induced peripheral blood neutrophil autophagy. Consistent with previous studies, the levels of IL-6, IL-8, IL-10, and MCP-1 were higher in CPP patients than in the control group [51, 52]. Singular treatments of silver needle warm acupuncture or MTrP intervention, as well as their combination, all effectively suppressed the CPP-induced elevation of plasma inflammatory factors, which aligns with the notion that acupuncture or MTrP intervention can mitigate the inflammatory response associated with pain [54-57]. These results suggested that silver needle warm acupuncture or MTrP intervention might influence CPP related increases in peripheral blood neutrophil autophagy and inflammatory factor levels, and the two treatment modalities had additive effects. Nevertheless, this study had certain limitations. For instance, the mechanisms should be further validated in larger sample sizes and more in-depth experimental investigations, which will be incorporated into our future research. In conclusion, single treatments of either silver needle warm acupuncture or MTrP intervention could potentially relieve CPP by modulating peripheral blood neutrophil autophagy and inflammatory factor levels. Moreover, the combination of warm silver needle acupuncture and MTrP intervention exhibited enhanced suppressive effects on CPP, suggesting a promising therapeutic approach for female patients with CPP.

### Acknowledgements

This study was supported by the project of Shanghai Science and Technology Innovation Action Plan (No. 19411952000), Health Science and Technology Project of Health Commission of Pudong New Area (PW2020A-26), Scientific Research Project of Hunan Provincial Health Commission (No. 202204052428) and Hunan University of Chinese Medicine - Hospital Collaborative Research Grant Program 2022-183, the Science and Technology Innovation Plan of Shanghai Science and Technology Commission (No. 22Y11900400).

### Disclosure of conflict of interest

None.

**Address correspondence to:** Shen Sun, Department of Anesthesiology, Shanghai Obstetrics and Gynecology Hospital, Fudan University, 128 Shenyang Road, Shanghai 200090, China. E-mail: sunshen1980@126.com; Jianhua Xia, Department of Anesthesiology, Shanghai Pudong New Area People's Hospital, 490 Chuanhuan South Road, Shanghai 200090, China. E-mail: xiajianhua@shp-dph.com

### References

- [1] Spruijt M, Kerkhof M, Rombouts M, Brohet R and Klerkx W. Efficacy of botulinum toxin A injection in pelvic floor muscles in chronic pelvic pain patients: a study protocol for a multicentre randomised controlled trial. *BMJ Open* 2023; 13: e070705.
- [2] Paspulati RM. Chronic pelvic pain: role of imaging in the diagnosis and management. *Semin Ultrasound CT MR* 2023; 44: 501-510.
- [3] Erin T and McClurg AB. Evaluation and medical management of chronic pelvic pain. *Semin Intervent Radiol* 2023; 40: 372-378.
- [4] Lam CM, Keim SA and Latif U. Novel implantation technique for pudendal nerve peripheral nerve stimulation for treatment of chronic pelvic pain. *Reg Anesth Pain Med* 2023; 48: 567-571.
- [5] Park S, Lim W and Song G. Delphinidin induces antiproliferation and apoptosis of endometrial cells by regulating cytosolic calcium levels and mitochondrial membrane potential depolarization. *J Cell Biochem* 2019; 120: 5072-5084.
- [6] Bar-Shalita T, Granovsky Y, Parush S and Weissman-Fogel I. Sensory Modulation Disorder (SMD) and pain: a new perspective. *Front Integr Neurosci* 2019; 13: 27.
- [7] Garcia K, Wray JK and Kumar S. Spinal cord stimulation. *StatPearls*; 2023.
- [8] Chiang MC, Bowen A, Schier LA, Tupone D, Uddin O and Heinricher MM. Parabrachial complex: a hub for pain and aversion. *J Neurosci* 2019; 39: 8225-8230.
- [9] Song DD, Li Y, Tang D, Huang LY and Yuan YZ. Neuron-glial communication mediated by TNF- $\alpha$  and glial activation in dorsal root ganglia in visceral inflammatory hypersensitivity. *Am J Physiol Gastrointest Liver Physiol* 2014; 306: G788-795.
- [10] Berta T, Strong JA, Zhang JM and Ji RR. Targeting dorsal root ganglia and primary sensory neurons for the treatment of chronic pain: an update. *Expert Opin Ther Targets* 2023; 27: 665-678.
- [11] Bian GH, Li ZS, Wang SM, Wang J, Sun P and Wang X. The origin and segmental distribution of primary afferent nerves in different parts of

## Acupuncture and myofascial trigger point intervention in CPP

- large intestine in cats by means of HRP. *Journal of Jinzhou Medical College* 1993; 3: 1-6.
- [12] Li RW, Li CH and Wang ZM. Segmental distribution of sensory and motor neurons at different levels in Zusanli point area. *Chinese Acupuncture* 2000; 3: 33-35.
- [13] Wang QC, Chen YM, Jia MJ and Zhai HL. Efficacy observation of chronic pelvic inflammation of different differentiated patterns/syndromes treated with acupoint embedding therapy. *Zhongguo Zhen Jiu* 2012; 32: 1081-1083.
- [14] Ma Y, Wang ZL, Sun ZX, Men B and Shen BQ. Efficacy observation on chronic pelvic pain syndrome of damp-heat stagnation pattern treated with acupoint catgut embedding therapy. *Zhongguo Zhen Jiu* 2014; 34: 351-354.
- [15] Ross V, Detterman C and Hallisey A. Myofascial pelvic pain: an overlooked and treatable cause of chronic pelvic pain. *J Midwifery Womens Health* 2021; 66: 148-160.
- [16] Huang QM, Wang J, Zhang JW and Wang JL. Treatment of lateral epicondylitis based on the principle of myofascial trigger points pain in 35 cases. *Clinical rehabilitation in China* 2005; 38: 64-65.
- [17] Melzack R, Stillwell DM and Fox EJ. Trigger points and acupuncture points for pain: correlations and implications. *Pain* 1977; 3: 3-23.
- [18] Ge HY, Arendt-Nielsen L and Madeleine P. Accelerated muscle fatigability of latent myofascial trigger points in humans. *Pain Med* 2012; 13: 957-964.
- [19] Hou CR, Tsai LC, Cheng KF, Chung KC and Hong CZ. Immediate effects of various physical therapeutic modalities on cervical myofascial pain and trigger-point sensitivity. *Arch Phys Med Rehabil* 2002; 83: 1406-1414.
- [20] Parisien M, Lima LV, Dagostino C, El-Hachem N, Drury GL, Grant AV, Huising J, Verma V, Meloto CB, Silva JR, Dutra GGS, Markova T, Dang H, Tessier PA, Slade GD, Nackley AG, Ghasemlou N, Mogil JS, Allegri M and Diatchenko L. Acute inflammatory response via neutrophil activation protects against the development of chronic pain. *Sci Transl Med* 2022; 14: eabj9954.
- [21] Zhang R, Sun C, Han Y, Huang L, Sheng H, Wang J, Zhang Y, Lai J, Yuan J, Chen X, Jiang C, Wu F, Wang J, Fan X and Wang J. Neutrophil autophagy and NETosis in COVID-19: perspectives. *Autophagy* 2023; 19: 758-767.
- [22] An Q, Yan W, Zhao Y and Yu K. Enhanced neutrophil autophagy and increased concentrations of IL-6, IL-8, IL-10 and MCP-1 in rheumatoid arthritis. *Int Immunopharmacol* 2018; 65: 119-128.
- [23] Karshikoff B, Martucci KT and Mackey S. Relationship between blood cytokine levels, psychological comorbidity, and widespreadness of pain in chronic pelvic pain. *Front Psychiatry* 2021; 12: 651083.
- [24] Rocha AL, Vieira EL, Maia LM, Teixeira AL and Reis FM. Prospective evaluation of a panel of plasma cytokines and chemokines as potential markers of pelvic endometriosis in symptomatic women. *Gynecol Obstet Invest* 2016; 81: 512-517.
- [25] Chmaj-Wierzchowska K, Kampioni M, Wilczak M, Sajdak S and Opalai T. The role of chemokine and non-chemokine factors in pain-related complaints in patients with endometrial cysts. *Ginekol Pol* 2015; 86: 94-99.
- [26] Li H, Yang H, Lu S, Wang X, Shi X and Mao P. Autophagy-dependent ferroptosis is involved in the development of endometriosis. *Gynecol Endocrinol* 2023; 39: 2242962.
- [27] Yang HL, Mei J, Chang KK, Zhou WJ, Huang LQ and Li MQ. Autophagy in endometriosis. *Am J Transl Res* 2017; 9: 4707-4725.
- [28] Cao Z. *Chinese gynecology & obstetrics*. People's Medical Publishing House; 2014.
- [29] Liu M, Tan W, Xiao C, Luo S, Zhang Y and Jin X. *Traditional Chinese obstetrics and gynecology*. People's Medical Publishing House; 2011.
- [30] Li GH, Zhao L, Lu Y, Wang W, Ma T, Zhang YX and Zhang H. Development and validation of a risk score for predicting postoperative delirium after major abdominal surgery by incorporating preoperative risk factors and surgical Apgar score. *J Clin Anesth* 2021; 75: 110408.
- [31] Ye Z. Clinical observation of myofascial trigger point warming acupuncture in the treatment of chronic pelvic pain in women. *Modern distance education of Chinese Medicine* 2023; 21: 123-125.
- [32] Jiang RA and Zhang JH. Clinical effect of myofascial trigger point warming acupuncture on chronic pelvic pain in women. *Guizhou Medicine* 2020; 8: 1300-1301.
- [33] Gu LL and Lin FP. A randomized controlled study of pelvic floor manipulation combined with myofascial trigger point acupuncture in the treatment of chronic pelvic pain. *Modern Medicine* 2021; 27: 151-152.
- [34] Chen T, Wang K, Xu J, Ma W and Zhou J. Electroacupuncture reduces postoperative pain and analgesic consumption in patients undergoing thoracic surgery: a randomized study. *Evid Based Complement Alternat Med* 2016; 2016: 2126416.
- [35] Ball E and Khan KS. Recent advances in understanding and managing chronic pelvic pain in women with special consideration to endo-

- metriosis. *F1000Res* 2020; 9: F1000 Faculty Rev-83.
- [36] Grinberg K, Sela Y and Nissanholtz-Gannot R. New insights about chronic pelvic pain syndrome (CPPS). *Int J Environ Res Public Health* 2020; 17: 3005.
- [37] Lamvu G, Carrillo J, Ouyang C and Rapkin A. Chronic pelvic pain in women: a review. *JAMA* 2021; 325: 2381-2391.
- [38] Chen CM, Lin SJ, Tsai YN, Su YC, Chen IH, Galeano Machuca MP and Tseng YH. Association between traditional Chinese medicine body constitution deviation and chronic pelvic pain: a cross-sectional study. *Taiwan J Obstet Gynecol* 2023; 62: 713-718.
- [39] Shi J, Tan X, Feng G, Zhuo Y, Jiang Z, Banda S, Wang L, Zheng W, Chen L, Yu D and Guo C. Research advances in drug therapy of endometriosis. *Front Pharmacol* 2023; 14: 1199010.
- [40] Xue JG and Fan Q. On the pathogenesis of chronic pelvic pain syndrome from "stasis heat". *Jiangsu Traditional Chinese Medicine* 2012; 12: 6-7.
- [41] Liang J, Chen Y and Ran Q. Clinical observation of Sanjie-zhentong capsule on chronic pelvic pain with qi stagnation and blood stasis syndrome. *Hebei Journal of Traditional Chinese Medicine* 2017; 12: 1797-1800.
- [42] Xiao Z and Zhu D. Female section Jinglun. People's Medical Publishing House; 2006.
- [43] Chen Z. Revised good remedies for women. Health Publishing House; 1956.
- [44] Lu NZ. Introduction to the academic characteristics of Lu's traumatology. *Shanghai Journal of Traditional Chinese Medicine* 2006; 2: 37-38.
- [45] Zhang T. Treatment of 160 cases of lumbago and leg pain with Changyin needle combined with bone-setting push and hold. *Shanghai Journal of Traditional Chinese Medicine* 1997; 10: 17-18.
- [46] Cheng K, Wei JZ and Shen XY. Temperature characteristic of moxibustion with warming needles made of different materials. *Journal of Chinese Medicine* 2011; 4: 703-707.
- [47] Yosef A, Allaire C, Williams C, Ahmed AG, Al-Hussaini T, Abdellah MS, Wong F, Lisonkova S and Yong PJ. Multifactorial contributors to the severity of chronic pelvic pain in women. *Am J Obstet Gynecol* 2016; 215: 760.e761-760.e714.
- [48] Phan VT, Stratton P, Tandon HK, Sinaii N, Aredo JV, Karp BI, Merideth MA and Shah JP. Widespread myofascial dysfunction and sensitisation in women with endometriosis-associated chronic pelvic pain: a cross-sectional study. *Eur J Pain* 2021; 25: 831-840.
- [49] Serrano-Imedio A, Calvo-Lobo C, Casañas-Martin C, Garrido-Marin A and Pecos-Martin D. Myofascial pain syndrome in women with primary dysmenorrhea: a case-control study. *Diagnostics (Basel)* 2022; 12: 2723.
- [50] Chen Z, Hu L, Liao Y, Zhang X, Yang Z, Hu C and Yu L. Different processed products of curcuma radix regulate pain-related substances in a rat model of Qi stagnation and blood stasis. *Front Pharmacol* 2020; 11: 242.
- [51] Cai T, Verze P, La Rocca R, Palmieri A, Tiscione D, Luciani LG, Mazzoli S, Mirone V and Malossini G. The clinical efficacy of pollen extract and vitamins on chronic prostatitis/chronic pelvic pain syndrome is linked to a decrease in the pro-inflammatory cytokine interleukin-8. *World J Mens Health* 2017; 35: 120-128.
- [52] Santanam N, Kavtaradze N, Murphy A, Dominguez C and Parthasarathy S. Antioxidant supplementation reduces endometriosis-related pelvic pain in humans. *Transl Res* 2013; 161: 189-195.
- [53] Armour M, Cave AE, Schabrun SM, Steiner GZ, Zhu X, Song J, Abbott J and Smith CA. Manual acupuncture plus usual care versus usual care alone in the treatment of endometriosis-related chronic pelvic pain: a randomized controlled feasibility study. *J Altern Complement Med* 2021; 27: 841-849.
- [54] Chen T, Zhang WW, Chu YX and Wang YQ. Acupuncture for pain management: molecular mechanisms of action. *Am J Chin Med* 2020; 48: 793-811.
- [55] Dou B, Li Y, Ma J, Xu Z, Fan W, Tian L, Chen Z, Li N, Gong Y, Lyu Z, Fang Y, Liu Y, Xu Y, Wang S, Chen B, Guo Y, Guo Y and Lin X. Role of neuro-immune crosstalk in mediating the anti-inflammatory and analgesic effects of acupuncture on inflammatory pain. *Front Neurosci* 2021; 15: 695670.
- [56] Kamali F, Sinaei E and Morovati M. Comparison of upper trapezius and infraspinatus myofascial trigger point therapy by dry needling in overhead athletes with unilateral shoulder impingement syndrome. *J Sport Rehabil* 2019; 28: 243-249.
- [57] Wang R, Li X, Zhou S, Zhang X, Yang K and Li X. Manual acupuncture for myofascial pain syndrome: a systematic review and meta-analysis. *Acupunct Med* 2017; 35: 241-250.