Evidence-Based Complementary and Alternative Medicine

Acupuncture for Pain: Implications and Applications

Lead Guest Editor: Jianliang Zhang
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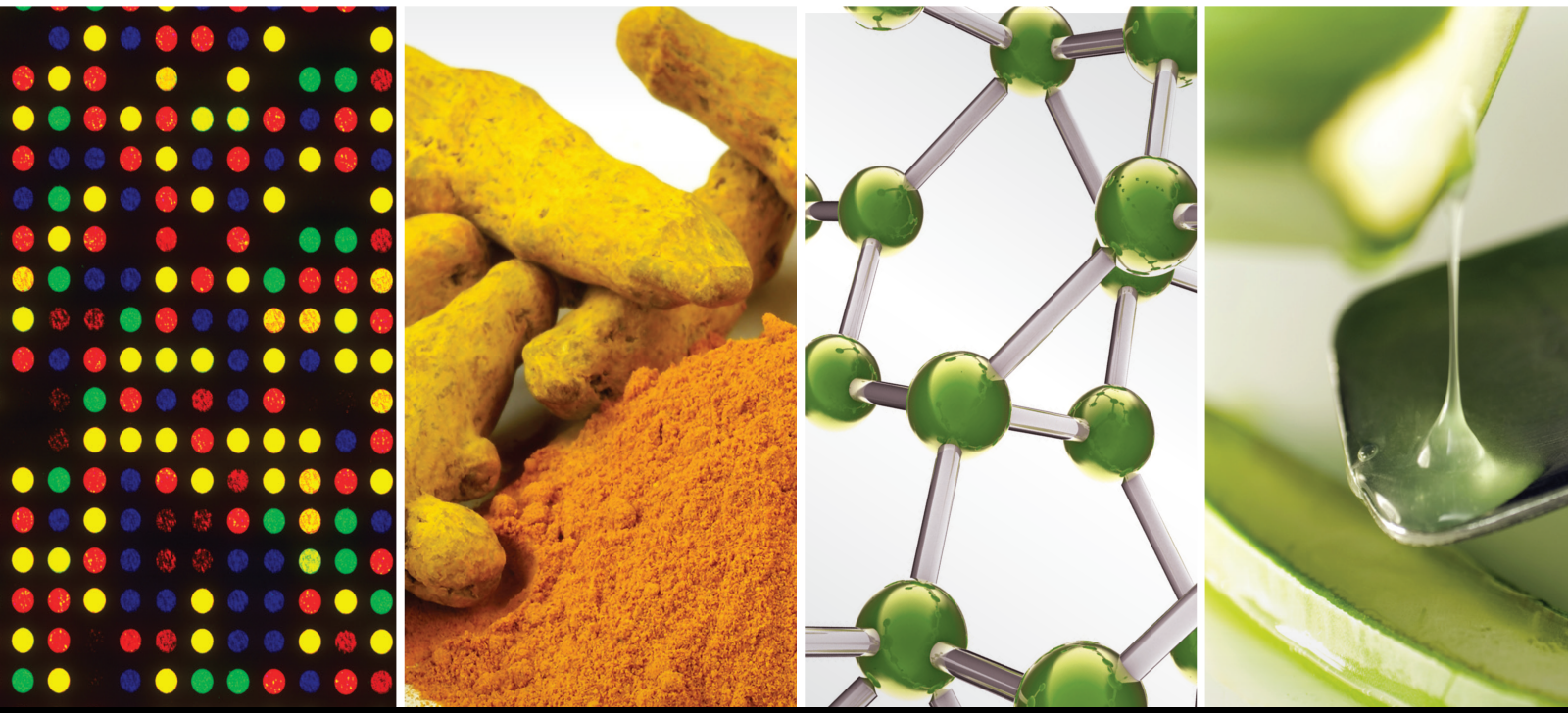

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Research Article

Electroacupuncture Alleviates Pain Responses and Inflammation in Collagen-Induced Arthritis Rats via Suppressing the TLR2/4-MyD88-NF-κB Signaling Pathway

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Background and Purpose. Electroacupuncture (EA) is effective on rheumatoid arthritis (RA), an autoimmune disease, but the mechanisms involved remain poorly understood. This study was designed to investigate the analgesic and anti-inflammatory effects of EA in a chronic inflammatory animal model of collagen-induced arthritis (CIA) and its underlying molecular mechanisms. *Experimental Approach*. For the male Sprague–Dawley (SD) rats were immunized with bovine type II collagen followed by a booster injection 7 days later. Two weeks after the first immunization, EA stimulation (2/100 Hz, 1 mA, lasting for 30 min/day) was delivered to Zusanli (ST36), and Sanyinjiao (SP6) or OxPAPC (TLR2/TLR4 inhibitor, 1.5 mg/kg) was injected by tail vein for 28 days. After intervention, the analgesic effect was evaluated from the aspect of pain responses including thermal withdrawal latency (TWL) and mechanical withdrawal thresholds (MWT). The anti-inflammatory effect was assessed by paw edema detection, histopathological analysis, and Meso Scale Discovery (MSD) testing of tumor necrosis factor-alpha (TNF-α), interleukin 1 beta (IL-1β), and interleukin 6 (IL-6). The underlying molecular mechanism was analyzed through western blotting and double-immunofluorescence labeling. *Results*. EA intervention and OxPAPC injection could relieve mechanical allodynia and thermal hyperalgesia caused by CIA. Paw edema and pathological damage of synovium were significantly ameliorated after EA intervention and OxPAPC injection. Furthermore, EA intervention and OxPAPC injection markedly reduced the contents of serum TNF-α, IL-1β, and IL-6, as well as the protein expression levels of synovial TLR2, TLR4, MyD88, and NF-κB p-p65. In particular, the expression of TLR2 and TLR4 on synovial fibroblasts and macrophages in synovium was significantly reduced by EA intervention. *Conclusions*. Repeated EA stimulation at ST36 and SP6 can effectively relieve joint pain and synovial inflammation caused by RA in CIA rats. The analgesic and anti-inflammatory effect of EA may be closely related to the inhibition of innate immune responses driven by the TLR2/4-MyD88-NF-κB signaling pathway in the synovium.

1. Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune polyarticular disease that can affect all ages, genders, and ethnicities [1]. RA has a worldwide prevalence of 0.5%–1% and is clinically characterized by marked synovial inflammation, joint swelling, joint pain, bone erosion, and progressive disability [2]. It severely degrades the sufferers' quality of life and is even life-threatening [3]. Existing antirheumatic drugs, including nonsteroidal anti-inflammatory drugs (NSAIDs), disease-modifying antirheumatic drugs (DMARDs), or bio-agents, only partially halt the RA progression and have many adverse effects [4, 5]. Growing evidence suggests that acupuncture, one of the non-pharmacological alternative therapies, is effective on RA [6–8]. It was reported that acupuncture significantly improved disease activity scores, pain and overall mobility, joint swelling, and health-related quality of life in patients with RA [9–11]. Although it is generally accepted that EA can be used as adjuvant therapy for the treatment of RA, its precise mechanisms remain to be elucidated.

RA is the consequence of dysregulation of the immune system and is characterized by an excessive autoimmune response to synovial membrane. The process involves the

activation of synoviocytes, inflammatory cells infiltration in synovium, and the increase of pro-inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α), interleukin 1 beta (IL-1β), and interleukin 6 (IL-6), leading to chronic localized synovial and systemic inflammation, joints pain, as well as cartilage and bone erosion [12]. At present, it is widely accepted that the cytokine network plays a vital role in RA pathogenesis [13], and anticytokines therapy in patients with RA has proven to be effective [14]. Moreover, EA has been previously reported to reduce the serum levels of pro-inflammatory cytokines in RA patients [15] and RA animal models [16, 17], which suggests the possible immunomodulatory mechanism of EA in alleviating RA.

Toll-like receptors (TLRs) are functionally key pattern recognition receptors (PRRs) in the innate immune system, which are mainly expressed in the innate immune cells and play a critical role in the initiation of inflammatory responses [18]. Numerous studies have demonstrated that TLRs and their downstream signaling molecules are involved in the development of autoimmune diseases such as RA [19, 20]. The human TLRs family includes 11 members, mainly distributing in cell membranous structures. Among them, TLR2 and TLR4 are closely associated with the pathogenesis of RA [18, 21]. Additionally, growing in vivo and in vitro studies have emphasized that TLR2 and TLR4 signaling play pivotal roles in the onset and maintenance of synovial inflammatory responses in RA [22–25]. However, it remains unknown whether the TLR2/4 signaling is the regulatory target through which EA exerts its antiarthritic effects on RA.

In the present study, we established a collagen-induced arthritis (CIA) model in rats to investigate the ameliorative effects of EA on RA synovitis and arthralgia through TLR2/4 and their downstream signaling cascades. Our findings highlighted the importance of the synovial TLR2/4-MyD88-NF-κB signaling pathway in the analgesic and anti-inflammatory effects of EA intervention for RA.

2. Materials and Methods

2.1. Animals. Animal care and experimental protocols were approved by the Ethics Committee of the Institute of Acupuncture and Moxibustion, China Academy of Chinese Medical Sciences (ethical approval number: D2017-08-16-1). All experimental animals were cared according to the guidelines provided by the U.S. National Institutes of Health for the Care and Use of Laboratory Animals. 200–250 g male Sprague–Dawley (SD) rats were purchased from Beijing Union Medical College and were housed (up to 5/cage) in standard plastic cages with water and standard mouse feed under controlled temperature (22–24°C), humidity (55 ± 5%), and 12-hr alternating light/dark cycle (lights were turned on between 7:00 a.m. and 7:00 p.m.).

2.2. Establishment of the CIA Model. The CIA model was established according to reference [26–28]. Briefly, bovine type II collagen (CII; Chondrex, Redmond, WA, USA) (dissolved in 0.1 M acetic acid) was emulsified 1 : 1 (v: v) with complete Freund's adjuvant (CFA; Sigma-Aldrich, Darmstadt, Germany) at a final concentration of 1 mg/mL. Rats were immunized with intradermal injection of the 300 μl CII emulsion at the base of the tail on day 0, followed by the same booster injection 7 days later. The animals with no signs of swelling in joints were removed. Controls were handled in the same way except that they were injected with 150 μl of 0.1 M acetic acid.

2.3. Experiment Design

2.3.1. Experiment 1. To determine the analgesic and anti-inflammatory effects of EA treatment on the CIA rats, we randomly divided 36 SD rats into three groups: control (normal), CIA (model only), and CIA + EA (model with EA treatment at ST36 and SP6 acupoints) ($n = 12$ each group).

2.3.2. Experiment 2. To assess the role of the TLR2/TLR4 signaling pathway in the maintenance of inflammation and joint pain in CIA rats, further 16 rats were randomly assigned to two groups after inducing the CIA model: the CIA + vehicle group (CIA rats treated with normal saline) and the CIA + OxPAPC group (CIA rats treated with the TLR2/TLR4 inhibitor OxPAPC) ($n = 8$ each group).

2.3.3. Experiment 3. To determine the expression of TLR2 and TLR4 in synovial macrophages and synovial fibroblasts in the synovium tissue, we evenly separated 12 SD rats into the same three groups as in Experiment 1. The rats in the CIA + EA group received EA intervention as in Experiment 1 for two weeks.

2.4. Electroacupuncture (EA) Intervention and Sampling in Experiment 1. Rats in the CIA + EA group received EA intervention at ST36 (located about 5 mm inferior to the capitulum fibulae and posterolateral to the hindlimb knee joint) and SP6 (located at the medial side of the hind leg, 10 mm directly above the tip of the medial malleolus) on day 14 after the initial immunization. Briefly, rats were lightly anesthetized with isoflurane (1.5% in oxygen) delivered via an anesthesia unit (Matrix Company, Midmark Animal Health, Versailles, OH, USA) and underwent EA stimulation by inserting needles (0.5 × 32 mm, Suzhou, China) at ST36 and SP6 in a depth of about 5 mm and 3 mm, respectively. The inserted needles were further connected to an electronic acupuncture treatment instrument (Hans-100A, Nanjing Jisheng Medical Technology, Co., Ltd., China). The EA parameters were alternating frequency of 2 Hz/100 Hz, a pulse width of 0.2–0.6 ms, and an intensity of 1 mA. The stimulation procedure was performed for 30 min every day for 28 consecutive days (Figure 1). Rats in the control group and the CIA group were anesthetized in the same manner as those in the CIA + EA group but did not receive EA stimulation.

Upon completion of EA intervention, all rats were anesthetized with pentobarbital sodium (50 mg/kg, i.p.) for blood collection and then sacrificed through decapitation

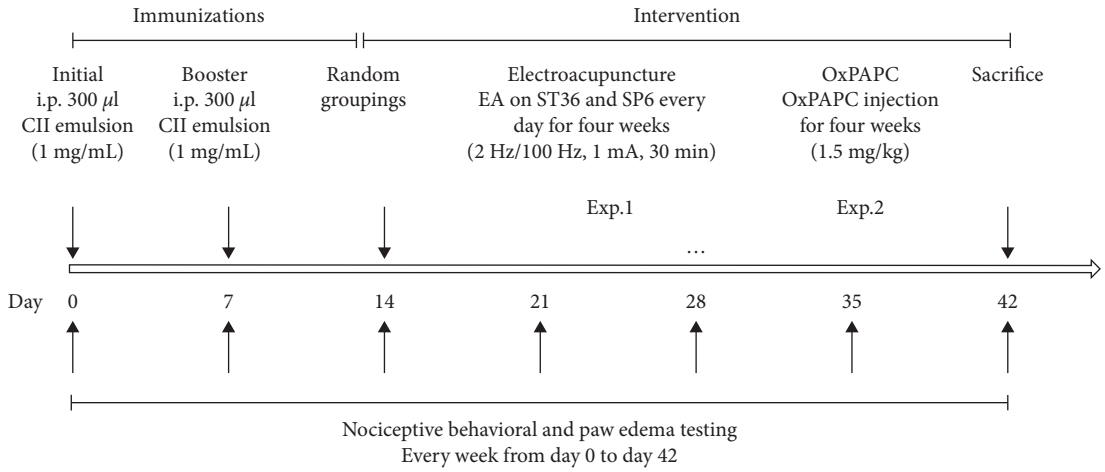

Figure 1: Experimental design for the effect of EA on CIA rats (experiment 1 and experiment 2). Male SD rats were initially immunized at the base of the tail on day 0 and boosted on day 7. From day 14 to day 42, rats in the CIA + EA group and the CIA + OxPAPC group received EA intervention or inhibitor injection every day, respectively. From day 0 to day 42, paw edema and pain responses were tested weekly in each group of rats.

(on day 42 after the first immunization). Blood ($n = 12$ per group) was taken via the tail vein and collected into procoagulation tubes (BD Vacutainer, Franklin Lakes, New Jersey, USA) for the separation of serum. Then, it was divided into aliquots and kept under −80°C for serological determinations. The ankle joints of hind paws ($n = 6$ per group) were separated for further histopathological analysis. The ankle synovial membrane tissues of hind paws ($n = 6$ per group) were isolated and kept under −80°C for further western blotting analysis.

2.5. Paw Edema Analysis. The paw edema of each rat's bilateral hind limbs (up to the ankle joint) was examined once a week from day 0 to day 42 (Figure 1) with a water displacement plethysmometer (Ugo Basile, Comerio, Varese, Italy). The mean values of water displacement volume were calculated from 3 measurements to judge the extent of paw edema [29].

2.6. Nociceptive Behavioral Tests. The pain responses of each rat's bilateral hind paws, including mechanical withdrawal threshold (MWT) and thermal withdrawal latency (TWL), were measured once a week from day 0 to day 42 (Figure 1). MWT was examined by electronic Von Frey (38450, Ugo Basile, Comerio, Varese, Italy). TWL was examined by a thermal plantar analgesia instrument (37370, Ugo Basile, Comerio, Varese, Italy) [30], with a heat intensity of 50 units and a cut-off time of 30 s. Mean MWT and TWL were calculated from 3 individual tests with a 5-min interval to represent the mechanical allodynia and thermal hyperalgesia.

2.7. Radiographic and Histopathological Analysis of Ankle Joints. For radiographic analysis, the hind limbs were imaged on a multimodality in vivo imaging system (Kodak Company, Rochester, NY, USA) shortly before the rats were sacrificed. The exposure time was 10 s, and the f-stop factor was 2.5.

For histopathological analysis, the protocol was performed as previously described [31]. Briefly, the fresh ankle joints were fixed with 4% paraformaldehyde (Servicebio, Wuhan, China) and then decalcified with 10% ethylene diamine tetraacetic acid (EDTA, Servicebio, Wuhan, China) at room temperature for 40 days. The processed joints were dehydrated, embedded, and sectioned at 4 μm thickness in a sagittal plane. Sections were then stained with hematoxylin-eosin (HE) for assessing EA's effect on the histopathological damage in joints. Images were acquired and observed under an upright light microscope (Nikon Inc., Tokyo, Japan). HE staining score was evaluated by an investigator who was blinded to the experimental protocol. The following morphological criteria were considered as follows [32]: score 0, no damage; score 1, edema; score 2, presence of inflammatory cells; score 3, bone resorption.

2.8. Serum Cytokine Levels Detection. Serum TNF-α, IL-1β, and IL-6 were measured by Meso Scale Discovery (MSD) electrochemiluminescence technology with a V-PLEX custom rat cytokine kit (K153AOH-1, MSD, Rockville, Maryland, USA) according to the manufacturer's instructions [33]. In short, the 96-well plate was washed 3 times with 150 μl wash buffer. After the washing, wells were incubated with 50 μl serum sample for 2 h at room temperature with shaking. Twenty-fiveμl of detection antibody solution was then added and incubated for 2 h at room temperature with shaking. After adding 150 μl of 2 × read buffer *T* to each well, the 96-well plate was detected with MESO QuickPlex SQ 120 machine (MSD, Rockville, Maryland, USA), and data were analyzed via MSD Discovery Workbench software version 4.0.12.

2.9. Western Blotting Analysis. For western blotting analysis, total proteins were obtained from the ankle synovium tissues with RIPA lysis buffer (Beyotime Biotechnology, Shanghai, China), containing 1% PMSF. Protein concentrations were quantitatively determined with a BCA protein assay kit

(Beyotime Biotechnology, Shanghai, China). Proteins were separated by 10% SDS-PAGE gel, transferred onto a 0.45 μm PVDF membrane (Millipore, Burlington, Massachusetts, USA), and blocked with 5% BSA (Amresco, Solon, Ohio, USA). After the blocking, the membranes were blotted with primary antibodies overnight at 4°C as follows: anti-TLR2 (sc-21760, Santa Cruz, Dallas, Texas, USA), anti-TLR4 (sc-293072, Santa Cruz, Dallas, Texas, USA), anti-MyD88 (4283, Cell Signaling Technology, Danvers, Massachusetts, USA), anti-Phospho-NF-κB p65 (3033S, Cell Signaling Technology, Danvers, Massachusetts, USA), and anti-β-actin (4970, Cell Signaling Technology, Danvers, Massachusetts, USA) and then incubated with the corresponding secondary antibodies (Jackson, ImmunoResearch Laboratories, West Grove, PA, USA) for 2 h at room temperature. Protein visualization was fulfilled with the enhanced chemiluminescence reagents (Millipore, Burlington, Massachusetts, USA) on a gel imaging system (Tanon Science and Technology Co., Ltd., Shanghai, China). Band density (quantification) was determined through TotalLab Quant analysis software (TotalLab Limited, England) after the subtraction of the background and normalization against β-actin.

2.10. Drug Administration in Experiment 2. Oxidized 1-palmitoyl-2-arachidonyl-sn-glycero-3-phosphorylcholine (OxPAPC) (tlrl-oxp1, InvivoGen, San Diego, USA), a dual TLR2 and TLR4 inhibitor, was dissolved in normal saline (1 mg/ml). Rats in the CIA + OxPAPC group and the CIA + vehicle group in Experiment 2 were administrated with OxPAPC (1.5 mg/kg) and normal saline, respectively, via the tail vein injection once a day for 4 consecutive weeks.

2.11. Double-Immunofluorescence Labeling in Experiment 3. Serial 4-μm-thick paraffin sections from CIA synovial tissues were deparaffinized in xylene and rehydrated through a graded ethanol series. Then, the sections were immersed in the 0.01 M citrate buffer (pH 6.0), and microwave irradiation was performed three times (8 min/time) for antigen retrieval. The sections were incubated in 5% goat serum for 1 h before immunostaining. In double-labeling experiments, the sections were incubated with anti-CD68 (pAb, 1:200, ab125212, Abcam, British; mAb, 1:100, NBP2-32831, NOVUS, USA), anti-Vimentin mAb (1:200, ab92547, Abcam, British; 1 : 200, ab20346, Abcam, British), anti-TLR2 pAb (1:150, 17236-1-AP, Proteintech, China), and anti-TLR4 mAb (1 : 100, sc-293072, Santa Cruz, USA) at 4°C overnight, followed by incubated with secondary antibodies, goat anti-rabbit IgG H&L(FITC) (1 : 200, ab6717, Abcam, British) or goat anti-mouse IgG H&L (Cy3) (1:200, ab97035, Abcam, British). The poststaining sections were examined with a full-spectrum scanning confocal microscope (FV1200, Olympus, Japan), and pairs of images were superimposed for colocalization analysis.

2.12. Statistical Analysis. Data were reported as mean ± standard deviation (SD) and were examined by one-way ANOVA, followed by the least significant difference (LSD) test for comparisons among multiple groups. The LSD test was run only if F-value achieved $P < 0.05$, and there was no significant variance in homogeneity. $P < 0.05$ was considered statistically significant. All analyses were performed using Statistical Package for the Social Sciences (SPSS) version 23.0 (SPSS Inc., Chicago, Illinois, USA).

3. Results

3.1. EA Intervention Reduced CIA-Induced Hind Paw Edema. The hind paw edema was employed for the assessment of EA's effect on RA progression. As expected, the hind paw edema in the CIA group obviously increased from day 14 after the first immunization and lasted to day 42 compared with that in the control group (Figure 2, $p < 0.01$). EA stimulation at ST36 and SP6 showed marked alleviation of paw edema in the CIA + EA group after two weeks of EA intervention compared with that in the CIA group (Figure 2, $p < 0.05$).

3.2. EA Intervention Attenuated Allodynia and Hyperalgesia in CIA. To explore the analgesic effects of EA, MWT and TWL were detected in all groups of rats. After successful induction of CIA (day 14 after the first immunization), MWT in the CIA group was much less than that in the control group with marked differences (Figure 3(a), $p < 0.01$). However, in comparison with the CIA group, MWT started to prominently recover in the CIA + EA group after two weeks of EA stimulation at ST36 and SP6 (Figure 3(a), $p < 0.05$). Similar results were corroborated in TWL detection (Figure 3(b), $p < 0.01$, $p < 0.05$). These results implicated that the long-term EA intervention may attenuate the arthritic inflammatory hyperalgesia in CIA rats.

3.3. EA Intervention Improved Histopathological Lesions in the Ankle Joint of CIA Rats. On day 42 after the first immunization, the visual redness and swelling of the hind paws in the CIA + EA group showed a great relief compared with those in the CIA group (Figure 4(a)).

As shown in the representative radiographs, bone erosion in the ankle joint was exacerbated in the CIA group compared to the control group whereas EA could distinctly reduce articular bone destruction and reverse the above trend in CIA rats (Figure 4(b)).

Consistent with the clinical behaviors, histopathological observations of ankle joints revealed signs of severest arthritis in the CIA rats. There was no evidence of inflammatory activity or joint erosion in the control group, while the massive inflammatory cell infiltration, synovial hyperplasia, and joint erosion were presented in the CIA group. The histological damage scores were higher in the CIA group (Figure 4(d), $p < 0.001$). After 28 days of EA intervention, these histopathological features significantly ameliorated in the CIA + EA group, and the HE staining score of the CIA + EA group also decreased remarkably (Figures 4(c) and 4(d), $p < 0.001$).

3.4. EA Intervention Showed the Anti-Inflammatory Effect on CIA Rats. It is well documented that pro-inflammatory

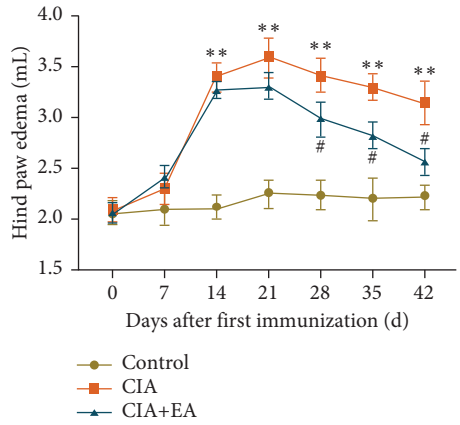

Figure 2: Measurement of the hind paw edema once a week from the day of the first immunization to the end of the EA intervention. (Data were expressed as mean ± SD, $n = 12$ per group, $^{**}p < 0.01$ compared with the control group, $^{\#}p < 0.05$ compared with the CIA group).

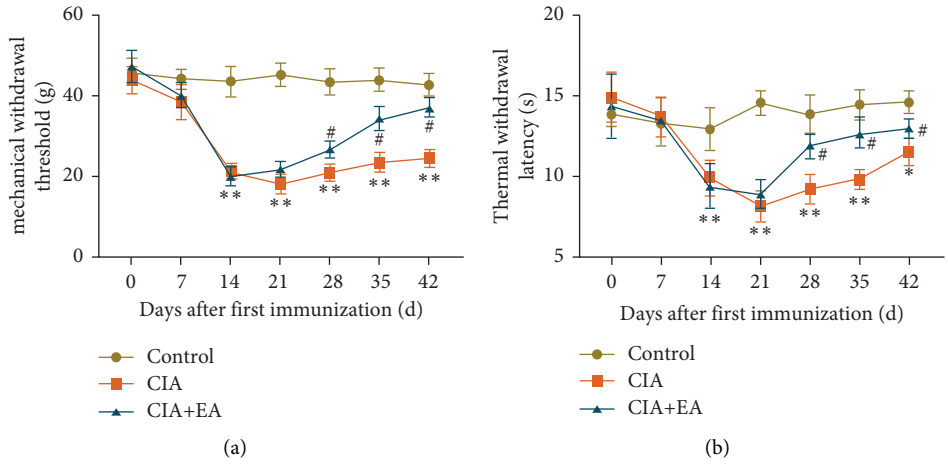

Figure 3: Measurement of the hind paw: (a) mechanical withdrawal threshold and (b) thermal withdrawal latency once a week from the day of the first immunization to the end of the EA intervention. (Data were expressed as mean ± SD, $n = 12$ per group, $^{*}p < 0.05$, $^{**}p < 0.01$ compared with the control group, $^{\#}p < 0.05$ compared with the CIA group).

cytokines play a vital role in the maintenance of chronic inflammation and tissue damage during RA progression. MSD test was conducted to assess the contents of serum TNF-α, IL-1β, and IL-6. As shown in Figure 5, compared with those in the control group, contents of serum TNF-α, IL-1β, and IL-6 noticeably elevated in the CIA group (5, $p < 0.001$). On the contrary, the serum levels of these cytokines significantly decreased in the CIA + EA group after 28 days' EA intervention (Figure 5, $p < 0.01$, $p < 0.001$).

3.5. EA Intervention Inhibited the Activation of TLR2/4-MyD88-NF-Kb Signaling Pathway in CIA. To further elucidate the underlying molecular mechanisms of analgesic and anti-inflammatory effects of EA intervention, we adopted western blotting to detect the expression levels of key proteins in the TLR2/4-NF-κB pathway in the ankle synovium. The results indicated that expression levels of TLR2, TLR4, MyD88, and NF-κB p-p65 in the CIA group were significantly upregulated by different degrees in comparison with those in the control group (Figure 6, $p < 0.05$, $p < 0.01$) whereas those in the CIA + EA group were remarkably downregulated compared with those in the CIA group (Figure 6, $p < 0.05$, $p < 0.01$). This illustrated that EA at ST36 and SP6 may ameliorate inflammation by inhibiting the TLR2/4-MyD88-NF-κB pathway.

3.6. Blockade of TLR2/4 Signaling Mimicked the Analgesic and Anti-Inflammatory Effects of EA in CIA Rats. To further assess whether the anti-inflammatory and analgesic effects of EA were mediated by the TLR2/4 signaling pathway, a dual TLR2 and TLR4 inhibitor, OxPAPC, was administered to CIA rats. Subsequently, behavioral tests and experiments such as and WB were conducted. The results indicated that the hind paw edema in the CIA + OxPAPC group decreased from day 28 to day 42 compared with that in the CIA + vehicle group (Figure 7(a), $p < 0.05$). Rats in the CIA + OxPAPC group had significantly increased MWT and TWL compared to rats in the CIA + vehicle group but

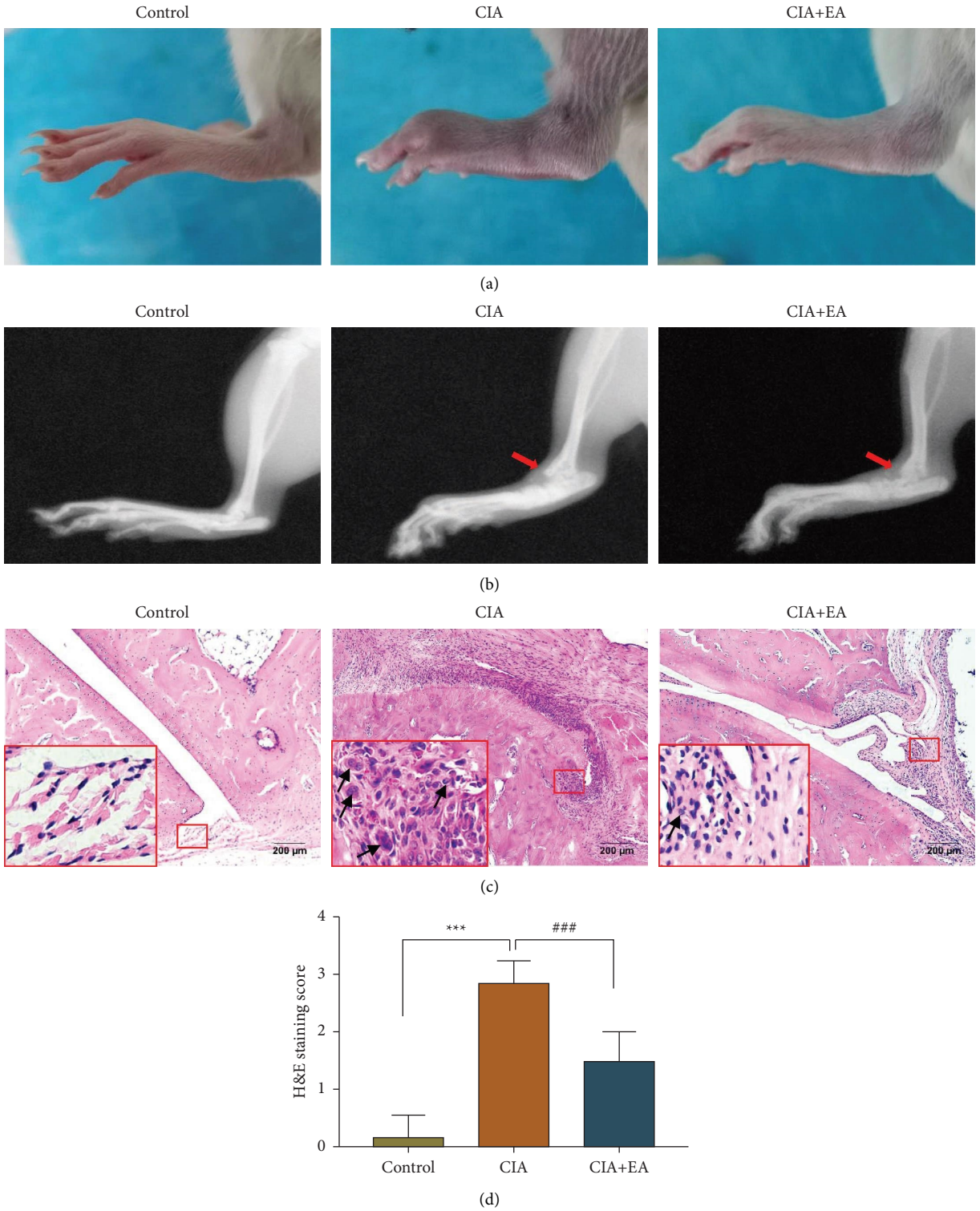

Figure 4: Macroscopic morphology, radiographic, and histopathological analysis of ankle joints. (a) Typical images of hind paws from each group after 28 days' EA intervention. $n = 12$ per group. (b) Representative radiographic images of hind paws from each group after 28 days' EA intervention. Red arrows indicate the bone erosion regions in the ankle joints, $n = 12$ per group. (c) HE staining of ankle joints from each group after 28 days' EA intervention. Black arrows indicate the infiltrated inflammatory cells, $n = 6$ per group. Scale bar: 200 μm. (d) HE staining score of each group. (Data were expressed as mean ± SD, $n = 6$ per group, $^{***}p < 0.001$ compared with the control group, $^{###}p < 0.001$ compared with the CIA group).

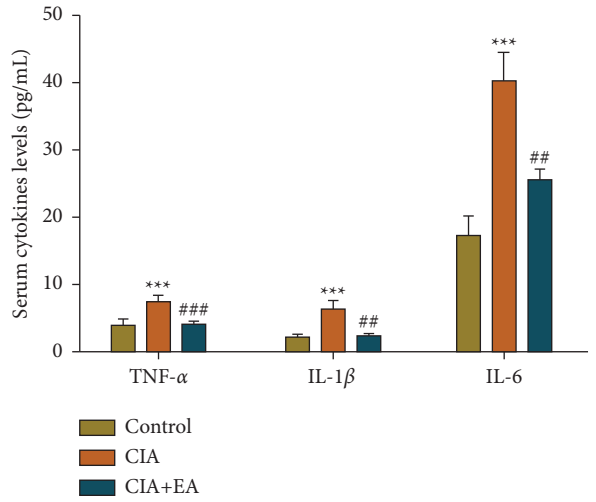

Figure 5: EA intervention inhibited expression of serum pro-inflammatory cytokines. The contents of TNF-α, IL-1β, and IL-6 were detected with MSD technology. (Data were expressed as mean ± SD, $n = 12$ per group, $^{***}p < 0.001$ compared with the control group, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ compared with the CIA group).

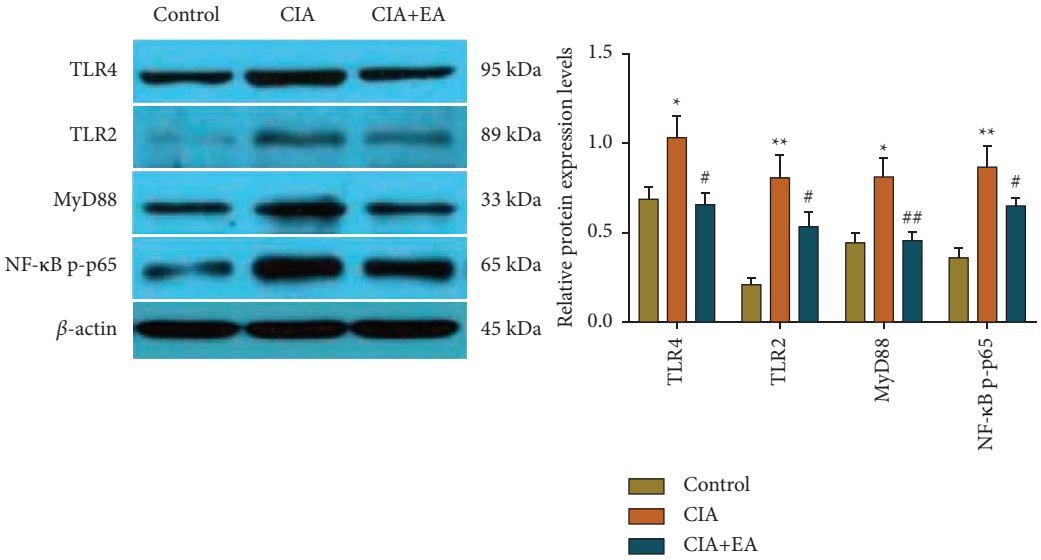

Figure 6: EA intervention inhibited TLR2/4-MyD88-NF-κB pathway. The expression levels of TLR2, TLR4, MyD88, and NF-κB p-p65 derived from ankle synovium were analyzed through western blotting. (Data were expressed as mean ± SD, $n = 6$ per group, $^{*}p < 0.05$, $^{**}p < 0.01$ compared with the control group, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ compared with the CIA group).

significantly lower serum levels of TNF-α, IL-1β, and IL-6 (Figures 7(b)–7(d), $p < 0.05$, $p < 0.01$). Histopathological observations showed visible inflammatory cell infiltration and synovial hyperplasia in the CIA + vehicle group, while the pathological changes in the ankle joint of rats in the CIA + OxPAPC group were significantly improved (Figure 7(e), $p < 0.01$). Sure enough, the expression levels of NF-κB p-p65 were notably downregulated in the CIA + OxPAPC group (Figure 7(f), $p < 0.05$). All these results suggested that OxPAPC could relieve the inflammation and joint pain in CIA rats, inversely verifying that the TLR2/4 signaling pathway plays an important role in the EA treatment of CIA.

3.7. EA Intervention Suppressed the Expression of TLR2 and TLR4 on Synovial Fibroblasts and Macrophages in Synovium. To characterize the TLR2 and TLR4 expressing cells in the joint synovium, tissue sections were double immunofluorescence stained for TLR2 or TLR4 and the macrophages marker CD68 or the fibroblasts marker Vimentin, respectively. Figure 8 shows representative staining patterns of CD68 or Vimentin and TLR2 or TLR4 expression in the synovium tissue. In all three groups, the majority of TLR2 and TLR4 expression was detected on macrophages and synovial fibroblasts in the lining layer and sublining layer of the synovium, and in particular, there were pronounced TLR2 and TLR4 expression in the synovial lining layer.

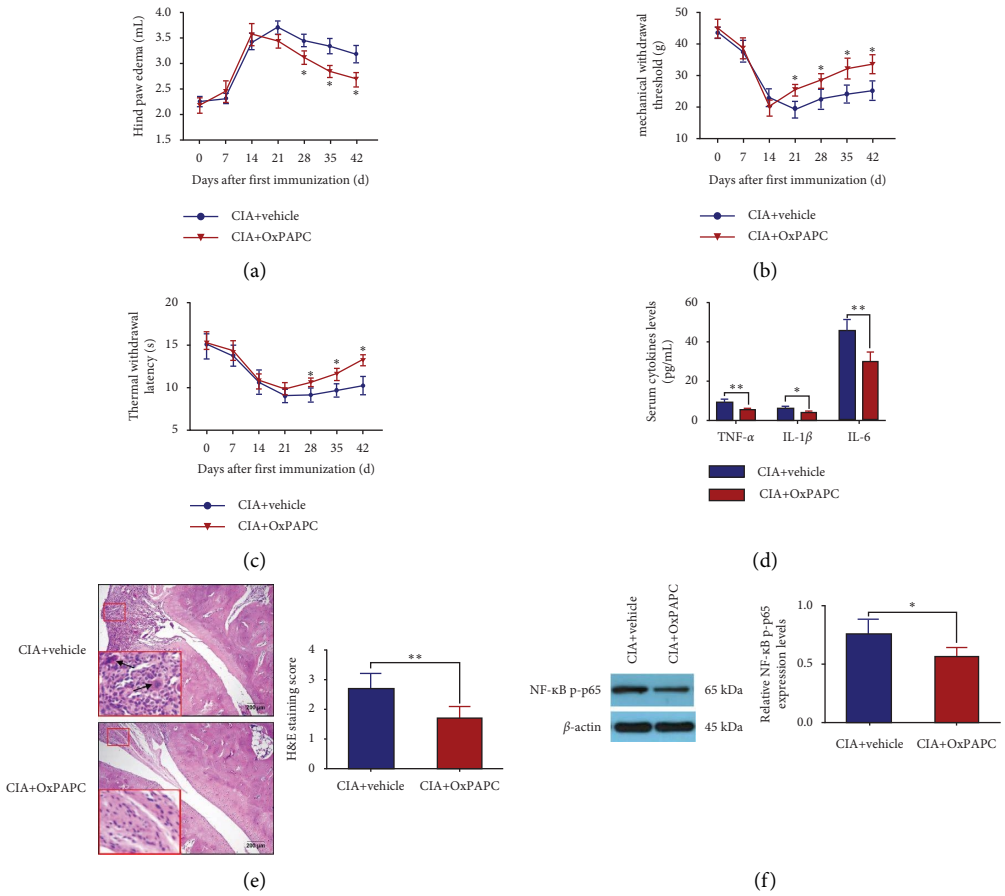

FIGURE 7: OxPAPC intervention relieved the inflammation and joint pain in CIA rats. (a) Measurement of the hind paw edema once a week from day 0 to day 42. Measurement of the hind paw (b) mechanical withdrawal threshold and (c) thermal withdrawal latency once a week from day 0 to day 42. (d) The contents of TNF-α, IL-1β, and IL-6 in serum. (e) Histopathological analysis of ankle joints. Scale bar: 200 μm. (f) The expression levels of NF-κB p-p65 in ankle synovium. (Data were expressed as mean ± SD, $n = 8$ per group in Figure (a–d), $n = 4$ per group in Figure (e and f), $^{*}p < 0.05$, $^{**}p < 0.01$ compared with the CIA + vehicle group).

Compared with the control group, the number of positive staining cells for TLR2 and TLR4 on synovial fibroblasts and macrophages in synovial lining and sublining layers was significantly increased in CIA rats (Figure 8). EA intervention significantly reduced the number of positive staining cells for TLR2 and TLR4 on synovial fibroblasts and macrophages in synovium tissues (Figure 8).

4. Discussion

In the present study, an experimental RA model of the rat was established through immunization with CII collagen, a major component of hyaline cartilage, combined with CFA, which is the most commonly used method for developing an autoimmune model of RA [26]. Our results showed that CIA rats exhibited severe evoked joint pain and paw edema compared with rats in the control group. Radiographic and histopathological analysis confirmed the obvious pathological changes such as degeneration of joint structures, synovial hyperplasia, inflammatory cell infiltration, and bone erosion in the arthritic joints of CIA rats. Also, the contents of pro-inflammatory cytokines TNF-α, IL-1β, and IL-6 in serum were significantly elevated, and the expression levels of TLR2, TLR4, MyD88, and NF-κB p-p65 were significantly upregulated in the synovium of CIA rats. These are consistent with the previous results obtained from patients with RA [34, 35] and experimental RA model animals [36–39], indicating that CIA rats showed significant inflammatory responses and hyperalgesia at arthritic joints, accompanied by the enhanced activation of the TLR2/4 signaling in the synovium.

Acupuncture is one of the oldest therapeutic strategies in the world and now has been widely reported to possess analgesic and anti-inflammatory effects in autoimmune diseases [40, 41]. In clinical practice, ST36 is the most frequently used acupuncture point for treating RA [42]. And in the studies of acupuncture effects on experimental RA animals, ST36 is also commonly used [43–45]. Meanwhile, a systemic review has shown that ST36 alone or combined with other acupoints is beneficial to the clinical status of RA [7]. Therefore, in this study, we selected two acupoints, ST36 and SP6, for EA intervention. The results showed that EA at ST36 and SP6 had a distinct anti-inflammatory and analgesic effect on CIA rats, as evidenced by reduced articular

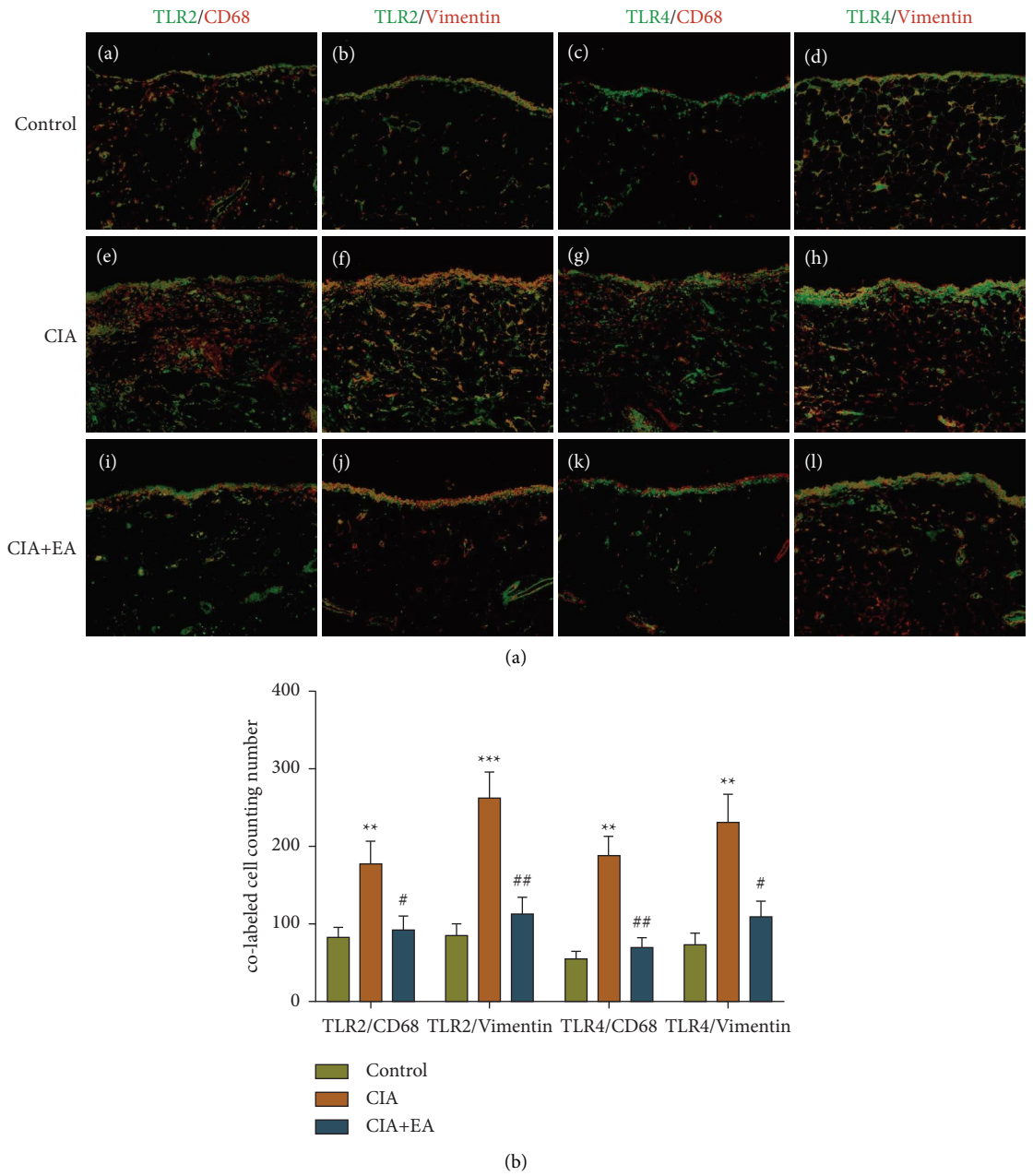

FIGURE 8: EA intervention decreased the expression of TLR2 and TLR4 on synovial fibroblasts and macrophages. (a) Synovium sections were double immunofluorescence stained for TLR2 or TLR4 and CD68 (macrophages marker) or Vimentin (fibroblasts marker), respectively; TLR2 and TLR4 immunopositive cells appear in green color, CD68 and Vimentin immunopositive cells appear in red color, and colabled cells appear in yellow color. The magnification of immunofluorescent images is 200x. (b) The number of double staining cells of TLR2 or TLR4 and CD68 or Vimentin. (Data were expressed as mean ± SD, $n = 4$ per group, $^{***}p < 0.001$, $^{**}p < 0.01$ compared with the control group; $^{\#\#}p < 0.01$, $^{\#}p < 0.05$ compared with the CIA group).

swelling, improved arthro-pathology, decreased levels of pro-inflammatory cytokines, and relieved mechanical allodynia and thermal hyperalgesia.

The synovium is the principal target tissue of inflammation in RA [46], and persistent chronic inflammation of the synovial membrane in RA causes inflammatory pain, which is known to be difficult to treat [47]. Emerging studies have indicated that EA has significant analgesic effects on inflammatory pain in CIA rats, which is related to the central descending pain inhibitory system mediated by adrenergic, cholinergic, and serotonergic receptors [48, 49]. However, it has been reported that pro-inflammatory cytokines, such as TNF-α, IL-1β, and IL-6, could directly increase the sensitivity and excitability of the primary afferents in the inflamed joints, leading to both peripheral and central sensitization [50, 51]. Our results also showed a positive correlation between nociceptive behavioral responses and articular inflammation in CIA rats. Therefore, inhibiting the secretion

of pro-inflammatory cytokines in RA can effectively relieve joint pain.

Over the past two decades, increasing data have supported the critical role of the innate immune system in the pathogenesis of RA [52, 53]. Specifically, TLRs, the key recognition structures of the innate immune system, can recognize microbial products and endogenous ligands released upon cell damage and necrosis and are expressed on cells within the RA joint [20, 54]. Meanwhile, a variety of endogenous TLR ligands, including heat shock proteins, high mobility group box-1 protein (HMGB1), host DNA, fibrinogen, and tenascin-C, have been identified in the synovium of patients with RA, predominantly TLR2 and/or TLR4 agonists [55, 56]. The functions of TLR2/4 signaling have been extensively studied in RA. A large body of evidence obtained from *in vivo* animal models and *in vitro* human explants has confirmed that the activation of TLR2 and TLR4 by endogenous TLR ligands in arthritic joints can trigger the innate immune response and initiate the production of pro-inflammatory cytokines, chemokines, and proteases, which are proposed to be responsible for the perpetuating inflammation and joints destruction of RA [22, 57–60]. Therefore, the pivotal role of TLR2/4 signaling in the pathogenesis of RA had been well established, and our study focused on the modification of the TLR2/4 signaling pathway in EA intervention.

At present, the development of promising therapeutic strategies for the treatment of RA targeting TLRs is emerging [61]. Several preclinical studies have shown that the blockade of TLR2 significantly inhibited the production of pro-inflammatory cytokines TNF-α, IL-1β, and IL-6 in cultured synoviocytes from RA patients [62]. Inhibition of TLR4 not only alleviated the severity of experimental arthritis and suppressed IL-1β expression in arthritic joints of CIA mice [63] but also ameliorated inflammatory symptoms in adjuvant-induced arthritis (AIA) rat model and inhibited the secretion of IL-6 and IL-8 in both serum of the AIA rats and human synovial fibroblasts [64]. In addition, a recent study indicated that blocking TLR4 could effectively attenuate monoiodoacetate-induced arthritis in rats by relieving joint pain and reducing the expression of TNF-α, IL-1β, and matrix metallopeptidase-13 (MMP13) [65]. Similar to the results of these studies, our findings suggested that the blockade of TLR2/4 signaling by the TLR2/4 inhibitor OxPAPC could effectively alleviate articular inflammation and joint pain. Meanwhile, evidence from animal models suggested that EA can also alleviate arthritis by inhibiting TLR2 and/or TLR4 signaling [66, 67]. In line with these findings, our results indicated EA exerted anti-inflammatory and analgesic effects by inhibiting the inflammatory responses driven by the TLR2/4-MyD88-NF-κB signaling pathway in the synovium of CIA rats.

The synovium is a connective tissue structure mainly comprised of resident macrophages and synovial fibroblasts [68]. The resident and infiltrating macrophages and the synovial fibroblasts are the principal innate immune effector cells of RA and are mainly activated by TLRs signaling [69, 70]. Biological agents targeting the pro-inflammatory cytokines TNF-α and IL-1β, predominantly produced by macrophages, have been proven clinically effective in RA [71]. Meanwhile, classical (M1, pro-inflammatory phenotype) macrophage activation occurs in the inflammatory environment of the RA joint dominated by TLRs signaling [72]. Numerous studies have confirmed that TLR2 and/or TLR4 were highly expressed on the synovial fibroblasts and triggered the production of the pro-inflammatory cytokines such as IL-6, chemokines, and tissue destroying mediators leading to inflammatory response and joints destruction [23, 59, 73–76]. In view of the important arthritic functions of TLRs signaling, especially TLR2 and TLR4 signaling in macrophages and synovial fibroblasts, we examined the effect of EA intervention on the expression of TLR2 and TLR4 on these two types of synovial cells of CIA rats. Our results showed that EA intervention markedly reduced the expression of TLR2 and TLR4 on macrophages and synovial fibroblasts in the synovial lining and sublining layers of CIA rats. In fact, the cellular and molecular mechanisms underlying the pathogenesis of RA are not fully understood by far, which undoubtedly increases the difficulty of elucidating the precise mechanism by which EA alleviates this disease. Inhibition of the innate immune response driven by TLRs signaling in the synovium seems to be the promising mechanism by which EA intervention alleviates the severity of RA, but the precise cellular and molecular mechanisms need to be further studied.

5. Conclusion

Taken together, the present findings indicate that EA markedly alleviated the severity of CIA in the rats by reducing paw swelling, serum levels of pro-inflammatory cytokines, and relieving joint pain. EA also can suppress the expression of TLR2/4-MyD88-NF-κB signaling pathway-related proteins in the synovium, especially the TLR2 and TLR4 expression on synovial fibroblasts and macrophages, and inhibition of TLR2/4 signaling could mimic the analgesic and anti-inflammatory effects of EA. These data suggested that the anti-inflammatory and analgesic effects of EA treatment on RA are closely related to the inhibition of innate immunity-mediated inflammatory response in the macrophages and synovial fibroblasts driven by TLR2/4-MyD88-NF-κB signaling pathway in the synovium.

Abbreviations

EA:	Electroacupuncture
CIA:	Collagen-induced arthritis
SD:	Sprague–Dawley
TNF-α:	Tumor necrosis factor-alpha
IL-1β:	Interleukin 1 beta
IL-6:	Interleukin 6
RA:	Rheumatoid arthritis
NSAIDs:	Nonsteroidal anti-inflammatory drugs
DMARDs:	Disease-modifying antirheumatic drugs
TLRs:	Toll-like receptors
MyD88:	Myeloid differentiation primary response 88
CII:	Bovine type II collagen

CFA: Complete Freund's adjuvant
MWT: Mechanical withdrawal threshold
TWL: Thermal withdrawal latency
HE: Hematoxylin-eosin.

Data Availability

All data generated or analyzed during this study are included in this article.

Ethical Approval

Animal care and experimental protocols were approved by the Ethics Committee of the Institute of Acupuncture and Moxibustion, China Academy of Chinese Medical Sciences (ethical approval number: D2017-08-16-1).

Conflicts of Interest

All authors declare that they have no conflicts of interest.

Authors' Contributions

Y-S Yang designed and supervised this study. S-Y Sun, Q-Q Yan, L-N Qiao, Y-N Shi, and L-H Tan conducted the experiments and analyzed the data. All authors took part in drafting the article or revising it, agreed to submit it to the current journal, and to be accountable for all aspects of the work. Shi-Yue Sun and Qi-Qi Yan contributed equally to this work.

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Review Article

Acupuncture and Moxibustion for Peripheral Neuropathic Pain: A Frequentist Network Meta-Analysis and Cost-Effectiveness Evaluation

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Purpose. Acupuncture and moxibustion techniques have been increasingly used to treat peripheral neuropathic pain (PNP). However, there is a paucity of comparative information and cost-effectiveness assessment for techniques on PNP management. *Patients and Methods*. Randomized controlled trials studying the acupuncture or moxibustion treatments on PNP were identified from electronic databases. The quality of the included studies and the potential risk of bias was evaluated using the ROB 2.0 assessment tool. The primary outcome was at least 20% pain relief. The treatment effects were pooled through a frequentist-based network meta approach. Subsequently, the cost-effectiveness measured by incremental cost per additional responder (ICPR) was calculated. *Results*. One three-arm trial and 15 two-arm trials comprising 1308 participants that satisfy the eligibility criteria were identified. Among the included studies, 12.5% were at low risk of bias, 68.75% had some concerns about the risk of bias, and 18.75% were at high risk of bias. The major sources of bias originated from the randomization processes of the studies. The patients were assigned to seven different acupuncture or moxibustion interventions and two pharmaceutical treatments. Except for acupoint injection, all the included acupuncture and moxibustion techniques showed superior improvements in PNP and were more cost-effective as compared to pharmaceutical treatments. Warm needling, fire needling, and moxibustion were the most effective treatments. Fire needling showed the lowest ICPR relative to the nonsteroidal anti-inflammatory drugs in the cost-effectiveness analysis of direct and indirect costs. *Conclusion*. Acupuncture and moxibustion techniques are beneficial and cost-effective approaches for easing PNP and hence can be considered for PNP management.

1. Introduction

Neuropathic pain (NP) is a common condition caused by different sources of lesions or diseases underlying the damages to the somatosensory nervous system. The pain is subclassified as peripheral and central based on different pathological and anatomical origins [1]. As for peripheral neuropathic pain (PNP), the inflammatory processes triggered after peripheral nerve lesion together with the substantial release of immune modulators can contribute to peripheral sensitization and nociceptors excitation [2]. According to the advanced classification defined by the International Association for the Study of Pain, chronic PNPs are as follows: trigeminal neuralgia, peripheral nerve injury, painful polyneuropathy, postherpetic neuralgia, and painful radiculopathy [3].

Until recently, there was still lacking reliable information regarding the epidemiology related to PNP or NP [4]]. Some epidemiological studies suggested that the incidence rate of NP was 8.2/1000 person-years (95% confidence interval, CI: 8.0–8.4) [5], and the overall prevalence was 1–17.9% [6]. However, peripheral and central NP together as the two fundamental conditions in pain is associated with serious social, psychological, and economic consequences. Anxiety, sleep disorders, and depression are common and severe in patients with NP, and patients' work time and the quality of life are significantly affected by NP as compared to the other conditions [7–9]. It is estimated that in the United States, the annual economic cost of chronic pain is at least $560–635 billion, including the incremental cost of health care ($261–300 billion) and the cost of lost productivity ($297–336 billion) [10]. A cohort study implementing the US health insurance claims database indicated that patients with NP are associated with an approximately 3-fold increase in healthcare costs as compared to those without NP [11]. The total cost of NP per patient was around €9,305–14,446 in Europe [9].

Multiple factors can sensitize nociceptors, such that no single pharmaceutical treatment is universally effective for PNP [2]. However, the potential therapeutic role of acupuncture and associated techniques in peripheral neuropathic pain (PNP) have been widely assessed experimentally and clinically exhibiting promising results [12–14]. Acupuncture and associated techniques are the most popular types of complementary alternative medicine available in China and the Western healthcare system and are believed to modulate local inflammatory reactions and the status of the whole body. Those techniques have been increasingly used to treat chronic pain related to PNP [15]. Currently, evidence about direct comparisons of clinical efficacy of those techniques on PNP and their associated cost-effectiveness assessment is still lacking. In this study, we conducted a two-step analysis. First, we performed a frequentist-based network meta-analysis to estimate the therapeutic effects of different acupuncture and moxibustion techniques. Second, a cost-effectiveness analysis (CEA) was performed to assess the economic feasibility of different acupuncture and moxibustion techniques.

2. Material and Methods

2.1. Network Meta-Analysis Process. The network meta-analysis was conducted according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines for network meta-analysis (PRISMA-NMA) and acupuncture (PRISMA-A), and a published research protocol on PROSPERO (CRD: 42020203315).

2.1.1. Search Strategies. The literature search was performed on the following electronic databases from inception to September 2020: Medline, Embase, Cochrane Library, Web of Science, China National Knowledge Infrastructure (CNKI), Wanfang database, WeiPu (VIP) database, and China Biology Medicine (CBM) database. The search terms included "acupuncture," "electro-acupuncture," "warming needling," "fire needling," "bloodletting," "auriculo-acupuncture," "moxibustion," "cupping," "collateral pricking," "needle knife," "neuropathic pain," and "randomized controlled trial." Titles and abstracts were screened by two authors independently; also, the bibliographies of articles were scanned for additional relevant studies.

2.1.2. Eligibility Criteria. (1) Randomized controlled trials or cohort studies that fulfilled the diagnostic criteria for PNP were included [3]; (2) studies conducted on humans; (3) assessment of acupuncture/moxibustion techniques; (4) each arm in a study should include only one intervention for PNP; (5) the data provided in the articles should be sufficient to estimate the risk differences (RDs) and the corresponding 95% CIs in both groups. Moreover, studies on herbal drugs or their associated products should be excluded. Only the most recent publication was preserved if the previous studies were conducted on the same population. Duplicated studies of previously published data were excluded. Case-control studies, case reports/series, letters, reviews, editorials, and article comments were not suitable in this analysis and should be excluded.

2.1.3. Outcome Measurements. The primary outcome was at least 20% relief of pain intensity. This could be assessed using pain evaluation scales (e.g., visual analogue scale, the McGill pain questionnaire, and symptoms scale) or pain threshold detectors.

2.1.4. Data Collection. Two authors (Liu and Li) extracted data from original studies independently using a predesigned data extraction form. The following information was collected: first author's names, publication years, regions,

ethnicities, sex, sample sizes, diagnoses, time of onset, interventions, treatment courses, and the number of drops. The discrepancies were resolved by referring to the original articles or consulting a superior author (Lin or Zheng).

2.1.5. Assessment of Bias and Evidence Quality. The ROB 2.0 assessment tool was used to evaluate the risk of bias. The assessment of each study was carried out independently by two researchers (Liu and Li) in the process of data extraction. The differences in the data from the two researchers were solved through discussion and negotiation; if the contradiction could not be solved after negotiation, then a superior researcher (Zheng) was consulted.

2.1.6. Network Meta-Analysis. Network meta-analysis was based on the frequentist method and calculated using the "netmeta" package (version 1.2-1) in R (version 3.6.3). The package constructed network models of direct or indirect comparisons of individual interventions. The heterogeneity of the treatment effects between studies was evaluated by the chi-square test of Q, τ^2, and I^2 metrics. A fixed-effects model for data synthesis was preferable if no heterogeneity was identified in the treatment effects between studies; otherwise, the random-effects model would be adopted. Data synthesis was carried out, and the treatment effects of interventions were examined (RDs and 95% CI) by comparing them to the reference treatment. For pairwise comparisons of the two treatments, a league plot of relative effect was presented. Sensitivity analysis was performed using a "split-node" method introduced by Dias et al. [16] to access the consistency between direct and indirect evidence. Publication bias was assessed through graphical inspection of the asymmetry of the funnel plot and evaluated by Egger, Begg–Mazumdar, and Thompson–Sharp methods.

2.2. Cost-Effectiveness Analysis

2.2.1. Referencing Resources. The cost of acupuncture and moxibustion treatments was extracted from a government document (Table 1. The reference prices of pharmaceutical medications were evaluated based on the information on the drugs retrieved from the local healthcare service pricing system (Table 2).

2.2.2. Cost Estimation. Net outpatient treatment costs, including direct and indirect, were estimated in a 1-week treatment period. The indirect costs include lost working income and the corresponding transportation expenses. The average income of citizens in Guangzhou city is 61,241 RMB (Chinese Yuan)/year in 2018 [17] (about 22.85 RMB/hour in an 8 h working period of a day). The transportation expense was estimated by assuming the use of public transport by the patients as 8 RMB each time they came to the clinic. We also assumed the patient spent 2 h on the road and another hour for the interview and treatment. The net indirect cost was 76.55 RMB every time a patient visited. For pharmaceutical treatments, the indirect cost was considered once a week for one-week replenishment in ordinary clinical settings. For nonpharmaceutical treatments, indirect costs should be incorporated into the costs per visit.

2.2.3. Cost-Effectiveness Assessment. The number needed to treat (NNT) values of each intervention were calculated by obtaining the reciprocal of the RDs synthesized in the network meta-analysis. Successively, the cost-effectiveness of the intervention was evaluated as incremental cost per additional responder (ICPR) as compared to a reference treatment that could be acquired by multiplying the NNTs with direct or total costs of the treatments. A smaller ICPR of the treatment indicated that it costs less to one extra responder, and thus, the treatment would be cost-effective.

3. Results

3.1. Characteristics of Studies. A total of 6751 studies were retrieved in the preliminary literature search. Of these, 16 studies [18–33] that satisfied the eligibility criteria were identified, and 1308 participants were included in the current study. The selection process of the studies was illustrated in Figure 1. All the included studies were conducted in China. The etiology that causes the PNP in the eligible studies includes sciatica, cervical spondylotic radiculopathy, shingles, postherpetic neuralgia, occipital neuralgia, cervical entrapment syndrome, and trigeminal neuralgia. The pain intensity was evaluated using scales or pain threshold detection. The characteristics of the included studies are summarized in Table 3.

3.2. Risk of Bias. The potential risks of bias were examined. The risk of bias in each domain and the bias rating of the individual study are depicted in Figure 2. The results indicated that randomization processes were the major sources of bias. The overall risk-of-bias judgment was as follows: 12.5% of the studies were at a low risk of bias, 68.75% of the studies had some concerns about the risk of bias, and the remaining 18.75% were at a high risk of bias.

3.3. Network Construction. The network of comparisons included 1 three-arm trial and 15 two-arm trials. Acupuncture and moxibustion techniques, such as normal acupuncture, acupoint injection, acupotomy (round-sharp needling), electrical needling, fire needling, moxibustion, and warm needling, were included in the current study. We also classified the pharmaceutical treatments into two categories, namely, anticonvulsants and nonsteroidal anti-inflammatory drugs (NSAIDs). The treatment effects are synthesized based on direct and indirect pairwise comparisons. The effects of NSAIDs were treated as a reference. The network correlation of a graphical indicator is shown in Figure 3.

3.4. Data Synthesis. The overall heterogeneity Q metric was 12.49 ($P = 0.1873$). Besides, the τ^2 metric was 0.0024 and the I^2 metric of the heterogeneity was 27.9% (95% CI: 0.0–65.4%).

Table 1: Price and dosage of acupuncture and moxibustion treatments.

Treatment	Price unit	Price (RMB)	Dosage	Treatment frequency (times a week)	Notes
Normal acupuncture	5 acupoints	18.98	10 acupoints	6	Charge 3.8 RMB for each extra acupoint
Acupoint injection	2 acupoints	16.50	5 acupoints	7	Charge 8.8 RMB for each extra acupoint
Acupotomy	1 site	55.00	2 sites	2	—
Electrical needling	2 acupoints	15.75	16 acupoints	7	Charge 10.5 RMB for every 2 extra acupoints
Fire needling	3 acupoints	16.50	6 acupoints	5	Charge 5.5 RMB for each extra acupoint
Moxibustion	2 acupoints	25.30	10 acupoints	6	Charge 6.3 RMB for each extra acupoint
Warm needling	5 acupoints	33.00	10 acupoints	7	Charge 3.3 RMB for every 5 extra acupoints

Table 2: Price and dosage of pharmaceutical medications.

Medication	Category	Specification	Dosage	Price (RMB)*
Diclofenac diethylamine emulgel	NSAIDs	20 g	2 g qid	17.18
Ibuprofen sustained release capsules	NSAIDs	0.3 g	0.3 g bid	0.65
Indometacin enteric-coated tablets	NSAIDs	25 mg	25 mg tid	0.15
Nabumetone capsules	NSAIDs	0.25 g	1 g qd	1.28
Nimesulide dispersible tablets	NSAIDs	0.1 g	0.1 g bid	1.73
Carbamazepine tablets	Anticonvulsants	200 mg	200 mg tid	0.84
Gabapentin capsules	Anticonvulsants	100 mg	1200 mg tid	0.46

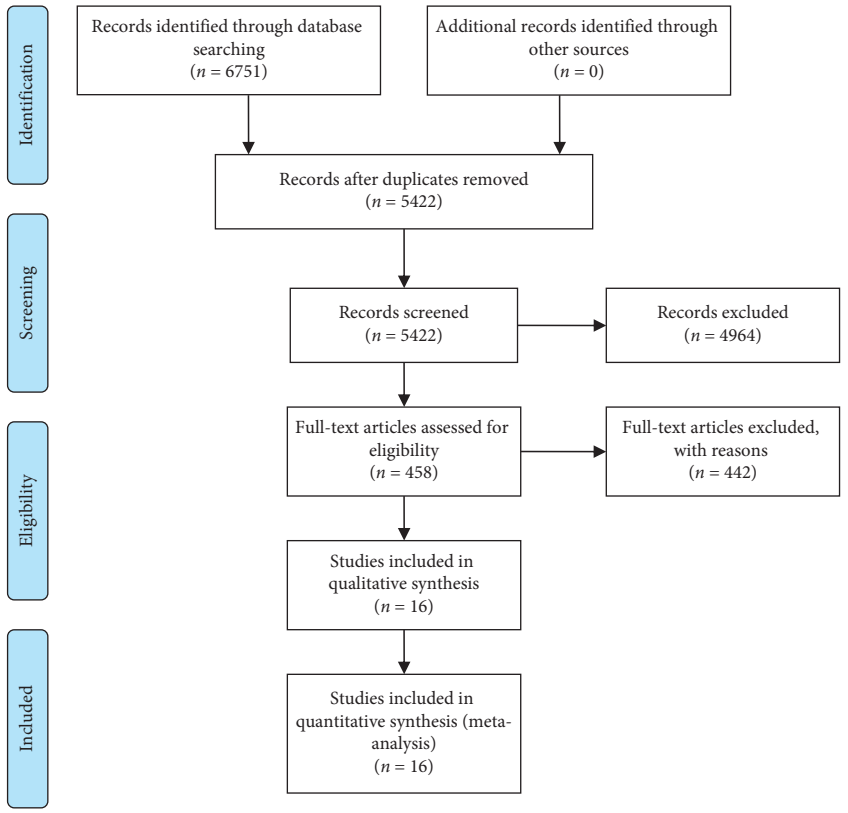

Figure 1: Flowchart of the selection process.

Table 3: Characteristics summary of included studies.

Author	Region	Diagnosis	Assessment tool	Arms	Intervention	Pain duration	Participants	Drop	Female/male	Responder/total	Treatment duration
Chen et al. [18]	China	Sciatica	Pain threshold	3	G1: warm needling G2: nimesulide dispersible tablets G3: acupoint injection	G1: 5.25 ± 3.59 y G2: 5.78 ± 4.87 y G3: 4.71 ± 3.96 y	G1: 30 G2: 30 G3: 30	G1: 0 G2: 0 G3: 0	G1: 8/22 G2: 9/21 G3: 10/20	G1: 27/30 G2: 22/30 G3: 19/30	10 d
Chen et al. [19]	China	PHN	VAS	2	G1: normal acupuncture G2: fire needling	G1: 1.35 ± 0.37 y G2: 1.42 ± 0.39 y	G1: 45 G2: 45	G1: 0 G2: 0	G1: 16/29 G2: 14/31	G1: 36/45 G2: 43/45	20 d
Gao [20]	China	Shingles	VAS	2	G1: electrical needling G2: indometacin enteric-coated tablets	G1: 3.32 ± 1.21 y G2: 3.12 ± 1.43 y	G1: 40 G2: 40	G1: 0 G2: 0	G1: 16/24 G2: 15/25	G1: 40/40 G2: 34/40	10 d
Han [21]	China	Cervical entrapment syndrome	VAS	2	G1: acupotomy (round-sharp needling) G2: normal acupuncture	G1: 3 m–11 y G2: 5 m–12 y	G1: 68 G2: 67	G1: 0 G2: 0	G1: 27/41 G2: 28/39	G1: 66/68 G2: 67/67	14 d
Hong [22]	China	Occipital neuralgia	VAS	2	G1: fire needling G2: carbamazepine tablets	G1: 3.8 ± 1.6 d G2: 3.7 ± 1.8 d	G1: 30 G2: 27	G1: 0 G2: 0	G1: 16/14 G2: 15/12	G1: 30/30 G2: 21/27	5 d
Li et al. [23]	China	PHN	VAS	2	G1: moxibustion G2: fire needling	G1: 5.90 ± 1.44 m G2: 6.07 ± 1.27 m	G1: 40 G2: 40	G1: 0 G2: 1	G1: 17/23 G2: 18/22	G1: 39/40 G2: 39/39	30 d
Li [24]	China	PHN	VAS	2	G1: indometacin enteric-coated tablets G2: normal acupuncture	G1: 4.05 ± 2.13 m G2: 4.13 ± 2.22 m	G1: 42 G2: 42	G1: 0 G2: 0	G1: 15/27 G2: 16/26	G1: 28/42 G2: 36/42	15 d
Lin et al. [25]	China	PHN	VAS	2	G1: moxibustion G2: ibuprofen sustained release capsules	G1: 5.90 ± 1.44 m G2: 6.07 ± 1.27 m	G1: 30 G2: 30	G1: 1 G2: 3	G1: 13/17 G2: 16/14	G1: 28/29 G2: 19/27	28 d
Liu [26]	China	PHN	VAS	2	G1: indometacin enteric-coated tablets G2: normal acupuncture	G1: 4.11 ± 1.69 m G2: 4.12 ± 1.61 m	G1: 40 G2: 40	G1: 0 G2: 0	G1: 18/22 G2: 17/23	G1: 32/40 G2: 38/40	15 d
Wang et al. [27]	China	PHN	VAS	2	G1: fire needling G2: normal acupuncture	G1: 1–10 m G2: 1–11 m	G1: 28 G2: 28	G1: 0 G2: 0	G1: 12/16 G2: 13/15	G1: 28/28 G2: 28/28	14 d
Xie and Chen [28]	China	Trigeminal neuralgia	VAS	2	G1: carbamazepine tablets G2: normal acupuncture	G1: 9.8 ± 2.6Y G2: 9.4 ± 2.7 y	G1: 63 G2: 63	G1: 0 G2: 0	G1: 28/35 G2: 27/36	G1: 47/63 G2: 57/63	1 m
Xu and Kang [29]	China	Occipital neuralgia	SF-MPQ	2	G1: moxibustion G2: normal acupuncture	NA	G1: 20 G2: 20	G1: 0 G2: 0	NA	G1: 20/20 G2: 18/20	10 d
Xu [30]	China	PHN	VAS	2	G1: fire needling G2: normal acupuncture	26 d–3 y	G1: 44 G2: 43	G1: 0 G2: 0	Total: 35/52	G1: 41/44 G2: 30/43	10 d
Yan et al. [31]	China	PHN	VAS	2	G1: fire needling G2: diclofenac diethylamine emulgel	G1: 180.41 ± 25.71 d G2: 175.50 ± 26.05 d	G1: 38 G2: 37	G1: 0 G2: 0	G1: 17/21 G2: 17/20	G1: 37/38 G2: 29/37	28 d
Zhou [32]	China	Occipital neuralgia	VAS	2	G1: normal acupuncture G2: nabumetone capsules	G1: 4.13 ± 1.22 d G2: 3.87 ± 1.39 d	G1: 60 G2: 60	G1: 2 G2: 5	G1: 33/27 G2: 29/31	G1: 55/58 G2: 40/55	5 d
Zhou et al. [33]	China	Cerical spondyliotic radicuolathy	Symptom scales	2	G1: warm needling G2: normal acupuncture	NA	G1: 30 G2: 30	G1: 0 G2: 0	G1: 8/22 G2: 12/18	G1: 30/30 G2: 26/30	10 d

PHN, postherpetic neuralgia; VAS, visual analogue scale; SF-MPQ, short-form McGill pain questionnaire; NSAIDs, nonsteroidal anti-inflammatory drugs; G1 or (2, 3), group 1 or (group 2, group 3); NA, not available; d, day; m, month; y, year.

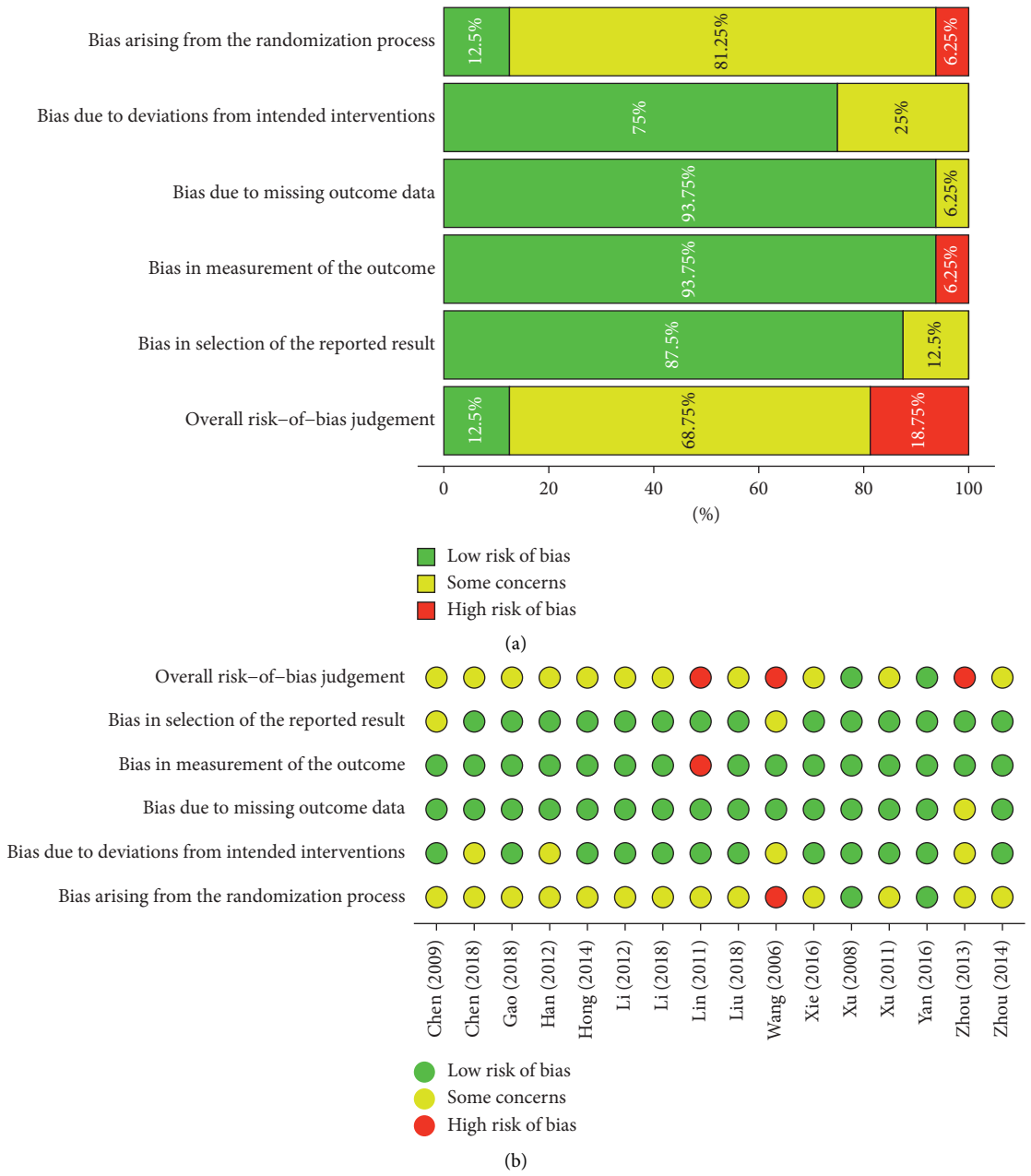

Figure 2: Risk of bias judgment in each domain (a) and risk of bias rating of individual study (b).

No heterogeneity was identified; thus, a fixed-effects model was used to estimate the pooled effect size for the RD of each treatment against the effect of NSAIDs as the reference (Figure 4). The effects of the treatment were scored as P values. In the current case, a small P-score indicates an improved treatment effect. Pairwise comparisons of the relative treatment effects were presented as a league plot (Figure 5). Typically, all the included acupuncture and moxibustion techniques showed superior improvements in PNP relief as compared to NSAIDs ($P < 0.05$), except acupoint injection. Anticonvulsants showed stronger effects (RD: 0.01 [−0.14; 0.16]) than NSAIDs; nevertheless, the effects were not significant ($P > 0.05$). Among the acupuncture and moxibustion techniques, warm needling (RD: 0.31 [0.17; 0.45]),6 fire needling (RD: 0.26 [0.17; 0.35]), and moxibustion (RD: 0.24 [0.15; 0.34]) were the most effective, but no significant differences were detected in the effects between these three interventions ($P > 0.05$).

3.5. Sensitivity Analysis. The split-node plot in Figure 6 shows the consistency between direct and indirect evidence, which indicated the robustness of our network results. However, there was only one direct comparison for each electrical needling (vs. NSAIDs) and acupotomy (vs. normal acupuncture), and estimated effects of these isolated nodes depend merely on the direct evidence, which the "split-node" method is incapable to assess.

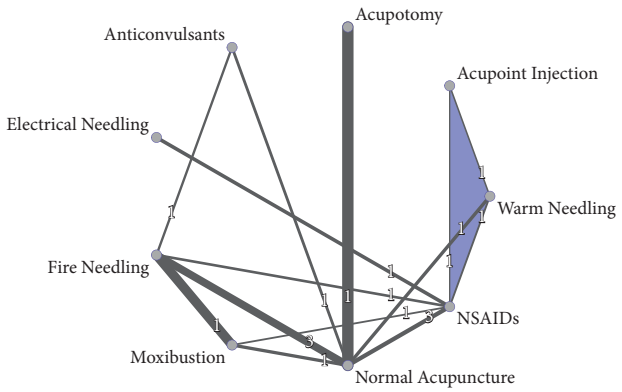

Figure 3: The network of direct comparisons of the treatments. Lines connecting two treatments indicate direct comparisons for those two arms of treatments. The width of the lines represents the inversion effect estimates. The blue shading area infers multiple arms of direct comparisons in a single study. NSAIDs, nonsteroidal anti-inflammatory drugs.

3.6. Publication Bias. The funnel plot was used to assess the publication bias (Figure 7), which showed the symmetry distribution of the comparison-specific effects of treatment pairs. Further tests of Begg, Egger–Mazumdar, and Thompson–Sharp tests showed no potential publication bias was identified ($P > 0.05$).

3.7. Cost-Effectiveness Analysis. The NNT exhibits one additional pain relief responder relative to NSAIDs, which can be calculated from the inversion of RDs (Table 4). In the situation when the treatment effect does not differ significantly from the reference, the zero is inevitably included in the 95% CI. Consequently, the NNT becomes infinite, containing two disjoint regions. To emphasize the continuity, we described the 95% CI of such a treatment effect in the format of "number needed to harm" to ∞ to "NNT" as recommended in a previous study [34]. The costs of treatments in 1 week are estimated in Table 5.

In the CEA of direct costs (Figure 8(a)), fire needling had the lowest ICPR (ICPR: 634.08 [469.90; 974.64] RMB/week) relative to NSAIDs, while that for warm needling was 822.48 (570.22; 1,475.00) RMB/week, normal acupuncture was 1,134.10 (796.82; 1,966.47) RMB/week, acupotomy was 1,284.27 (827.84; 2,862.53) RMB/week, moxibustion was 1,855.12 (1,327.45; 3,079.06) RMB/week), and electrical needling was 3,896.34 (2,163.42; 19,580.54) RMB/week).

Similar results are shown in the CEA of total costs (Figure 8(b)). Fire needling still had the lowest ICPR (2,104.97 [1,559.91; 3,235.51] RMB/week) relative to NSAIDs, followed by acupotomy (ICPR: 2,178.01 [1.403.94; 4,894.60] RMB/week), warm needling (ICPR: 2,556.92 [1,772.70; 4,585.51] RMB/week), normal acupuncture (ICPR: 3,419.91 [2,402.84; 5,929.95] RMB/week), moxibustion (ICPR: 3,731.07 [2,669.81; 6,192.69] RMB/week), and electrical needling (ICPR: 7,238.25 [4,019.00; 3,6374.82] RMB/week).

Conversely, there is indiscriminate harm or benefit of (in)direct ICPR in anticonvulsants and acupoint injection relative to NSAIDs. Patients might not benefit significantly from anticonvulsants and acupoint injection as compared to NSAIDs.

4. Discussion

We presented a comprehensive systematic review and network meta-analysis that focuses on acupuncture and moxibustion treatments for PNP. Herein, seven widely applicable acupuncture and moxibustion techniques and two categories of pharmaceutical therapies have been assessed. However, NSAIDs, one of the included categories of pharmaceutics in the present study, are not recommended in the guidelines, but are still prescribed in Chinese clinics. Nonetheless, 6 of the included studies involved NSAID treatments. Since analgesics, such as NSAIDs have only ceiling effect against the pain of neuropathic origin, they constitute the reference group in our analyses. Furthermore, techniques applying heat, such as warm needling, fire needling, and moxibustion, showed the highest therapeutic rankings in our network meta-analysis. Except for acupoint injection, we observed that the acupuncture and moxibustion techniques exhibited higher efficacy as compared to pharmaceutics. Previous meta-analyses have shown controversial results. Dimitrova et al. [37] concluded that acupuncture is beneficial in some peripheral neuropathies, while Ju et al. [38] speculated that the data are insufficient to conclude the effects of acupuncture for PNP. Despite different primary outcomes and diverse conclusions, both studies emphasized the demand for additional rigorously designed trials to clarify the actual therapeutic effects of acupuncture in PNP treatment. Similarly, the included trials, with diverse qualities, in the current study were exclusively conducted in China. The risk-of-bias assessment addressed some of the great concerns of the risk of bias, among which randomized processes were the major sources of bias.

A secondary analysis of the assessment of the cost-effectiveness correlation was performed among the included treatments. Based on the perspective of healthcare providers, at least one visit per week is necessary for acupuncture and moxibustion treatment, while one visit a week for the replenishment of pharmaceutical drugs is feasible in most clinical settings. Hence, the cost-effectiveness of different treatments was assessed in a 1-week treatment period. We found that fire needling is the most cost-effective treatment for PNP, especially for direct costs. Accounting for the total cost of each treatment from the perspective of patients' treatment burdens, fire needling still exhibited maximal cost-efficiency. Notably, acupotomy became the second-ranked cost-efficient therapy in our CEA of total costs that required only a few visits per week. PNP is a chronic condition that needs prolonged treatment. Potential risks of adverse effects and abuse concerns should be tagged, especially in the second and third-line therapies for PNP containing opiates or neurotoxin [39]. Acupuncture and moxibustion techniques are widely used in pain management, which cause fewer side effects than other pharmaceutical alternatives. Our results suggested that patients would have better responses to acupuncture and

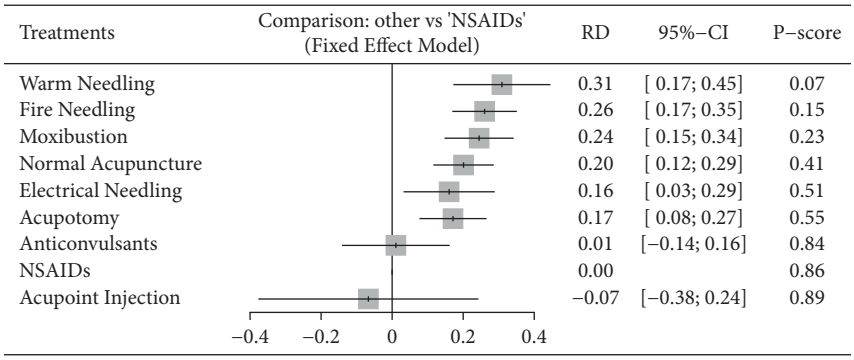

Figure 4: Forest plot of treatment effects vs. NSAIDs (risk difference). The smaller *P*-score value indicates a better effect ranking. NSAIDs, nonsteroidal anti-inflammatory drugs; RD, risk difference; 95% CI, 95% confidence interval.

Acupoint Injection								
-0.24 [-0.55; 0.07]	*Acupotomy*							
-0.08 [-0.41; 0.26]	0.16 [0.03; 0.30]	*Anti-Convulsants*						
-0.23 [-0.56; 0.11]	0.01 [-0.15; 0.17]	-0.15 [-0.35; 0.05]	*Electrical Needling*					
-0.33 [-0.64; -0.02]	-0.09 [-0.16; -0.02]	-0.25 [-0.38; -0.12]	-0.10 [-0.26; 0.06]	*Fire Needling*				
-0.31 [-0.63; 0.00]	-0.07 [-0.15; 0.00]	-0.23 [-0.37; -0.10]	-0.08 [-0.25; 0.08]	0.02 [-0.03; 0.06]	*Moxibustion*			
-0.27 [-0.58; 0.04]	-0.03 [-0.07; 0.01]	-0.19 [-0.32; -0.06]	-004 [-0.19; 0.11]	0.06 [0.01; 0.11]	0.04 [-0.02; 0.11]	*Normal Acupuncture*		
-0.07 [-0.38; 0.24]	0.17 [0.08; 0.27]	0.01 [-0.14; 0.16]	0.16 [0.03; 0.29]	0.26 [0.17; 0.35]	0.24 [0.15; 0.34]	0.20 [0.12; 0.29]	*NSAIDs*	
-0.38 [-0.67; -0.08]	-0.14 [-0.27; -0.01]	-0.30 [-0.48; -0.12]	-0.15 [-0.34; 0.04]	-0.05 [-0.18; 0.08]	-0.06 [-0.20; 0.07]	-0.11 [-023; 001]	-0.31 [-0.45; -0.17]	*Warm Needling*

Figure 5: League plot of relative effects (RDs) among nine interventions. The treatment in the corresponding column is compared to the treatment in the corresponding row. Values are the RDs and related 95% CI. Bold fonts and orange-shaded cells represent significant relative effects ($P < 0.05$).

moxibustion treatments in lowering pain intensity. The clinical and economic feasibility of therapy is a critical factor for healthcare providers and patients. We also found that fire needling was the most cost-effective among the included therapies.

We have assessed a total of 7 widely applicable acupuncture and moxibustion techniques that elaborate specific manipulations to achieve their clinical effects. Normal acupuncture is the most fundamental acupuncture technique that simply inserts fine needles (acupuncture needles) into the skin or acupoints. Sensations of soreness, heaviness, numbness, or distension around the insertion points through the twisting of the body of the needles, a process called "deqi," are believed to increase the therapeutic effects [30]. Greater effects are often achieved by applying additional sources of stimulations on the needles, such as electrical currents or heats, namely, electrical needling and warm needling. In electrical needling, micro-electric currents are applied on the needle resulting in circuit loops formation and subsequent stimulations on the needling sites [31]. Warm needling is a combination of acupuncture and moxibustion techniques. A burning small moxa tower affixed on the handle of the acupuncture needle, and heat generated from the burning moxa would radiate superficially or be conducted through the needle body to between tissues underneath the skin [32]. Fire needling involves a slightly different technique compared to normal acupuncture, that is, rapid insertion of red-hot specialized needles that are made of tungsten-manganese alloy into acupoints and lift without leaving the needles on the insertion points [33]. Acupoint injection is a kind of therapeutic method that injects medicine or nutrient supplements into acupoints subcutaneously or intramuscularly [34]. The moxibustion technique involves stimulating acupoints or areas of the body by radiating heat and volatile chemicals released from a burning moxa stick [35]. Acupotomy (round-sharp

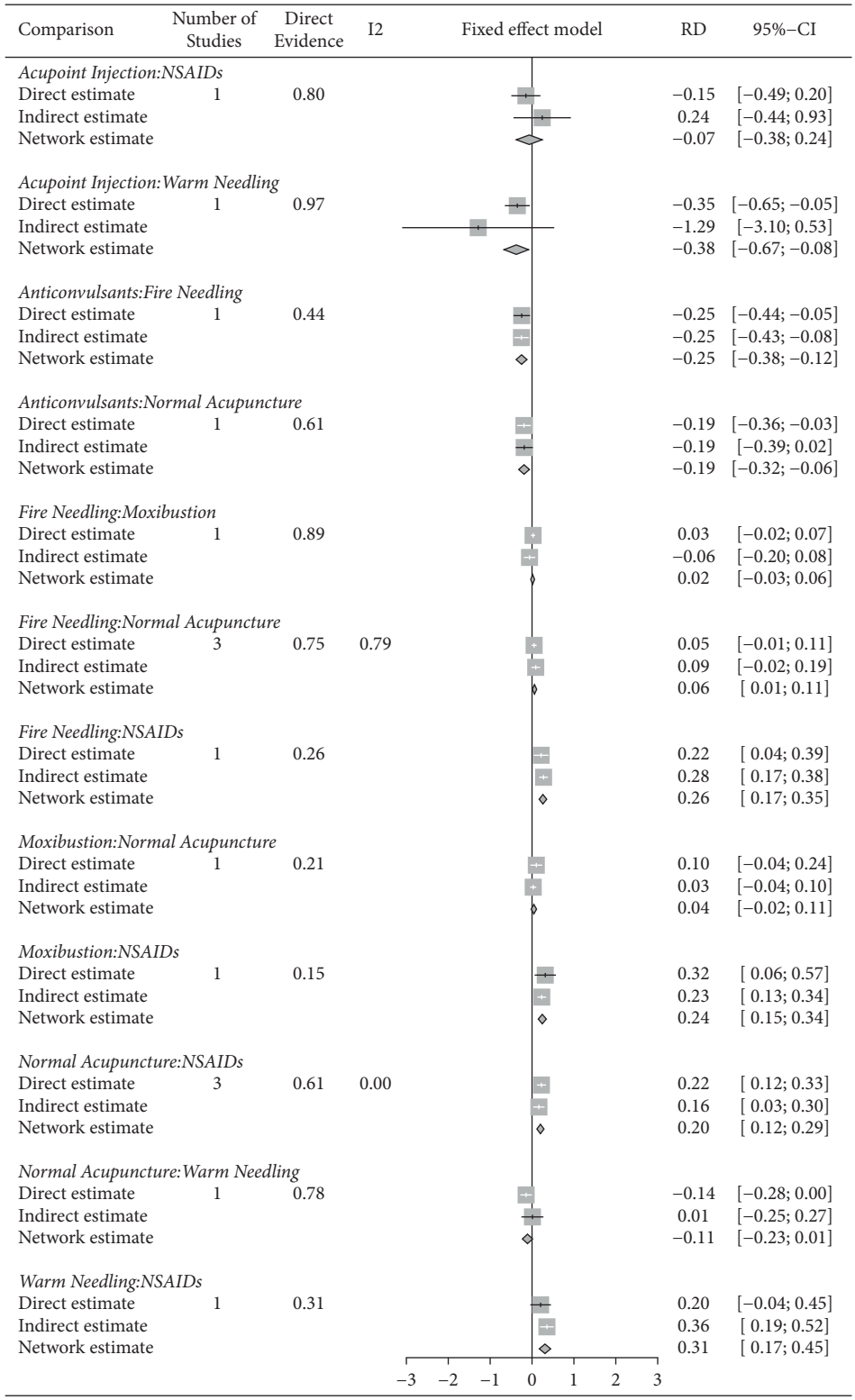

Figure 6: Split-node plot of treatment pairs.

needling) is developed from an ancient acupuncture technique that integrates modern anatomy and sports medicine theories. A needle with a sharp round tip was inserted deeply into the skin, and by manipulating the sharp edge at the end of the needle tip, compressions or adhesions can be resolved [36].

Diagnosis and assessment of NP are challenging due to the absence of pain biomarkers. The diagnosis of NP rarely

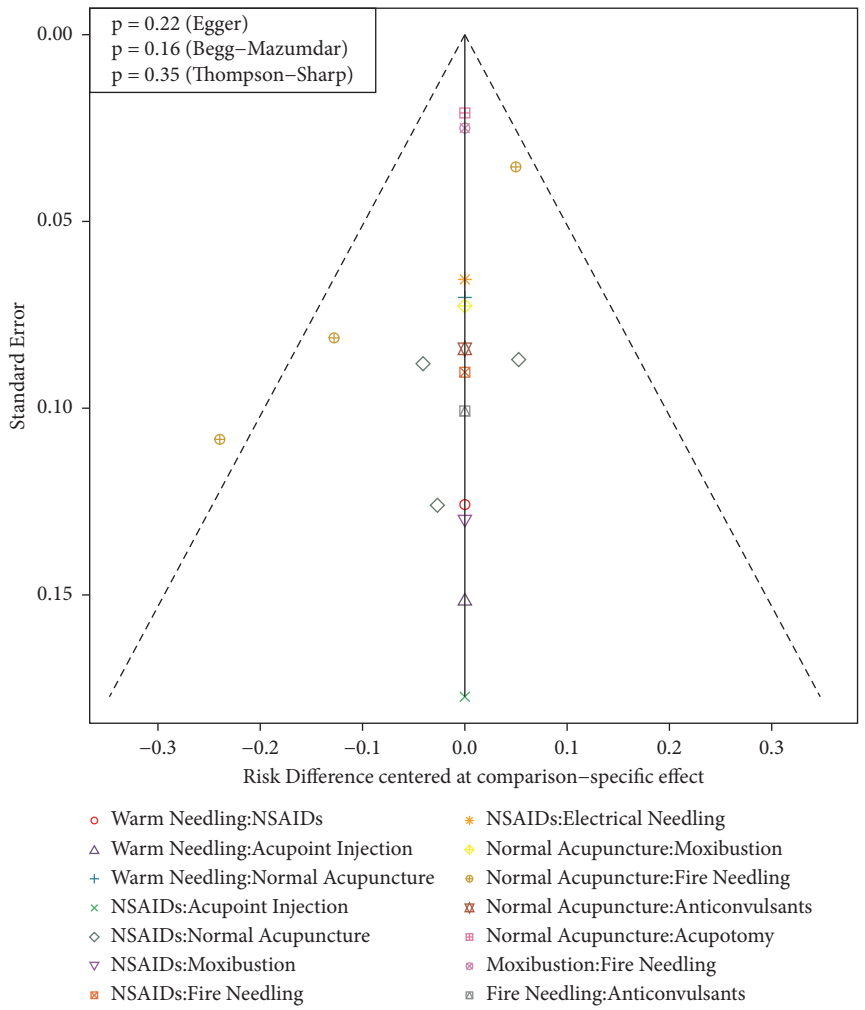

Figure 7: Funnel plot of comparison-specific effects of treatment pairs. Symbols indicate comparisons of different treatment pairs with an indicator on the upper-right corner; *P* values of statistical tests for publication bias are shown on the upper-left corner. NSAIDs, nonsteroidal anti-inflammatory drugs.

Table 4: Risk difference and number needed to treat for each intervention.

Treatments	RD [95% CI]		NNT [95% CI]	
NSAIDs	Reference		Reference	
Normal acupuncture	0.20	[0.12; 0.29]	4.98	[8.63; 3.50]
Anticonvulsants	0.01	[−0.14; 0.16]	95.08	[7.09 (H) to ∞ to 6.17(B)]
Acupoint injection	−0.07	[−0.38; 0.24]	−14.96	[2.65 (H) to ∞ to 4.12 (B)]
Acupotomy	0.17	[0.08; 0.27]	5.84	[13.01; 3.76]
Electrical needling	0.16	[0.03; 0.29]	6.24	[31.34; 3.46]
Fire needling	0.26	[0.17; 0.35]	3.84	[5.91; 2.85]
Moxibustion	0.24	[0.15; 0.34]	4.08	[6.78; 2.92]
Warm needling	0.31	[0.17; 0.45]	3.24	[5.80; 2.24]

NSAIDS, nonsteroidal anti-inflammatory drugs; RD, risk difference; NNT, number needed to treat; 95% CI, 95% confidence interval; (H), number needed to treat to be harmful; (B), number needed to treat to be beneficial.

relies on other diagnosis techniques except for clinical criteria, and the crucial point is to differentiate pain caused by nerves lesion from other types of pain. In our current study, the assessment of pain is based on tools for common situations such as visual analogue scale, short-form McGill pain questionnaire, pain threshold, and symptom scales. However, as stated before, apart from pain, NP or PNP patients are often accompanied by psychological conditions and lower quality of life. Future research should emphasize whether acupuncture and the associated techniques can

Table 5: Treatment costs in a one-week period.

Treatments	Direct cost/week (RMB)	Total cost/week (RMB)	Average cost/week (RMB)
Nonpharmaceutical treatments			
Normal acupuncture	227.88	687.18	
Acupoint injection	300.30	836.15	
Acupotomy	220.00	373.10	
Electrical needling	624.75	1160.60	
Fire needling	165.00	547.75	
Moxibustion	454.20	913.50	
Warm needling	254.10	789.95	
Pharmaceutical treatments			
NSAIDs			Direct: 24.08 Total: 100.63
Diclofenac diethylamine emulgel	48.10	124.654	
Ibuprofen sustained release capsules	9.10	85.65	
Indometacin enteric-coated tablets	3.15	79.70	
Nabumetone capsules	35.84	112.39	
Nimesulide dispersible tablets	24.22	100.77	
Anticonvulsants			Direct: 63.84 Total: 140.39
Carbamazepine tablets	11.76	88.31	
Gabapentin capsules	115.92	192.47	

NSAIDs, nonsteroidal anti-inflammatory drugs.

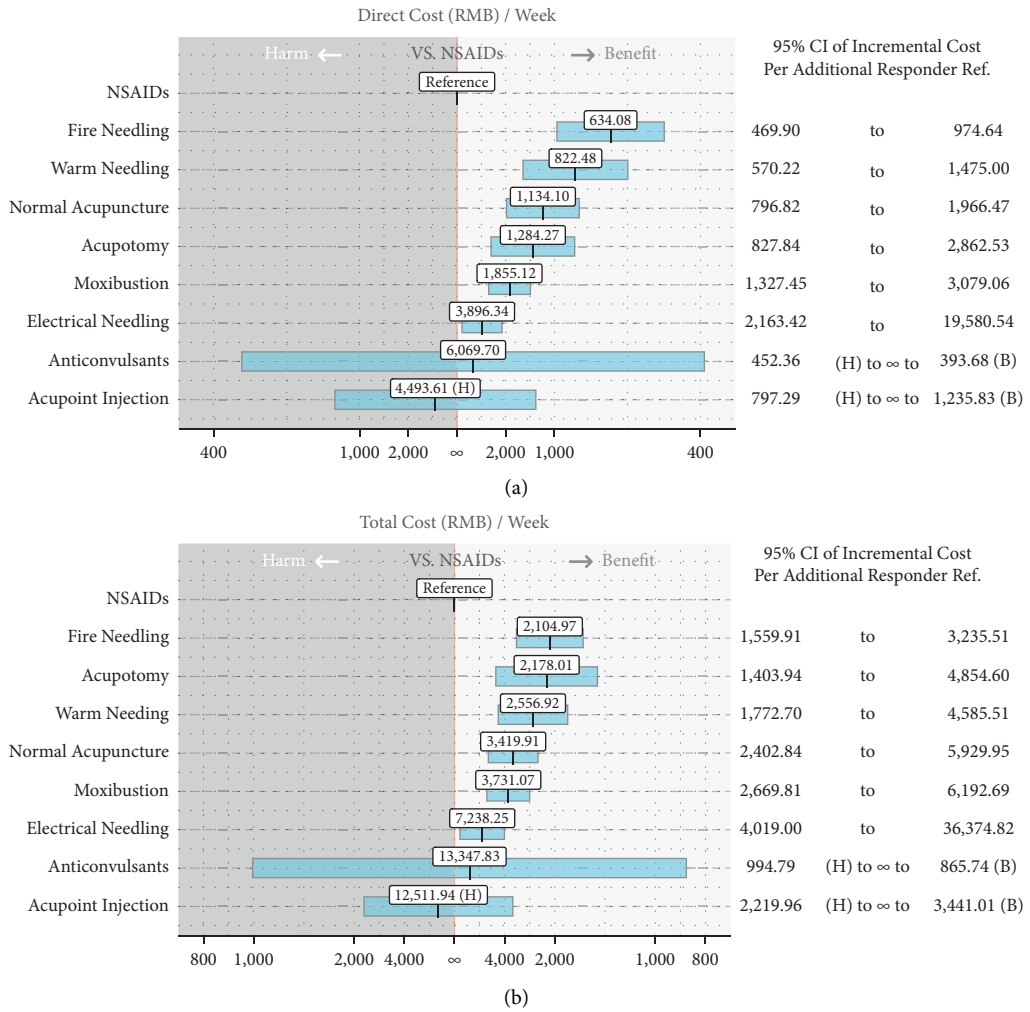

Figure 8: Incremental direct (a) and total (b) costs per additional responder related to NSAIDs in the 1-week treatment period. The treatment in the corresponding column is compared to the treatment in the corresponding row. Values are the RDs and related 95% CI. Bold fonts and orange-shaded cells represent significant relative effects ($P < 0.05$). 95% CI, 95% confidence interval; Ref., reference cost (NSAIDs); (H), cost to be harmful; (B), cost to be beneficial.

improve the psychological status and the quality of life among NP patients. Screening tools that are specialized for assessing NP and the associated psychometric properties. Many of them are available, like the Leeds assessment of neuropathic symptoms and signs, neuropathic pain questionnaire, PainDetect, ID-Pain, and DN4, which were developed and validated [4].

The primary limitation of the present study is the small number of studies that fulfill the selection criteria and small sample sizes of the included studies, leading to insufficient direct comparisons between treatment pairs in the network construction. Agents, such as gabapentinoid, tricyclic antidepressants, and norepinephrine reuptake inhibitors, are currently recommended as first-line analgesics for NP management [40]. The efficacy of anticonvulsants was indifferent to the efficacy of NSAIDs on PNP and might be underestimated in the present analysis, and more upcoming researches are needed to be included to produce more precise inferences. There was only one direct comparison for each electrical needling and acupotomy therapy and the robustness of their network effects should be interpreted with cautions; further studies on different comparisons about these two therapies are warranted. Our network meta-analysis involved only mono-therapy interventions. Herein, we did not assess the treatments and additive effects of combination therapies of acupuncture and moxibustion with other therapies, which might be a common clinical application. Moreover, studies on PNP due to various causes and different disease duration and treatment periods were also included in this meta-analysis. These might introduce sources of confounding to our results.

5. Conclusion

In summary, our findings revealed that approaches for easing PNP including acupuncture and moxibustion techniques are cost-effective as compared to pharmaceutics except for acupoint injection. The current results also suggested that acupuncture and moxibustion techniques involving heat modalities provide preferable treatment responses in PNP relief.

Abbreviations

NP: Neuropathic pain
PNP: Peripheral neuropathic pain
CEA: Cost-effectiveness analysis
RD: Risk difference
CI: Confidence interval
NNT: Number needed to treat
ICPR: Incremental cost per additional responder
NSAIDs: Nonsteroidal anti-inflammation drugs.

Data Availability

All data supporting our findings are available within the manuscript.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Weixuan Zhao and Haoming Huang contributed equally to the study.

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Research Article

Efficacy of Fu's Subcutaneous Needling on Myofascial Trigger Points for Lateral Epicondylalgia: A Randomized Control Trial

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Lateral epicondylalgia (LE), a common overuse syndrome of the extensor muscle and tendons on the lateral epicondyle, causes persistent severe musculoskeletal pain on the outer part of the elbow. Fu's subcutaneous needling (FSN), a newly invented subtype of acupuncture and dry needling, is a new trend and potential treatment of LE by targeting the myofascial trigger points (MTrPs). However, no scientific evidence is available to support this method. This study aims to evaluate the distal FSN treatment on the LE by measuring pain-related scales, such as visual analog scale (VAS), pressure pain threshold (PPT), muscle tissue hardness (TH), pain-free grip (PFG), and the functional outcome by a patient-rated tennis elbow evaluation (PRTEE) questionnaire study. A total of 60 LE patients were randomly divided into FSN ($n = 30$) and transcutaneous electrical nerve stimulation (TENS, $n = 30$) as the control group. Every subject was treated with three regimens and followed up for 15 days. Results showed that FSN has an immediate effect on VAS, PPT, TH, and PFG. Moreover, sustained effects on pain relief were followed up to 15 days. Pain remission was consistent with long-term PRTEE results. Overall, FSN is a safe and efficient therapy option for LE, significantly improving pain relief and activity difficulty with immediate, short-term, and long-term effectiveness. This trial is registered with ClinicalTrials.gov NCT03605563.

1. Introduction

Lateral epicondylalgia (LE), also known as tennis elbow, is one of the musculoskeletal disorders and is the most common cause of elbow pain due to overuse of the extensor muscle and tendons [1]. The prevalence of LE is approximately 1% to 3% of the overall population; however, LE is found in up to 23% of male tennis athletes [2, 3]. The pain and tenderness caused by LE are highly related to repeated and forceful contractions of the wrist and fingers muscles, which is commonly seen in griping and holding continuously for a long time [4]. Lateral elbow tendinopathy is a common condition and is used to assess the severity of LE despite the absence of pathological bone lesion of the elbow in radiographic imaging with large intrasubstance tears identified by musculoskeletal sonography [5]. The thick common extensor tendon, bone spurs, and color Doppler activity of the elbow are often found in LE patients [6].

Although most patients recover within one year, some patients have a long disease course up to two years [7]. Conventional treatments, including local injections with anesthetics [8], steroids [9], and platelet-rich plasma [10], extracorporeal shock waves [11], transcutaneous electrical nerve stimulation (TENS) [12], acupuncture [13, 14], and dry needling [15, 16] are widely used. However, most of these treatments only provide short-term pain relief. The most effective treatment for LE remains to be inconclusive.

Fu's subcutaneous needling (FSN) is a new technique originated from Chinese acupuncture, which utilizes the dual structure trocar and chooses the site away from the lesion, targeting the myofascial trigger points (MTrPs) in the taut band with needling insertion and sway movement in the superficial fascia. FSN has the advantages of few needle requirements and no need to penetrate to the deep layers of the derma and muscle generally. FSN has an immediate and long-lasting effect on pain control of musculoskeletal diseases through the swaying and reperfusion approach [17, 18]. For example, FSN has also been reported as an effective treatment for low back pain [19, 20]. MTrP in the extensor carpi radialis brevis and extensor digitorum communis muscle can reproduce the pain of LE [21]. As a new technique of acupuncture and dry needling, FSN has been recently applied to treat the chronic recurrent LE clinically without adverse side effects. However, the scientific evaluation of FSN on short- and long-term effectiveness is still lacking.

This study utilized subjective and objective outcome measurements, such as visual analog scale (VAS), pressure pain threshold (PPT), muscle tissue hardness (TH), pain-free grip (PFG), and patient-rated tennis elbow evaluation (PRTEE) questionnaire, to assess the efficacy of the FSN treatment compared with TENS on the LE patients.

2. Materials and Methods

2.1. Study Subjects. A total of 60 participants were recruited in accordance with a randomized, controlled, open-label experiment. This study was approved by the Institutional Review Board of the China Medical University Hospital. All patients gave their written informed consent to participate in this study, and the research was conducted in accordance with the principles of the Declaration of Helsinki.

The inclusion criteria are as follows: (1) age over 20; (2) diagnosed with LE for more than one month and subjective VAS >5; and (3) local tenderness on lateral epicondyle with exacerbating pain under isometric resistant test for supination of the forearm. Meanwhile, the exclusion criteria include the following: (1) patients who previously received operations for the neck, upper back, or four limbs; (2) currently undergoing different therapies for LE; and (3) equipped with a pacemaker, diagnosed with epilepsy, or other conditions, such as skin injury, contributing to the inapplicability of electric patch.

Every subject was randomly allocated into two groups: an experimental group who will undergo FSN treatment, and a placebo group who will undergo TENS treatment (Figure 1). The entire course lasts for two weeks. A total of three treatment sessions will be performed in the first week, with assessment before each treatment session and immediately after treatment as well as the 1st and 2nd week for follow-up. All the treatments were conducted by the same acupuncturist who worked in the medical center in Taiwan for more than five years.

2.2. Fu's Subcutaneous Needling (FSN). The forearm of the experimental group relied on using a disposable Fu's subcutaneous needle (Nanjing FSN Medical Co., Ltd., Jiangsu, China) to treat the radial aspect of the forearm extensor muscle (Figure 2(a)). The puncture site was on the midpoint of the extensor muscle of the forearm, in which the tip of the needle was pointed toward the lateral epicondyle (Figure 2(b)) and inserted into the subcutaneous layer with the entire needle body (Video S1).

Receding the core needle and then fixing the protuberance of the soft tube seat in the slot of the core seat prevent the exposure of the needle tip outside followed by starting a swaying movement. The tip of the needle should be maintained at the same horizontal level in swaying by using the thumb and the middle finger holding the core base, and the index and ring fingers are separated on the left and right side of the middle finger to sway in a seesaw-like sector one after the other (Figure 2(b) and Video S2). The time and frequency of swaying are 50 times within 30 s. The subjects would be asked to perform wrist extension movement with resistance for 10 s after swaying and then rest for another 10 s (Figure 2(c)). The cycle is repeated up to three times for 1 min. The subjects would then be asked to simulate actions of wringing a towel (forearm supination and pronation) for 10 s and rest for 10 s, also repeating the cycle up to three times for 1 min (Figure 2(d)). The needle is then removed after completing the two movements, namely the "reperfusion approach," with the subcutaneous embedding of FSN.

2.3. Transcutaneous Electrical Nerve Stimulation. The forearm of the placebo subjects was treated with a transcutaneous electrical nerve stimulator (Well-Life Healthcare Limited, Taiwan, Figure 3(a)), with 2 electrodes attached to acupoints, namely TE5 (*Waiguan*) and LI11 (*Quchi*), according to the guidance of the WHO (Figure 3(b)), the two most commonly used acupoints for LE treatment by acupuncturists [22]. The treatment parameters were set to a pulse of width 200 μs, a frequency of 200 Hz, and a continuous wave for 20 min.

2.4. Outcome Measurements

(1) VAS is a subjective pain intensity questionnaire for pain severity [23]. Patients evaluated the score of pain severity from no pain (score zero) to intolerable pain (score ten) before and after treatment.

(2) PPT is a semiobjective quantification tool. Pressure algometry is used to measure the PPT on MTrPs by following Fischer's standard method [24, 25].

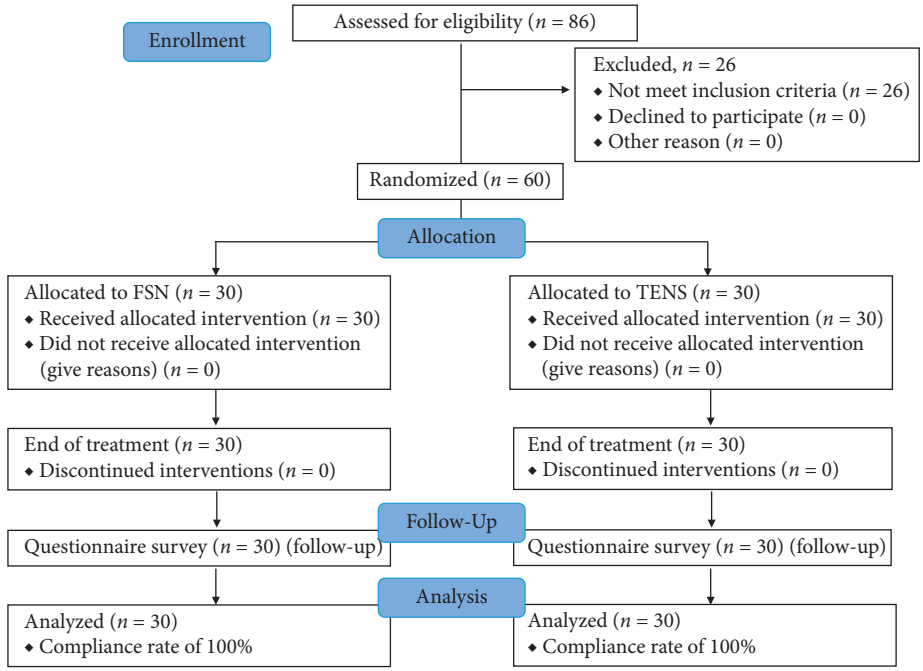


Figure 1: Study flow chart.

(3) The PFG test is commonly performed using a grip dynamometer to measure the amount of force of grasp (kg) generated by LE patients upon the onset of pain [26]. The test started with a normal arm, wherein subjects holed the grip dynamometer (Jamar® Plus + Digital Hand Dynamometer, Performance Health, IL US) with a relax and extension status and slowly began griping until the onset of pain. No pain of the test result was recorded as the maximum grip strength. The testing was repeated three times with 1 min rest intervals. The same procedure was performed on the affected arm. The average of PFG was then calculated and recorded.

(4) TH assessment of the forearm muscle revealed the capability of muscles against deformation during activities. [27] Patients with LE often suffered from the stiffness of the extensor muscle near the elbow joint, and their activities are limited. TH is determined by using a myometer (OE-220, purchased from ITO CO., Ltd., Tokyo, Japan), a noninvasive and objective electronic device, thus providing an accurate diagnosis for clinicians to determine worse situations involving the muscle [28].

(5) The PRTEE questionnaire was regularly used to measure perceived pain and disability [29]. A total of 15 self-reported questionnaires involved in pain, usual activities, and specific activities scaled from 0 (no pain or difficulty) to 10 (worst ever or unable to perform). The subjects were asked to answer the questionnaire before the treatment on day 1 and were then followed up on days 8 and 15 post treatment.

2.5. Statistics. Statistically significant differences ($P < 0.05$) among the results were revealed by using Statistical Package for Social Science (SPSS 18.0) for Windows. Data were expressed as mean ± standard deviation. The analysis of baseline characteristics of age, sex, VAS, PTT, TH, PFG, and PRTEE was conducted by analysis of variance (ANOVA). The within-group analysis of all variables was conducted by paired sample t-test for inferential statistics, while the between-group analysis of the variables was performed by independent two-sample t-test.

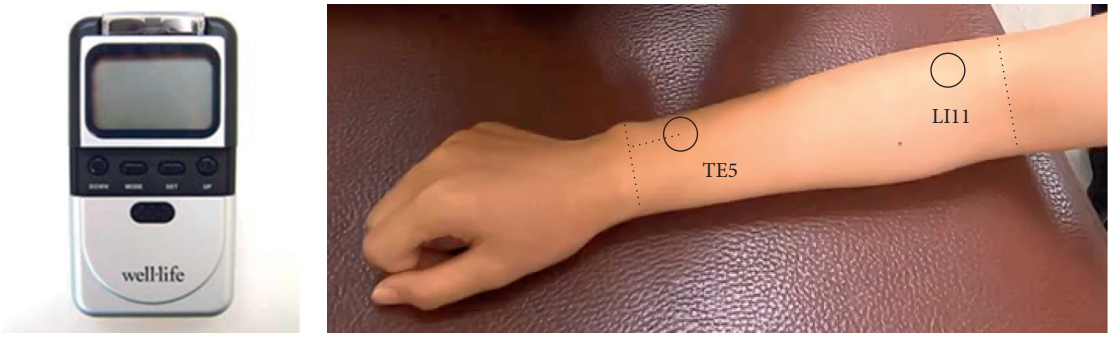
3. Results and Discussion

3.1. Baseline Characteristics of the Subjects of the Two Groups in the Study. All subjects were randomly divided into FSN and TENS groups, and the baseline characteristics are shown in Table 1. The mean age was 47 in both groups. A total of 21 males and 39 females were enrolled and assigned randomly. No significant difference was found between the two groups of age, gender, pre-Tx value of VAS, PPT, TH, PFG, and PRTEE. Thus, this study is a well-randomized prospective investigation for the advanced measurement of effectiveness.

3.2. Immediate Effect of FSN and TENS. The pain-related scales, such as VAS, PPT, TH, and PFG, are compared in pre-and post-treatment to evaluate the immediate effectiveness of FSN (Table 2). VAS (pre-Tx, 6.06 ± 1.43; post-Tx, 3.56 ± 2.16, $P < 0.01$), PPT (pre-Tx, 16.60 ± 3.80; post-Tx, 20.71 ± 6.69, $P < 0.01$), and PFG (pre-Tx, 16.66 ± 7.76; post-Tx, 20.62 ± 9.67, $P < 0.01$) were significantly improved in the FSN group in day 1 (Figures 4(a)–4(d)). Meanwhile, only VAS (pre-Tx, 5.70 ± 1.19; post-Tx, 4.45 ± 1.37, $P < 0.01$) and PFG (pre-Tx, 16.52 ± 5.99; post-Tx, 17.68 ± 6.28, $P = 0.01$) were significantly improved for TENS (Figures 4(a) and

(a) (b) (c) (d)

Figure 2: Illustration of the operations of the Fu's subcutaneous needling. (a) Fu's subcutaneous needle. (b) Puncture site away from the elbow, and starting a swaying movement. (c) Wrist extension movement. (d) Wringing a towel.



(a) (b)

Figure 3: Illustration of the transcutaneous electrical nerve stimulations (a) and electrodes attached sites on TE5 and LI11 (b).

Table 1: Baseline characteristics and clinical evaluation indicators of the subjects in the two groups.

Groups	FSN	TENS	P value
Number of subjects	30	30	
Age (years)	47.97 ± 11.85	47.10 ± 12.15	0.80
Range of age (years)	26–70	28–70	
Sex			
Male ($n = 21$)	11	10	0.90
Female ($n = 39$)	19	20	0.90
Pre-Tx VAS (score 0–10)	6.06 ± 1.43	5.70 ± 1.19	0.43
Pre-Tx PPT (N)	16.60 ± 3.80	16.11 ± 3.79	0.54
Pre-Tx TH (%)	54.68 ± 7.43	55.70 ± 7.85	0.90
Pre-Tx PFG (Kg)	16.66 ± 7.06	16.52 ± 5.99	0.89
Pre-Tx PRTEE (score)	35.97 ± 20.13	37.90 ± 19.45	0.66

Data were expressed as mean ± SD; P value was tested with ANOVA. FSN: Fu's subcutaneous needling; TENS: transcutaneous electrical nerve stimulations; VAS: visual analog scale; PPT: pain pressure threshold; TH: tissue hardness of muscle; PFG: pain-free grip; PRTEE: Patient-Rated Tennis Elbow Evaluation Questionnaire.

Table 2: The immediate effects of the two groups.

	FSN			TENS		
	Pre-Tx	Post-Tx	P value	Pre-Tx	Post-Tx	P value
Day 1						
VAS (1–10)	6.06 ± 1.43	3.56 ± 2.16	< 0.01	5.70 ± 1.19	4.45 ± 1.37	<0.01
PPT (N)	16.60 ± 3.80	20.71 ± 6.69	< 0.01	16.11 ± 3.79	17.92 ± 3.95	0.06
TH (%)	54.68 ± 7.43	51.93 ± 6.65	0.06	55.70 ± 7.85	54.37 ± 7.46	0.11
PFG (Kg)	16.66 ± 7.76	20.62 ± 9.67	< 0.01	16.52 ± 5.99	17.68 ± 6.28	0.01
Day 2						
VAS (1–10)	4.45 ± 2.11	2.38 ± 2.03	< 0.01	4.93 ± 1.10	3.65 ± 1.51	<0.01
PPT (N)	18.52 ± 6.14	23.63 ± 6.90	< 0.01	17.69 ± 4.29	17.98 ± 4.27	0.32
TH (%)	54.81 ± 7.56	44.88 ± 8.81	< 0.01	55.01 ± 6.93	52.77 ± 8.51	0.06
PFG (Kg)	18.96 ± 8.22	22.92 ± 9.43	< 0.01	16.66 ± 6.09	17.96 ± 6.26	0.04
Day 3						
VAS (1–10)	3.07 ± 2.17	1.69 ± 1.18	< 0.01	4.67 ± 1.21	3.58 ± 1.39	<0.01
PPT (N)	19.73 ± 7.02	23.32 ± 6.33	< 0.01	17.33 ± 4.12	18.77 ± 4.38	0.08
TH (%)	54.47 ± 8.81	48.80 ± 7.69	< 0.01	53.47 ± 9.15	52.14 ± 6.67	0.18
PFG (Kg)	20.28 ± 9.31	23.39 ± 8.71	< 0.01	17.00 ± 6.25	19.19 ± 5.60	<0.01

Data were expressed as mean ± SD; P value was analyzed by paired t-test. FSN: Fu's subcutaneous needling; TENS: transcutaneous electrical nerve stimulations; VAS: visual analog scale; PPT: pain pressure threshold; TH: tissue hardness of muscle; PFG: pain-free grip; PRTEE: Patient-Rated Tennis Elbow Evaluation Questionnaire.

4(d)). VAS, PPT, TH, and PFG were significantly improved in the FSN group on days 2 and 3 ($P < 0.01$ in all tests, Figures 4(a)–4(d)). Only VAS (days 2 and 3) and PFG (days 2 and 3) were significantly improved in the TENS group (Figures 4(a) and 4(d)). These results indicated that FSN had an instant pain relief effect for LE with a decrease in VAS and TH and an increase in PPT and PFG compared with TENS.

3.3. Short- and Long-Term Effects of FSN and TENS. Patients were followed up on days 8 and 15 after treatment to evaluate the short- and long-term effects and investigate whether pain relief is a sustained effect by FSN (Table 3). VAS in the FSN group was scored from 6.06 ± 1.43 in day 1 pretreatment to 2.10 ± 1.57 in day 8 ($P < 0.01$) and 1.82 ± 1.52 in day 15 ($P < 0.01$, Figure 5(a)). Meanwhile, VAS in the TENS group also demonstrated significant improvements on days 8 and 15 ($P < 0.01$) compared with day 1 pretreatment. Except for TH, PPT and PFG revealed significant improvements on days 8 and 15 ($P < 0.01$) compared with day 1 pretreatment in both groups (Figures 5(b)–5(d)).

To further understand the superiority of FSN to TENS on LE treatment. The improved VAS of patients in FSN on days 8 and 15 was significantly higher than that in TENS (day 8, 3.96 ± 1.46 in FSN compared with 1.67 ± 1.21 in TENS, $P < 0.01$; day 15, 4.24 ± 1.45 in FSN compared with 1.88 ± 1.23 in TENS, $P < 0.01$, Table 4). No significant improvement was noted in PPT on day 8 or 15 of FSN or TENS. The improved value of PFG in FSN was significantly higher than that in TENS ($P < 0.01$). These results indicated the sustained effect of FSN on pain relief.

3.4. Improving the Perceived Pain and Disability by FSN. The PRTEE questionnaire was utilized for assessment to understand FSN for achieving LE-induced pain remission and disability improvement (Table 5). A significant decrease in score is notably observed from 35.97 ± 20.13 in day 1 pretreatment to 22.57 ± 14.57 in day 8 ($P < 0.01$) and to 15.23 ± 12.16 in day 15

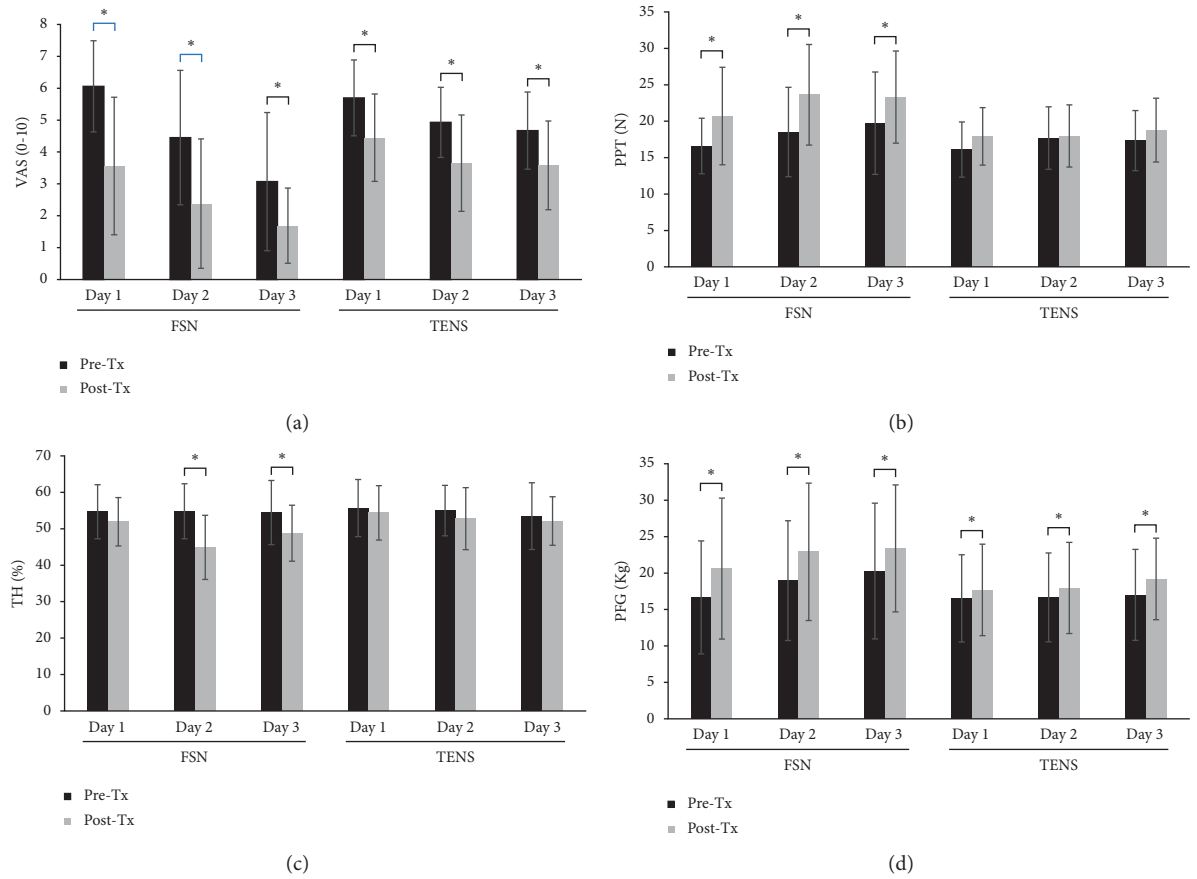


Figure 4: Comparison of the immediate effects of the two groups. The pre- and post-treatment values of VAS (a), PPT (b), TH (c), and PFG (d) were measured in 3 treatment sessions in both groups. *showed the $P < 0.05$. VAS: visual analog scale, PPT: pressure pain threshold, TH: tissue hardness, PFG: pain-free grip.

Table 3: The short-term and long-term effects of FSN-improved value are compared to those of TENS.

	Pre-Tx in day 1	Day 8	P value[a]	Day 15	P value[b]
VAS (0–10)					
FSN	6.06 ± 1.43	2.10 ± 1.57	<0.01	1.82 ± 1.52	<0.01
TENS	5.70 ± 1.19	4.03 ± 1.30	<0.01	3.92 ± 1.29	<0.01
PPT (N)					
FSN	16.60 ± 3.80	21.67 ± 6.60	<0.01	22.18 ± 6.78	<0.01
TENS	16.11 ± 3.79	18.87 ± 4.16	<0.01	19.83 ± 4.44	<0.01
TH (%)					
FSN	54.68 ± 7.43	54.94 ± 7.96	0.45	53.58 ± 7.74	0.27
TENS	55.70 ± 7.85	53.36 ± 7.17	0.10	53.51 ± 5.95	0.09
PFG (Kg)					
FSN	16.66 ± 7.76	21.02 ± 8.96	<0.01	23.54 ± 9.75	<0.01
TENS	16.52 ± 5.99	19.08 ± 7.18	<0.01	19.83 ± 6.63	<0.01

Data were expressed as mean ± SD; P value was analyzed by paired t-test. [a]Comparison of the value in pre-Tx and in day 8 of the FSN or TENS group. [b]Comparison of the value in day 8 and in day 15 of the FSN or TENS group. FSN: Fu's subcutaneous needling; TENS: transcutaneous electrical nerve stimulations; VAS: visual analog scale; PPT: pain pressure threshold; TH: tissue hardness of muscle; PFG: pain-free grip.

($P < 0.01$, Figure 6) by FSN. Moreover, a significant decrease in the score is observed from 37.90 ± 19.45 on day 1 to 31.67 ± 14.00 on day 8 (total of 6.23 difference, $P = 0.01$) and to 26.27 ± 15.77 on day 15 (total of 11.63 difference, $P < 0.01$, Figure 6) by TENS. Overall, both treatment groups were effective. However, the improvement of FSN is more than TENS in days 8 and 15 compared with day 1 pretreatment (improvement score followed up to day 8, 13.40 ± 15.83 in FSN and 6.23 ± 16.82 in TENS, $P < 0.01$; day 15, 20.74 ± 14.56 in FSN and 11.63 ± 16.28 in TENS, $P < 0.01$, Table 6). These results demonstrated that FSN not only has a sustained pain relief effect but also improved the disability in LE patients.

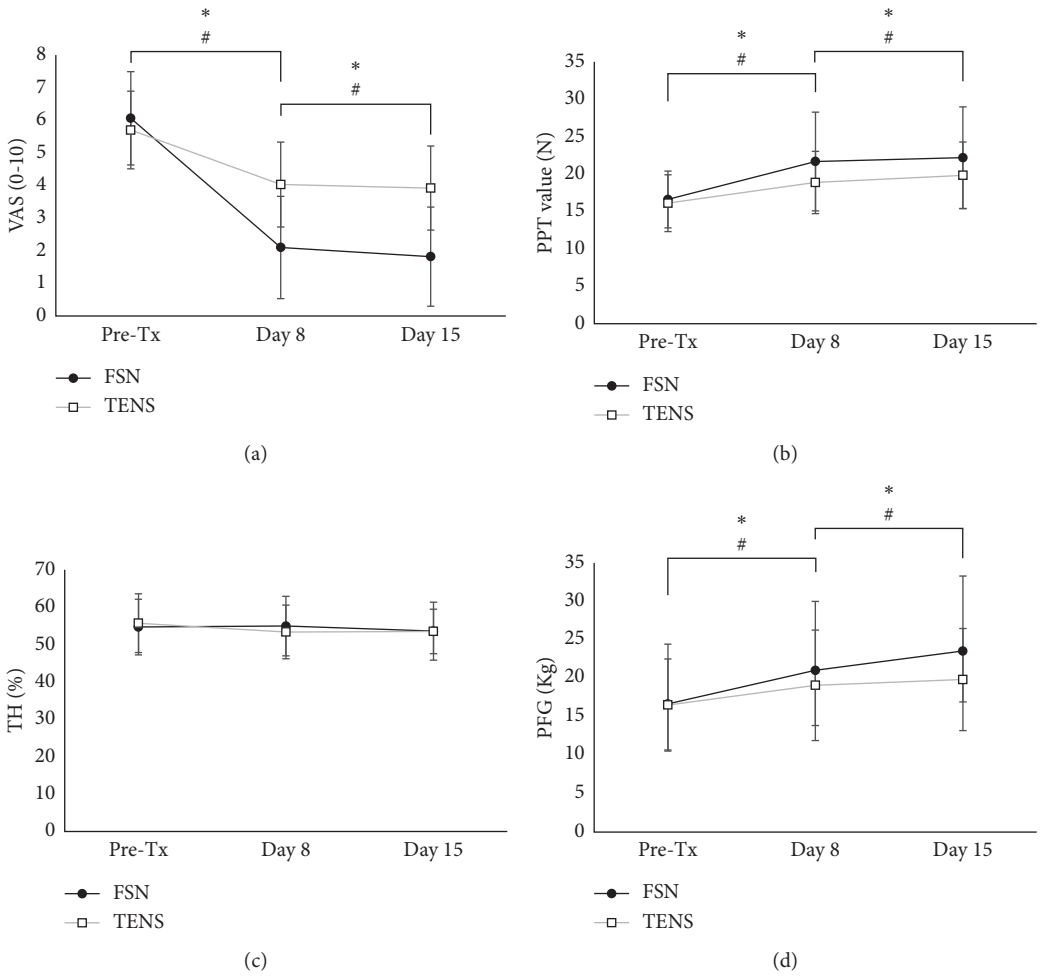


Figure 5: Comparison of the short-term and long-term effects of the two groups. The value of VAS (a), PPT (b), TH (c), and PFG (d) was measured in day 1 pretreatment and followed up to day 8 and day 15 in both groups. * and #showed the $P < 0.05$ in FSN or TENS, respectively. VAS: visual analog scale, PPT: pressure pain threshold, TH: tissue hardness, PFG: pain-free grip, FSN: Fu's subcutaneous needling, TENS: transcutaneous electrical nerve stimulations.

Table 4: The improvement effects of FSN compared to those of TENS.

	FSN	TENS	*P* value
Day 8			
VAS (0–10)	3.96 ± 1.46	1.67 ± 1.21	<0.01
PPT (N)	5.07 ± 4.45	2.76 ± 3.98	0.05
PFG (Kg)	4.36 ± 8.32	2.56 ± 6.28	0.07
Day 15			
VAS (0–10)	4.24 ± 1.45	1.88 ± 1.23	<0.01
PPT (N)	5.58 ± 4.82	3.72 ± 4.13	0.11
PFG (Kg)	6.68 ± 8.13	3.31 ± 6.35	<0.01

Data were expressed as mean ± SD; *P* value was analyzed by the *t*-test. FSN: Fu's subcutaneous needling; TENS: transcutaneous electrical nerve stimulations; VAS: visual analog scale; PPT: pain pressure threshold; PFG: pain-free grip.

Table 5: The effectiveness of FSN and TENS on PRTEE score.

Group	Pre-Tx in day 1	Day 8	*P* value	Day15	*P* value
FSN	35.97 ± 20.13	22.57 ± 14.57	<0.01	15.23 ± 12.16	<0.01
TENS	37.90 ± 19.45	31.67 ± 14.00	0.01	26.27 ± 15.77	<0.01

Data were expressed as mean ± SD; *P* value was analyzed by the *t*-test. FSN: Fu's subcutaneous needling; TENS: transcutaneous electrical nerve stimulations; PRTEE: Patient-Rated Tennis Elbow Evaluation Questionnaire.

4. Discussion

Nonsurgical therapies are the leading options for treating LE patients. FSN and TENS are compared in this study by measuring pain-related scales, such as VAS, PPT, PFG, and TH, and the pain relief and improvement on disability after three treatment sessions are estimated. The results indicate that FSN reduced the elbow pain and improved the disability caused by LE. Remarkable progress in immediate remission of VAS, PPT, TH, and PFG within three days is also observed. FSN also had short- and long-term benefits on VAS and PFG compared with TENS. Subjective questionnaire research showed an impressive improvement on LE-specific pain and disability. These results indicated that FSN is an effective treatment for LE.

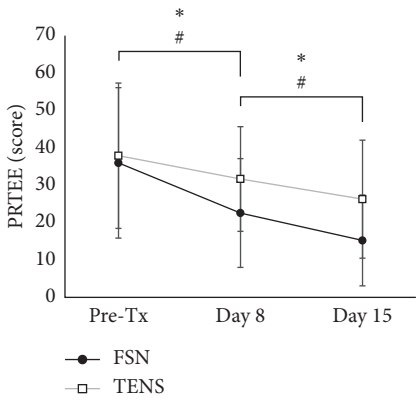


Figure 6: Comparison of the improvement effect of the two groups. The score of the PRTEE questionnaire was evaluated before the treatment on day 1, followed up on day 8 and day 15 post treatment in both groups. * and #showed the $P < 0.05$ in FSN or TENS, respectively. PRTEE: Patient-Rated Tennis Elbow Evaluation Questionnaire; FSN: Fu's subcutaneous needling; TENS: transcutaneous electrical nerve stimulations.

Table 6: The improvement effect of PRTEE in FSN compared to that in TENS.

	FSN	TENS	P value
Day 8	13.40 ± 15.83	6.23 ± 16.82	<0.01*
Day 15	20.74 ± 14.56	11.63 ± 16.28	<0.01*

Data were expressed as mean ± SD; P value was analyzed by the t-test. FSN: Fu's subcutaneous needling; TENS: transcutaneous electrical nerve stimulations; PRTEE: Patient-Rated Tennis Elbow Evaluation Questionnaire.

TENS was used clinically to reduce pain by activating large-diameter afferents to reduce nociceptive input via inhibitory neurotransmitters as gate control theory [30, 31] by the electrical stimulation electrodes attached to the affected sites. This mechanism resulted in immediate pain relief of MTrP-induced pain [32], but no benefit was found for reducing the muscle tissue hardness or recovering the function as observed on PPT and TH in the present study. FSN could reduce muscle tissue hardness and increase the PPT by up to two weeks. These results suggested that FSN has better benefits than TENS on pain relief by improving muscle functions. Unlike TENS treated on TE5 and LI11 near the lateral epicondyle, FSN is performed on the midpoint of the forearm extensors with some distance from the lateral epicondyle, demonstrating a more significant outcome than treating the symptom at the pain site. Thus, the etiology of pain may come from MTrPs, not the proximal sites where the pain occurred [33].

The MTrP-associated muscle, namely "tightened muscle," is essential for diagnosis and has been the treatment target of FSN because most pathological tightened areas were located in the muscle belly, and these areas were generally felt similar to a sheet or a zone, not a point [33]. Two decades ago, FSN was first discovered in muscle disease research [34]. Parallel or vertical needling has the same effectiveness with swaying movement in superficial fascia as first demonstrated in 2007 [35]. The mechanism of action of conventional acupuncture is hypothesized to be a mechanical coupling between the needle and connective tissue starting from needle insertion and rotation and then transmits the signal to connective tissues via mechanotransduction [36]. Some researchers had attempted to explain the phenomenon of acupuncture, that is, piezoelectric effects [37, 38]. They assumed that the electric current was initially generated by pressing the skin and then transduced to connective tissues by needle insertion and rotation. Several biological organic compounds, such as collagen, have piezoelectric properties associated with their crystalline structure [39, 40]. The liquid semicrystalline state of collagen polymers exhibited piezoelectric polarization [40]. However, demonstrating the mechanism of the piezoelectric effect during acupuncture is difficult. Dr. Lin recently developed an in-glove sensor to monitor piezoelectricity from the acupuncture performer [41]. This device may be used for demonstrating piezoelectricity in acupuncture in the future.

The swaying movement of FSN in a seesaw-like sector could stretch loose connective tissues to modulate homeostasis [42, 43]. Extruding, stretching, and swaying by FSN could change the liquid crystalline nanoarchitectures of loose connective tissues to release bioelectricity following transduction piezoelectricity to reverse piezoelectricity in the lesions. This mechanism could open the ion channel to relieve muscle contracture and ischemia and restore muscle function [44]. The reperfusion approach is another feature of FSN. The LE subjects would be asked to simulate actions of wringing a towel (supination and pronation of the forearm) during the treatment. This action would largely increase the bloodstream and congestion regions to promote tissue recovery. Combining swaying and reperfusion is essential for FSN to relieve pain and disability due to the pathological tightened muscle.

Moreover, the diagnosis of the location of the pathological tightened muscle is crucial. The midpoint of the forearm extensors in this study was punctured with some distance from the lateral epicondyle, which is the common site of pathological tightened muscles. Dr. Simons (2002) revealed the etiological mechanism of taut band induced by MTrPs [45], and the application of needling therapy on the active MTrP via breaking the energy crisis and resetting the MTrP circuits was suggested [46]. Some tensed and shortened sarcomeres could cause the extension of nearby sarcomeres. LE may not arise from the lateral epicondyle. The study of PPT on MTrPs in 550 healthy children with ages ranging from 4 to 11 revealed that PPT on the midpoint of the muscle belly was substantially decreased than lateral epicondyle at the elbow and the muscle-tendon junction after the age of 9 years [47]. Curing a disease should focus on its etiology and finding a treatment strategy rather than the name [48]. Therefore, this study focused on the pathological tightened muscle of LE rather than the lateral epicondyle at the elbow.

The mechanical pain is due to microwound accumulation from an imbalance in global muscle recruitment or local muscle overuse [49]. The muscle tissue involved in unscrewing the cap or wringing a towel is still in an overtense state without movement, which is the target of FSN

treatment. Reperfusion action to perform wrist extension movement with resistance and wringing a towel in FSN increased the PFG, indicating that FSN-improved muscle function and pain relief immediately. This effect was also observed in one- and two-week follow-ups. These pieces of evidence support that FSN is a good treatment strategy for LE.

5. Conclusions

This study provides evidence to support the effectiveness of FSN as a therapy for LE with immediate and sustained effects to relieve pain. Furthermore, the data from the questionnaire study suggest a significant and positive effect of FSN for LE treatment. Thus, FSN is a good treatment option for LE.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Authors' Contributions

Ching-Hsuan Huang and Chun-Yen Lin contributed equally to this work.

Acknowledgments

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Supplementary Materials

Video S1: procedure of FSN performed by a doctor. Video S2: swaying and wrist extension movement. (*Supplementary Materials*)

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Review Article

Effective Oriental Magic for Analgesia: Acupuncture

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Pain is a kind of complex physiological and psychological symptom, which makes the person debilitated and uncomfortable. Some persistent pain is unbearable for the patients, reducing the quality of life and bringing considerable pressure to the individuals and society. Pain killers seem to be effective in analgesia for patients, but their safety and addiction are crucial issues. From the theory of traditional Chinese medicine (TCM), the blocked meridian is the main cause of pain, and effective acupuncture can play a positive analgesic effect. Acupuncture that can date back thousands of years is one of the ancient medical practices in China. Its safety and effectiveness are respected. Based on its superior safety and inferior side effects, it has been gradually recognized as a therapeutic intervention method for complementary medicine, which is also generally used to treat multiple pain diseases. It is shown by modern medical studies that neurotransmitters are the material basis for the acupuncture effect, and the effect of acupuncture analgesia is related to changes in neurotransmitters. However, the specific mechanism has not been elucidated. This review aims to comprehensively discuss the historical evolution of acupuncture analgesia, clinical research of acupuncture analgesia, comparison of acupuncture and drug therapy, the neurotransmitter mechanism of acupuncture analgesia, the effect of acupuncture manipulation on analgesia, and bibliometric analysis of acupuncture treatment for pain, to explore the superiority and related mechanism of acupuncture analgesia from different aspects, and to provide a more effective treatment for alleviating patients' pain.

1. Introduction

Pain is often the main compelling reason for seeking medical attention, and it can seriously affect the quality of life [1, 2]. Pain is a complex process propagated by many systems [3]. After the nociceptive stimulus is transformed into a nerve impulse, the pain sensation and pain response are produced by integrating and processing all aspects of the central nervous system (CNS) [4]. There are numerous methods to relieve pain. So far, the most effective drugs to relieve pain are opioids, and the most widely used drugs are nonsteroidal anti-inflammatory drugs (NSAIDs); at the same time, addiction, drowsiness, and other side effects could not be ignored [5, 6]. Abuse of painkillers may cause serious damage to brain function [7]. The severe opioid crisis is a tough issue that deserves more attention. It is urgent to search for some safe therapeutic approach to deal with the present emergency [8]. Due to the lack of analgesic drugs with positive curative effects but no apparent side effects, the research of alternative treatment methods has further promoted [9].

Acupuncture is also the mainstay of pain management [10]. As a therapeutic intervention approach for alternative medicine, acupuncture has gained popularity around the world. It is commonly known that acupuncture is the effective pain management from China. Today, many types of research have confirmed that acupuncture has a positive analgesic effect in treating pain [11]. A significant quantity of clinical trials has shown that the neuromodulation of acupuncture can control pain and inflammation in many diseases [12]. It has an excellent curative effect in relieving headache, neuropathic pain, lumbago, knee osteoarthritis

(KOA), and other pain diseases [2]. Some international clinical guidelines suggest that the effect of acupuncture on pain is equivalent to traditional pharmacology or interventional technology [13]. Due to its positive effect and superior safety, it is more acceptable to the general population [14].

In 1997, the National Institutes of Health (NIH) recognized that acupuncture alleviates pain. Although basic research has attempted to explain the underlying mechanisms of its effects, these mechanisms have not yet been determined [15]. Acupuncture mainly exerts an analgesic effect by activating acupoints with special anatomical locations [16]. Through the stimulation of acupoints, it can produce analgesia mainly by activating neurotransmitters [17]. Although multiple types of research have attempted to elucidate its analgesic mechanism, the mechanism is still unclear. From a clinical perspective, some rigorous large-scale, multicenter randomized controlled trials ought to be implemented to investigate the mechanism of acupuncture analgesia. This review intends to introduce the history of acupuncture analgesia, the clinical research status of acupuncture analgesia, comparison of acupuncture and drug therapy, its neurotransmitter mechanism, and limitation.

2. Historical Evolution of Acupuncture Analgesia

In ancient times, the Chinese discovered that using stones (those sharp stones are the prototype of the current needle) to press on the painful parts of the body can relieve the pain. Along with the science and technology development, needle material has changed from stone and bone to gold, silver, copper, and iron. At present, the most popular one is stainless steel [18]. Acupuncture did not have a theoretical basis until the appearance of Huangdi Neijing (The Yellow Emperors Internal Classic) more than two thousand years ago, and the acupuncture theory is mainly recorded in its Su Wen chapter [19]. Acupuncture treatment of pain disorders has also been documented since the Huangdi Neijing period [20]. In the Ming Dynasty, the development of acupuncture reached its climax. Many doctors have offered different opinions on acupuncture and published plenty of masterpieces, but the well-known work was Compendium of Acupuncture and Moxibustion by Dr. Yang Jizhou. In his book, he recorded the manipulation and indications of acupuncture and elaborated on acupuncture analgesia in detail.

Acupuncture is an effective method of therapeutic intervention for alternative medicine that has been widely accepted around the world. Acupuncture was introduced to the West as early as the 16th century, and it was not until the 19th century that it began to be used in clinical practice by Western medicine. Acupuncture analgesia was once introduced in the condition of the use of acupuncture in surgical anesthesia. The first operation of acupuncture analgesia was reported in 1958 in China [21], and it was the result of integrated TCM and Western medicine. Until 1971, Dimond witnessed the application of acupuncture analgesia during surgery and made the first report about acupuncture analgesia in JAMA [22]. In 1973, the Lancet published an article about acupuncture analgesia. Until 1997, NIH publicly recognized acupuncture's efficacy and potential therapeutic effects in alleviating pain and multiple other disorders.

From the perspective of TCM, acupuncture is based on the theory of meridians and uses the needle to penetrate specific acupoints of the human body to cure diseases. The potential rationality of acupuncture is that diseases related to Qi (energy as considered in TCM) can be treated by stimulating related acupoints [23]. In the view of TCM, Qi is essential energy flowing through meridians and can participate in various homeostatic regulations in the human body [24]. Moreover, the analgesic effect can be achieved by stimulating Qi inside the body [25]. From the perspective of modern medicine, the underlying physiological mechanisms of acupuncture analgesia can be roughly separated into peripheral and central mechanisms, and the most complete system involved in pain management is the endogenous opioid system [17]. Changes in neurotransmitters may also be relevant to analgesia [26]. Segmental inhibition of the spinal cord produced by acupuncture may elevate the pressure pain threshold [27]. Within the modern medical system, the acupuncture theoretical system has been well-established, and scientists have made adequate progress in acupuncture for the treatment of pain disorders.

3. Clinical Research Condition of Acupuncture Analgesia

Since its origin in China, acupuncture has been used in more than 180 countries and regions. Forty-three diseases used for acupuncture treatment have been acknowledged by the World Health Organization (WHO) [17]. The indications of acupuncture for curing pain diseases are becoming more extensive, and the spectrum of diseases is constantly completed. Since the development of evidence-based medicine in the discipline of acupuncture, many acupuncture clinical studies have been implemented to confirm the availability of acupuncture analgesia [28, 29].

The option of the proper control group is beneficial for improving the feasibility of acupuncture research [30]. However, acupuncture is a complex intervention. It is rough to interpret the acupuncture efficacy in a single control group. The choice of the control group remains a methodological challenge in the design of acupuncture RCTs [31]. Therefore, the ideal control group is more conducive to the progress of acupuncture research. In clinical research on acupuncture treatment of pain, three control groups are usually selected. The groups include (i) needling fake acupoints (offset the correct acupuncture point); (ii) true acupoints, but using a thin needle or a very shallow depth of acupuncture (minimize the acupuncture stimulation); and (iii) placebo needle that does not penetrate the skin. Many clinical pain studies on acupuncture have shown that the analgesic effect of the acupuncture group is superior to the standard control group [32]. In acupuncture randomized studies, the curative effect of the acupuncture group and the minimal acupuncture group is better than the waiting list in

the alleviation of osteoarthritic pain [33]. The placebo effects always occur at the same time as the acupuncture effects. High-quality clinical evidence indicates almost no apparent difference between true and sham acupuncture in the management of chronic low back pain [34]. In partial clinical trials of acupuncture, sham acupuncture is as effective as real acupuncture [32]. A large-scale RCT divided 857 individuals equally into the optimal acupuncture, the shallow acupuncture, and the sham acupuncture groups to treat cervical spondylosis-related neck pain. It was found that the optimal acupuncture group had the best effect, followed by the shallow acupuncture group [35]. The analgesic effects of the acupuncture and the placebo groups are superior to those of the no acupuncture groups, but placebo analgesia has only a tiny to moderate effect [36]. Some research has shown that acupuncture is available for numerous chronic pain diseases such as nonspecific musculoskeletal pain, osteoarthritis, and omalgia [37]. The effectiveness of acupuncture was better than the sham and no acupuncture group, alleviating the pain cannot be elucidated by the placebo effect barely [37]. It can also be found that acupuncture in different acupoints has a positive analgesic efficacy on sciatica treatment, and the effectiveness is better than the sham acupuncture group [38]. In alleviation of knee osteoarthritis (KOA), acupuncture has a noticeable higher cure rate than sham acupuncture, it is worth mentioning that over time, the utility of EA seems to be higher than other control groups [39]. This may be related to the frequency of acupuncture and the intensity of stimulation. The research on EA has declared that low-frequency electricity is more valid for nociceptive pain; instead, high-frequency electricity has a potent therapeutic effect for neuropathic pain [40]. Nevertheless, the frequency of acupuncture seems challenging to quantify. Although the analgesic effect of the acupuncture group was superior to the control groups, it cannot be neglected that on the management of some pain (pain in labor, migraine, and neuropathic pain), low-quality evidence suggests that acupuncture does not differ much from usual care [41–43]. Since a large number of the current RCTs provide conflicting and inconclusive evidence, the methodological shortcomings of RCTs are recurrent [43, 44].

In terms of reducing the pain effectively, acupuncture combined with other therapies also has a powerful effect. Compared with a single therapy, acupuncture combined with medical training therapy is more effective than routine care [45]. Based on traditional Western medicine, EA can relieve severe cancer pain and reduce the dose of opioid analgesics [46]. Traditional acupuncture combined with drugs is more effective than drugs alone in treating migraine [47]. Studies have shown that different acupuncture methods (actually stimulating acupoints but with different intensities) also have an analgesic effect. Twist ankle acupuncture (WAA) and auricular acupuncture (AA) have curative effects on cancer pain, and the combination of different acupuncture methods has a better analgesic effect [48]. Patients with chronic low back pain experienced a notable decrease in VAS scores after treatment with thread embedding acupuncture (TEA) combined with acupuncture, indicating that acupuncture combined with TEA has better analgesic efficacy than acupuncture alone [49].

In summary, acupuncture analgesia is widely believed to be superior to placebo needles, but a large amount of low-quality evidence has been provided clinically due to the shortcomings in RCT methodology and deficiency of rigorous trial design. However, in almost all clinical trials, the acupuncture group was superior to the usual care group, which provides abundant evidence that the effect of acupuncture analgesia is credible. Since the placebo effect is ubiquitously present in RCTs and seems to have a great connection with patient expectations, which seems inevitable and can only minimize the placebo effect, therefore further studies are needed to confirm that acupuncture is not a powerful placebo. Due to the wide range of indications, there are lots of clinical studies on MA and EA. Research on other acupuncture therapies is still insufficient. However, based on the current indications for these acupuncture treatments, to further enhance the analgesic efficacy, acupuncture combined with these therapies should also be advocated. Crucially, rigorous clinical trials must be carried out so that effective interventions for acupuncture analgesia can reasonably be provided.

4. Acupuncture vs Analgesic Drugs

Compared with addictive opioids, as a valid agent for pain management, nonsteroidal anti-inflammatory drugs are one of the most widely prescribed drugs [6]. However, attention should be paid to gastrointestinal complications and cardiovascular diseases associated with long-term use [50, 51]. There is no substitute for NSAIDS in some diseases, such as rheumatoid arthritis, but the major cardiovascular events they entail are even harder to avoid [52]. To minimize the harm caused by its side effects, several strategies have been adopted. Therefore, there is a need for a treatment that allows for effective pain management with fewer side effects for long-term use. Acupuncture can provide analgesia by releasing endogenous opioids with a few side effects and high safety and could be an effective alternative therapy for analgesia to the clinical use rate of drugs to avoid potential adverse events caused by medicines [53, 54].

The analgesic effect of acupuncture in certain pain disorders is as effective as NSAIDs, even has a faster and longer-lasting action in acute pain analgesia. Cho et al. divided 45 patients suffering from chronic neck pain into three groups, and after three weeks of treatment, there was no difference in VAS scores between the acupuncture-treated group and the NSAIDs-treated group, and acupuncture could provide excellent analgesia [55]. Murugesan et al. divided 157 patients with symptomatic irreversible pulpitis into three groups according to the mode of emergency pain management, and the acupuncture combined with placebo tablet group had longer-lasting analgesia and faster efficacy initiation than the sham acupuncture combined with ibuprofen group [56]. Not only acupuncture but also other acupuncture methods derived from traditional acupuncture can reduce drug dependence. Zhang et al.

conducted a randomized, sham-controlled prospective study and found that auricular acupressure was analgesic and did not cause any adverse events in the treatment of KOA, and was effective in reducing the use of NSAIDs. A Systematic Review and Meta-Analysis conducted by Wu et al. found that patients treated with transcutaneous electric acupoint stimulation (TEAS) were less dependent on opioid analgesics after surgery compared to controls [57]. A large retrospective cohort study by Timothy et al. found that acupuncture shows significant advantages in complete opioid discontinuation compared with NSAIDs. Acupuncture can be used as a complementary therapy in combination with NSAIDs for better analgesia. In their study, Dingemann et al. divided 46 patients with postoperative swallowing pain into three groups, each receiving NSAIDs, and showed that the analgesic effect of the acupuncture group was better than that of the drug treatment group [58]. Interestingly, acupuncture may be the best option for pain relief when a patient cannot take NSAIDs or other analgesic medications, exerting analgesic effects even faster than medication. In treating patients with NSAIDs-tolerant dysmenorrhea, Iorno et al. showed a significant reduction in the duration of dysmenorrhea and the use of NSAIDs by acupuncture intervention, with a 74% reduction in NSAIDs [59]. Kaynar et al. found that the analgesic effect of acupuncture in urolithiasis-driven renal colic pain relief was superior to that of diclofenac and acetaminophen after ten minutes, and there was no significant difference in the analgesic effect of the three types of therapy after 120 minutes [60].

As a safe analgesic therapy with low side effects and low economic burden, acupuncture has a lower incidence of adverse events than drug therapy. It even shows excellent superiority in the treatment of some diseases. For patients who cannot use NSAIDs or other analgesics, acupuncture can be an effective alternative for better pain relief. However, the effectiveness of acupuncture for analgesia is not limited to traditional MA or EA. Other related acupuncture modalities based on acupuncture theory have also demonstrated great superiority. When combined with drugs to treat pain diseases, acupuncture can enhance the analgesic effect and reduce patients' dependence on drugs and even reduce the side effects of drugs. Therefore, acupuncture may play an essential role in formulating the analgesic treatment plan, effectively ensuring patients' quality of life and lowering the ratio of drug use (Figure 1).

5. Mechanism of Neurotransmitters in Acupuncture Analgesia

Acupuncture analgesia is a comprehensive effect of transmitting the signals generated by acupuncture induction to relevant regions of the spinal cord and brain, thereby increasing and decreasing neurotransmitters to achieve the purpose of analgesia [16]. Some neurotransmitters (opioid peptides, γ-aminobutyric acid, norepinephrine, and 5-hydroxytryptamine) have been found to exert analgesic effects by modulating the prescribing pain modulatory pathway (Figure 2) [53]. Glutamate likewise plays a considerable role in pain modulation (Figure 2) [61].

5.1. Opioid Peptides. Numerous animal and clinical trials have demonstrated that acupuncture is an excellent means of analgesia. EA stimulation can release endogenous opioid peptides for positive pain management [5, 17]. Their receptors are similarly involved in pain mechanism modulation [62]. The endogenous opioid mechanism is the most well-recognized neuronal mechanism of acupuncture analgesia. There are mainly four opioid peptides: enkephalins, endomorphins, dynorphins, and nociceptin, and their δ, μ, and κ-opioid receptors and nociceptin peptide receptor [16]. Elucidating the endogenous response to pain is essential to optimize therapeutic action and minimize side effects [63]. EA at various frequencies may provoke different endogenous opioid mechanisms, and the healing effect of low-frequency EA is better than that of high-frequency EA [64]. In the collagenase-induced osteoarthritis (CIOA) rat model, 2 Hz EA has a better analgesic effect than 100 Hz EA [65]. The activation of the opioid peptide mechanism may be related to frequency. EA may have a superior effect by virtue of its controllable frequency advantage. Low-frequency EA can promote β-endorphin expression to relieve neuropathic pain [66]. At 2 Hz frequency, EA induces the release of β-endorphin, endomorphin, and met-enkephalin combined with μ- and δ-opioid receptors to achieve pain management. At 100 Hz frequency, dynorphin can be released and activation of κ-opioid receptors [67]. At different frequencies, EA may activate opioid receptors in different parts of the brain. At 2 Hz frequency, the μ-opioid receptors binding potential of the anterior cingulate cortex was significantly increased [68]. In the goat experiment, compared with other frequencies, EA at 60 Hz increased the pain threshold most [69]. All of this indicates that the effect of electroacupuncture is closely relevant to frequency.

In the EA treatment of inflammatory pain, activation of the peripheral cannabinoid CB2R may increase β-endorphin levels in inflamed tissues and combine to activate μ-opioid receptors to achieve analgesia [70]. Through activating μ-opioid receptors, the expression level of netrin-1 can be reduced to alleviate neuropathic pain caused by RTX [71]. Animal experiments on labor pain management have shown that EA can enhance the protein activation of κ-opioid receptors, but mainly in the lumbar spine [72]. This suggests that acupuncture analgesia may be related to the choice of acupoints. Different receptors in specific regions of the CNS may mediate different frequencies of EA.

The study of endogenous opioid peptide mechanisms is beneficial for elucidating acupuncture placebo analgesic utility. Placebo analgesia was also associated with endogenous opioid peptides [73]. The analgesic effect of the placebo can be blocked by opioid antagonists such as naloxone [74]. Studies are suggesting that the placebo effect may be related to patient expectations [75]. The placebo effect triggered by expectation can activate opioid neurotransmission and thus play an analgesic role [76]. Opioid antagonists can block pain modulation elicited by patient expectations [77].

5.2. γ-Aminobutyric Acid. γ-Aminobutyric acid (GABA) mainly plays an inhibitory role in the CNS. EA analgesic mechanism is closely related to GABA expression. It is well

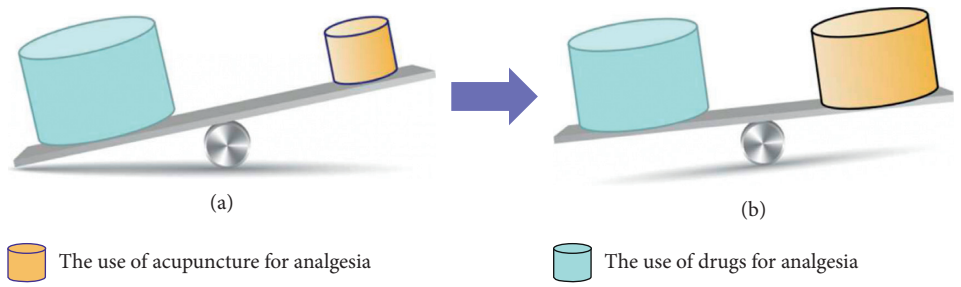


Figure 1: Changes in the use rate of acupuncture and drugs in the treatment of pain diseases. (a) In the past, drugs were widely used as the first choice for analgesia. (b) Based on the superiority and safety of acupuncture, acupuncture is now also used extensively as an effective analgesic therapy for various diseases. It is worth mentioning that acupuncture reduces the abuse rate of analgesics and may be able to replace painkillers for patients with some pain diseases or unable to use drugs.

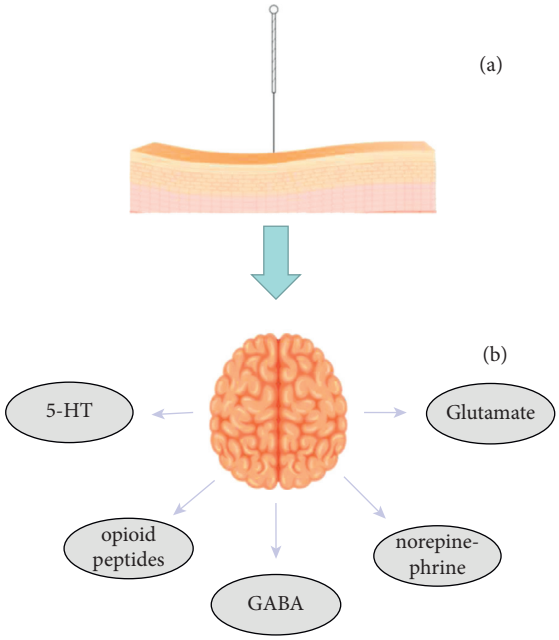


Figure 2: Release of neurotransmitters after acupuncture. (a) The corresponding stimulation is produced after acupuncture into the acupoint. (b) The signals induced by acupuncture are transmitted to the relevant regions of the brain, so as to release the above five neurotransmitters, and achieve the effect of analgesia through the interaction between different neurotransmitters.

known that GABAA and GABAB can participate in pain regulation. GABAA and GABAB are the main subtypes involved in EA analgesia [78]. In the animal experiment of KOA mice, the signal pathway mediated by GABA can be involved in EA to improve pain [79]. PAG is one of the main centers of descending pain suppression system [80]. GABA released in the PAG may involve pain management. Under EA treatment, the glutamate declined in the hippocampus, and the GABA enhanced in the PAG, due to the increase of GABA receptors, 15 Hz EA but not 2 Hz or 50 Hz can relieve mechanical and thermal hyperalgesia pain [81]. In the experiment of EA treatment of neuralgia in rats, under 2 Hz and 15HZ EA, the level of GABAA receptor in the spinal cord of rats was higher than that of the sham acupuncture group [82]. After EA treatment, the GABA concentration in DRG increased, and EA can alleviate incisional neck pain by upregulating GABA expression in DRGs [83]. EA seems to upregulate the GABA expression and its receptors in spinal cord DHs to have a good analgesic effect on rats with incision neck pain [84]. GABA may be involved in compensatory enhanced acupuncture analgesia. After EA treatment, increased GABA exerts an analgesic effect in FM patients [85]. GABAA receptor γ-2 subunit participates in EA alleviating neuropathic pain [86]. Other types of research have proved that EA can induce the release of endogenous endorphins and inhibit the release of GABA by activating μ-opioid receptors of GABAergic neurons [87]. Consequently, GABA participates in the pain management of EA, and the frequency and area of EA may also affect the production of GABA.

5.3. Norepinephrine. Studies have shown that norepinephrine combined with α2-adrenoceptor can achieve analgesic effects [88]. EA can downregulate pain inhibitory pathways by enhancing the release of norepinephrine [89]. The involvement of norepinephrine in pain relief is mediated by the stimulation of adrenergic receptors on inflammatory cells that release β-endophilin to achieve analgesia [90]. The analgesic effect of noradrenergic in the dorsal horn of the spinal cord is probably through activation of the inhibitory factor α2-adrenergic receptor [91]. EA controls the transmission of pain messages by activating projection to spinal noradrenergic neurons [92]. Tolerance is similarly observed in EA analgesia. Cross-tolerance may develop between norepinephrine and EA, probably owing to the large secretion of norepinephrine in the brain, acting through α-receptors, against EA analgesia [93]. Its tolerance should be thoroughly studied to maximize the analgesic effect. Further research is needed on the mechanism of action of norepinephrine.

5.4. 5-Hydroxytryptamine. 5-Hydroxytryptamine (5-HT) is generally renowned as serotonin. It is the neurotransmitter of the descending inhibitory system of the brainstem involved in analgesia. 5-HT secretion increased in the brain during acupuncture analgesia [94]. It is mainly produced in the rostroventromedial medulla (RVM) to the spinal cord and exerts bidirectional modulatory effects in descending facilitatory and inhibitory pathways [53]. 5-HT is a pain

mediator in the periphery, which can decrease the secretion of pain-related factor 5-HT after acupuncture [95]. 5-HT of descending pain regulatory system participates in acupuncture analgesia. Different types of research have shown that surgical pain can be alleviated by upregulation of 5-HT receptors (5-HT1AR and 5-HT2AR) by EA [96]. In a rat model of recurrent migraine, 5-HT levels in the plasma of the EA-treated group were higher in the RVM and trigeminal nucleus caudalis regions than those of the other treated groups [97]. After EA treatment, the number of neurons and the relative protein expression of 5-HT7R in migraine rats were significantly decreased [98]. Related rats' pain experiments showed that 5-HT1AR and 5-HT3AR could participate in mediating EA analgesia [99]. Osteoarthritis-induced pain can be suppressed by EA enhancement of spinal 5-HT2A/2C receptor activity [100]. In the model of CIOA rats, 5-HT1R and 5-HT3R can reduce the analgesic effect of 2 Hz EA [65]. EA at 100 Hz reduced pain and upregulated 5-HT expression in DRN of reserpine injected rats [101]. Different matching points may have different efficacies. Liu et al. [102] measured the concentration of 5-HT and 5-HT4R in chronic visceral hypersensitivity rats after EA stimulation by ELISA, and the results suggested that EA could improve the pain threshold, decrease the concentration of 5-HT, and increase the concentration of 5-HT4R. However, it makes no difference in the concentration of 5-HT3R. These studies clearly show that serotonin is involved in acupuncture analgesia, but the corresponding matching points should be selected for better analgesic effects. Moreover, EA analgesia at different frequencies may be antagonized or enhanced by 5-HT. The choice of the corresponding frequency is also particularly significant.

5.5. Glutamate. The most widely spread neurotransmitter in the CNS is glutamate, which plays a vital role in excitatory ascending pathways. It is a crucial excitatory neurotransmitter that efferents from the dorsal horn of the spinal cord [103]. Glutamate and its receptors N-methyl-D-aspartate receptor (NMDAR) participate in the transmission and integration of pain messages at the spinal level [16]. Glutamate can induce CNS sensitization by activating its receptors to act as an analgesic. Acupuncture can achieve an analgesic effect by downregulating glutamate in the ascending excitation pathway [53]. Glutamatergic pathways may induce acupuncture analgesia [104]. Central sensitized NMDAR may participate in spinal cord pain [105]. Studies have suggested that EA combined with NMDA antagonists produces a stronger antihyperalgesic effect [106]. In the CCI-induced neuropathic pain model, glutamate is reduced in the hippocampal region of PAG rats under EA analgesic treatment [81]. Although the acupuncture method is different, it seems to play the same analgesic effect. In a rat model of neuropathic pain, WAA reduced pain sensitivity possibly by inhibiting the expression of Glu and P-NMDAR1 in the spinal dorsal horn [107]. The modulation of glutamate and its receptor content by acupuncture is beneficial for optimizing the analgesic effect of acupuncture.

In conclusion, the frequency of EA is fixed and controllable. The release of neurotransmitters seems to be related to a fixed frequency, with different frequencies releasing different neurotransmitters. The analgesic effect is closely related to the quantity of acupuncture stimulation. Most of the current studies on acupuncture analgesia are based on animal models, and the choice of acupuncture points in different models is diverse. Whether the different anatomical locations affect the release of different neurotransmitters remains to be clarified. The neurotransmitters released are different when acupuncture is applied to rapid analgesia and slow analgesia. How to shift the analgesic phase and release neurotransmitters to maximize the analgesic effect remains to be further investigated. There are other mechanisms of acupuncture analgesia. How to combine multiple mechanisms to achieve the best analgesic effect deserves our attention.

6. Effect of Acupuncture Manipulation on Analgesia

Plenty of clinical trials and molecular mechanism studies have proved that acupuncture analgesia is indeed effective. But the analgesic effect of acupuncture still has limitations, for instance, placebo as mentioned above effect and the inadequacy of RCTs design such as the design of an effective control group. The specificity and quantity of acupuncture also deserve further exploration.

The manipulation of acupuncture closely relates to physicians' personal clinical experience, and it is hard to achieve standardization. Heterogeneity among practitioners may bias the analgesic effect of acupuncture. There are high-quality meta-analyses of acupuncture trials for chronic pain that demonstrate a more significant variation in treatment efficacy among different practitioners than would be expected by chance [108]. Between the differences in the manipulation of practitioners, the efficacy achieved with acupuncture may also be biased. Acupuncture techniques have various characteristics and have commonalities, but their effectiveness is different [109]. Li et al. [110] found that different acupuncture manipulations may have different effects on blood perfusion. Using five different acupuncture methods to treat patients with KOA found that EA and fire acupuncture were more effective than other methods [111]. The right acupoint is significant to acupuncture. In treating patients with ischemic stroke, changes in brain activity by fMRI contrast between acupuncture Wai Guan (SJ5) and sham acupoints indicated a remarkable decrease in the BOLD signal of the right BA5 after acupuncture SJ5 compared with sham acupoints [112]. In the formalin-induced pain rat model, acupunctures ST36, SP9, and BL60 had a better analgesic effect than the control group, and BL60 had the most significant effect [113]. Studies have shown that acupoints have specificity in treating migraine, and the effect of acupuncture at SJ5, GB34, and GB20 is better than that of the control group [114]. Bias due to the specificity of acupuncture, such as the choice of practitioners, acupuncture methods, and acupoints, should be minimized,

and a large number of studies are necessary to confirm the specificity of acupuncture.

The quantity of acupuncture is related to the number of acupoints, the number of needles, the frequency of acupuncture, and the duration of treatment [11, 115]. High-quality RCTs suggest that an eight-week thrice-weekly EA may have the optimum effectiveness in reducing pain in patients with KOA [39]. Meta-analyses suggest that acupuncture is a positive medical method for limb pain, but it takes five or eleven weeks to achieve maximum effect [11]. The curative effect of acupuncture can be achieved by rotating the needle. After acupuncture at SJ5, it was shown by fMRI that the BOLD signal was higher when the needle was rotated than when it was not rotated [112]. The underlying mechanism of the persistent effect of acupuncture remains unclear.

7. Bibliometric Analysis of Acupuncture in the Treatment of Pain

There are a few bibliometric analyses on acupuncture analgesia. In recent years, studies related to acupuncture analgesia have gradually increased. We attempted to analyze the progress and research trend of acupuncture analgesia by bibliometric method to observe the discipline's current research hotspots and frontier areas. All data were retrieved from the Web of Science on December 31, 2021, with the search terms (acupuncture) AND (pain). We selected publications from the last decade, with publication dates from January 1, 2011, to December 31, 2021. A total of 4781 articles were included in the bibliometric analysis after excluding articles that could not be used as full-text duplicate publications. We used VOSviewer v.1.6.17 to carry out descriptive statistical analysis on publications from journals, keywords, authors, and countries. We used Excel 2019 to analyze the trend of literature published in the past decade.

7.1. Analysis of Annual Publications. A total of 4781 papers were identified for this study. The number of articles published each year is shown in Figure 3. Although the number of publications fluctuated, it generally showed an increasing trend yearly, from only 304 in 2011 to 687 in 2020. Compared with 2020, the number of publications in 2021 reduced to 620.

7.2. Analysis of Journals. 4781 articles on acupuncture treatment for pain came from 1043 journals. Among them, Evidence-Based Complementary and Alternative Medicine published the largest number of publications (7.8%), followed by Medicine (4.8%) and Acupuncture in Medicine (4.8%; Table 1). Table 1 lists the impact factor (IF) and publishing countries of the top ten journals. Most of the journals are published in England and the United States. The top ten journals with the highest IF are BMC Complementary and Alternative Medicine, with an IF of 3.659. Figure 4 shows the bibliometric coupling of journals. This indicated the degree of association between different journals.

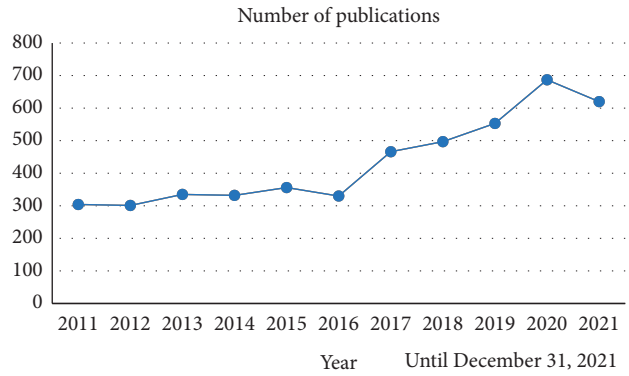


Figure 3: Changes in the number of acupuncture for pain diseases' literature publications from 2011 to 2021 until December 31, 2021.

7.3. Analysis of Keywords. Based on the frequency of keywords, the current research area can be identified. The keywords in 4781 articles were analyzed by VOSviewer, and a total of 11,483 keywords were mentioned in all articles of which 80 were mentioned more than 85 times. We divided these keywords into four groups by VOSviewer. As shown in Figure 5, different colors represent different groups and research directions. Group 1 represented clinical studies, in which a total of 27 keywords were mentioned, of which the most frequent keyword was management (689 times), followed by low back pain (433 times). Group 2 represented mechanism studies, in which a total of 16 keywords were mentioned, of which the most frequent keyword was acupuncture (2674 times), followed by pain (1420 times). Group 3 represented pain therapy, in which a total of 32 keywords were mentioned, of which the most frequent keyword was prevalence (339 times), followed by complementary (253 times). Group 4 represented research methodology, in which a total of 5 keywords were mentioned, of which the most frequent keyword was systematic review (262 times), followed by meta-analysis (166 times).

7.4. Analysis of Authors. We found a total of 18,841 authors by VOSviewer, of whom 27 authors have more than 25 articles. From Figure 6, we found that the top five authors in terms of the number of articles published were Lao Lixing (46 articles), Lee Myeong soo (45 articles), Liu Cunzhi (42 articles), Ha In-hyuk (41 articles), Park Hi-joon (40 articles), and Macpherson Hugh (40 articles). We used VOSviewer to analyze the author's publication year, and the lighter the color is, the closer the publication year is to the present.

7.5. Analysis of Countries. A total of 91 countries have published publications on acupuncture treatment for the pain of which 32 countries have published more than 20 articles. As shown in Figure 7, the top five countries in terms of the number of publications were China (1577 articles), the United States (1205 articles), South Korea (465 articles),

Table 1: The 10 journals publishing the highest number of articles about acupuncture treatment for pain.

Ranking	Journal title	Records (n)	IF2020[a]	Country	% (of 4781)
1	Evidence-Based Complementary and Alternative Medicine	375	2.629	United States	7.8
2	Medicine	233	1.889	United States	4.8
3	Acupuncture in Medicine	232	2.267	England	4.8
4	Journal of Alternative and Complementary Medicine	155	2.579	United States	3.2
5	Trials	135	2.279	United States	2.8
6	Complementary Therapies in Medicine	88	2.446	United States	1.8
7	BMC Complementary and Alternative Medicine	81	3.659	England	1.6
8	PLoS One	75	3.24	United States	1.5
9	Journal of Pain Research	71	3.133	England	1.4
10	BMJ open	68	2.692	England	1.4

Notes: [a]IF in Table 1 according to Journal Citation Reports (2020).

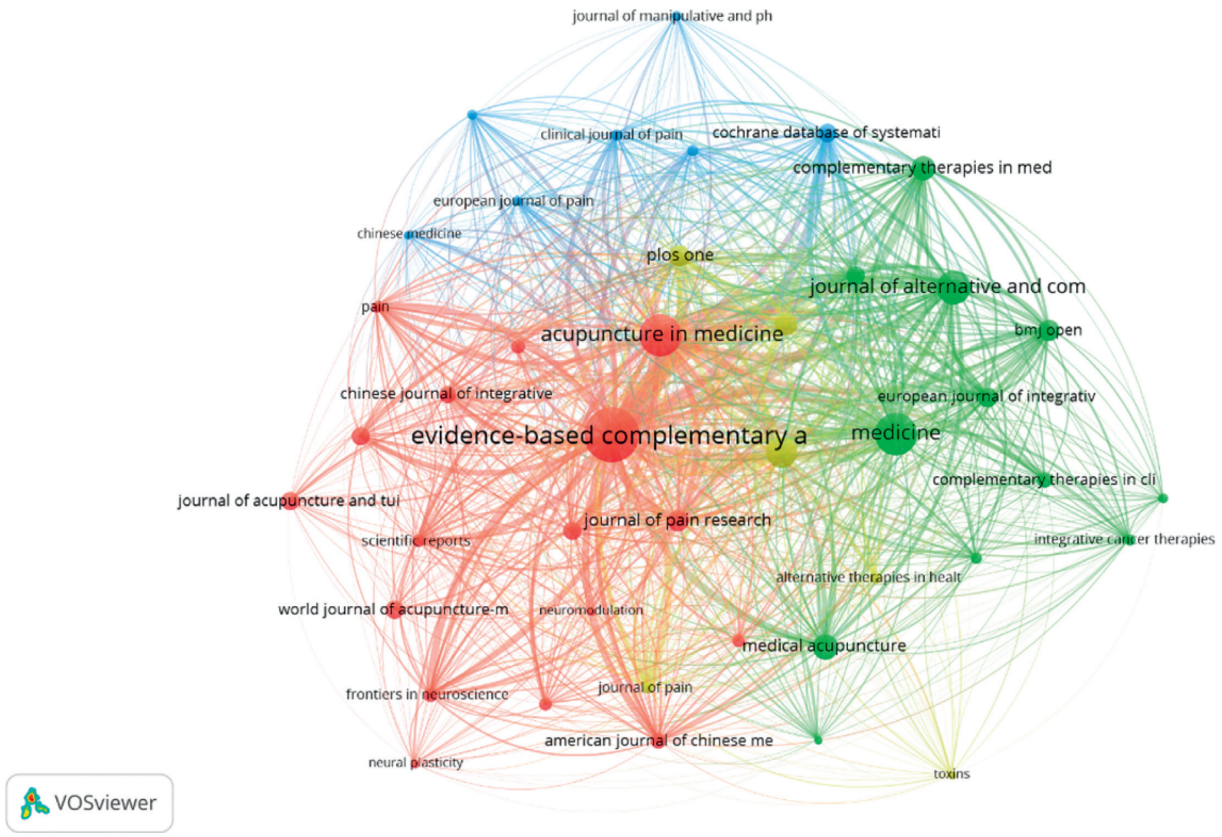


Figure 4: Bibliometric coupling of journals.

England (330 articles), and Germany (258 articles). As the birthplace of acupuncture, China had the largest number of publications, although the bibliometric analysis in this paper did not include Chinese journals.

8. Discussion

Acupuncture is an effective method of analgesia. Since acupuncture analgesia was discovered, its effectiveness has been confirmed by numerous clinical and molecular mechanism studies. Acupuncture has been widely applied to alleviate several pain diseases and incorporated into guidelines, and it is an effective method of coping with the opioid crisis and is widely respected due to its safety, low price, and low addiction. The use of acupuncture helps to increase the levels of neurotransmitters in the body, which can be targeted for the treatment of different pain disorders according to different activation mechanisms while maximizing the analgesic effect according to their quantity requirements. However, acupuncture analgesia still suffers from the following deficiencies: (1) Acupuncture can be used to treat various pain diseases, but the spectrum of diseases has not been well defined, and its indications should be further expanded. (2) The design of RCTs related to acupuncture is still flawed, and how to improve the design methods of RCTs to avoid bias is also an urgent issue to be solved. The placebo effect seems to affect the essence of acupuncture analgesia, and avoiding the placebo effect requires further examination. (3) Whether acupuncture can be an effective alternative therapy to drugs in the treatment of

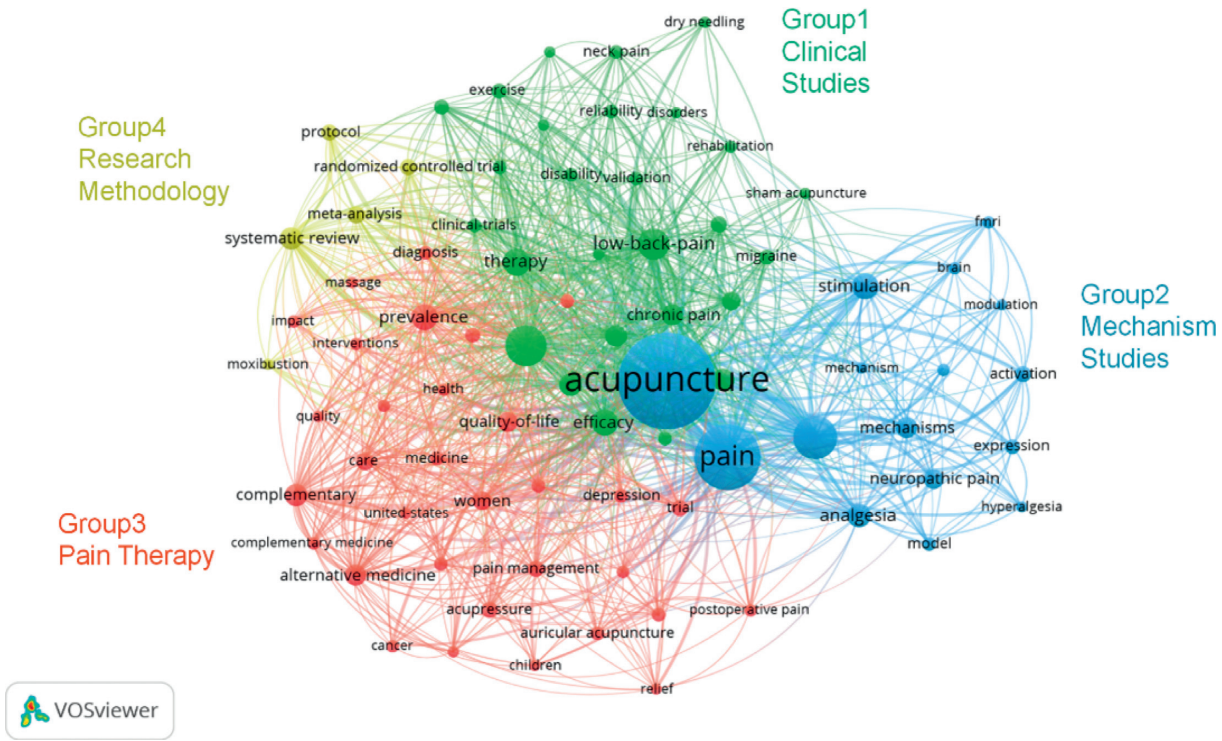
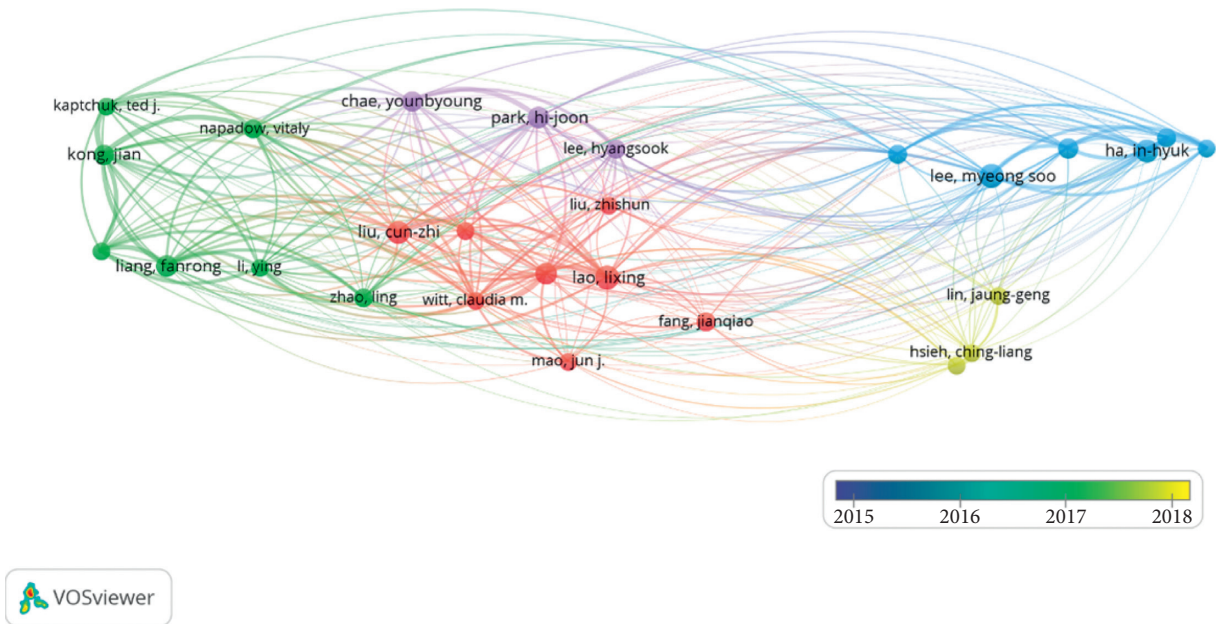
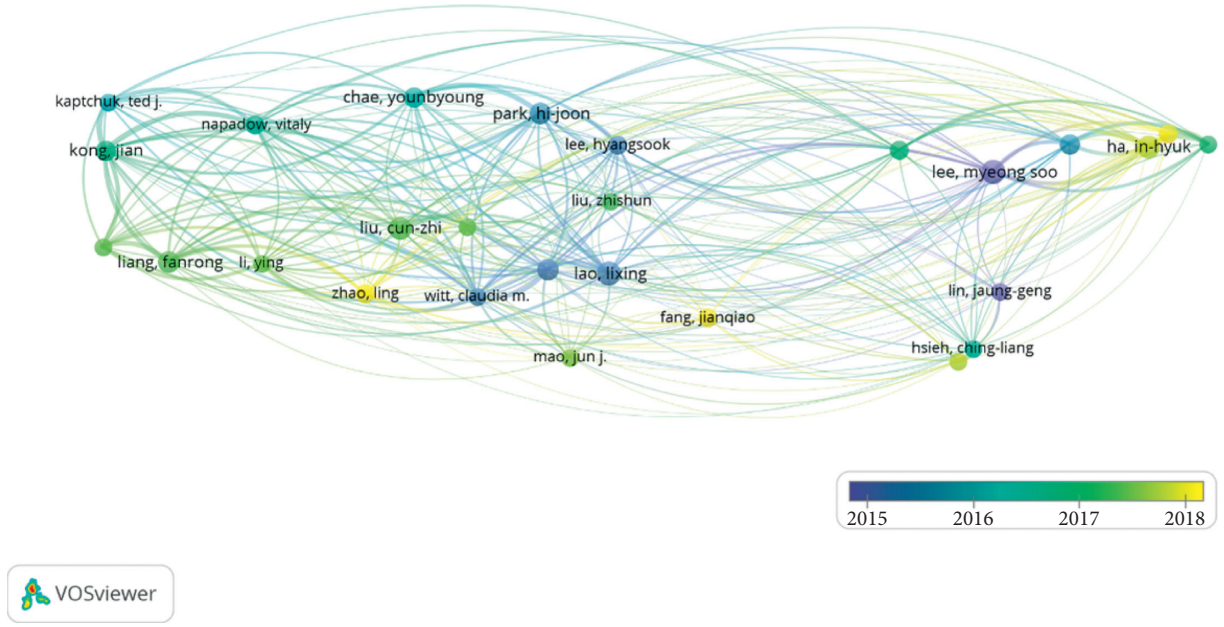


Figure 5: Network map of keywords of acupuncture and pain (divided into four groups).



(a)

Figure 6: Continued.



(b)

Figure 6: Network map of authors. (a) Authors with more than 25 articles. (b) Year of author's publication in the journal.

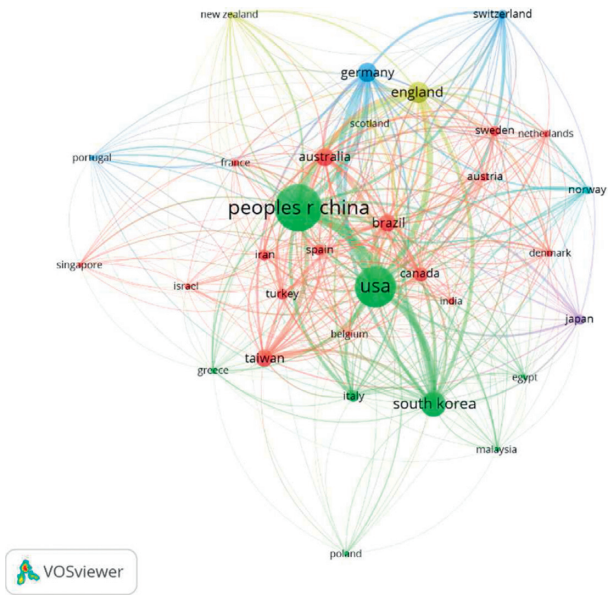


Figure 7: Network map of countries with at least 20 publications.

certain diseases or to develop individualized treatment plans based on acupuncture analgesia. (4) The analgesic mechanisms of the different acupuncture modalities have not been elucidated, but most of them seem to be based on the same neurotransmitter mechanism. Different frequencies of acupuncture can lead to the activation of neurotransmitters at different sites. The related mechanism has not been clarified. The mechanism of action of different neurotransmitters for analgesia also needs to be further demonstrated. (5) The choice of different acupoints would induce different analgesic efficacy, and the variability between acupoints remains to be clarified, and the choice of the correct acupoint should be based on a large body of evidence. (6) How to avoid bias produced by acupuncture specificity remains to be addressed, and the criteria for acupuncture quantification are unclear. (7) Through the literature measurement tool, we can clarify the current research trend and the cross-research between disciplines and even countries, which is significantly beneficial to the research progress of acupuncture treatment of pain, but there are still deficiencies. The literature selected in this paper is in English, while other languages are not included, which may lead to bias in some aspects. If it includes the literature in Chinese or other languages, it may make the analysis results more comprehensive. Second, the results of the bibliometric analysis may be subjective, and the results may be different due to the different settings of software applications. However, the analysis of keywords and publications in this paper can accurately determine the current prominent researchers and research hotspots, which is conducive to the induction and research of acupuncture treatment for pain. In a word, effective analytical methods and considerable basic and clinical research are still vital to confirm the related mechanism of acupuncture analgesia, thereby providing an effective means of diagnosis and treatment for the medical service.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Authors' Contributions

Menglong Zhang, Lei Shi, Shizhe Deng, and Bomo Sang contributed equally to this study.

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Research Article

Acupuncture Alleviates Menstrual Pain in Rat Model via Suppressing Eotaxin/CCR3 Axis to Weak EOS-MC Activation

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Introduction. Emerging data show that chemokine-mediated inflammation is involved in the occurrence and maintenance of pain. Recent evidence suggests that eotaxin levels rise when dysmenorrhea happens. The purpose of this study is to investigate whether eotaxin/CC chemokine receptor 3 (CCR3) axis, a key regulatory pathway for eosinophils (EOS) recruitment, is involved in acupuncture analgesia for dysmenorrhea. *Methods*. After the cold congealing dysmenorrhea (CCD) rat model prepared, animals received perpendicular needling (PN) and transverse needling (TN) at SP6, respectively, for 20 min. The CCR3 agonist CCL11 was administered 30 min prior to acupuncture. Pain behavior was assessed via a writhing response. The uterine contraction test was detected by an electrophysiological method. Eotaxin, histamine (HIS), and interleukin-6 (IL-6) levels were evaluated by ELISA. The expression of CCR3 and histamine H1 receptor (H1R) was analyzed by RT-qPCR and Western blot. The expression of EOS, mast cells (MCs), eosinophil peroxidase (EPO), and eosinophil cationic protein (ECP) was assessed by hematoxylin-eosin staining (HE), Toluidine Blue staining (TB), and immunohistochemistry, respectively. *Results*. Acupuncture prominently attenuated the menstrual pain in CCD rats, particularly TN technique. Electrophysiological recording data showed that the increased uterine contractility was ameliorated by acupuncture. In addition, TN decreased the release of eotaxin, HIS, IL-6, and the expression of CCR3 and H1R. HE, TB staining, and immunohistochemistry experiments showed that the increased expression of EOS, MCs, EPO, and ECP in uterine tissues was reversed by TN. Furthermore, we found that the effects of TN against CCD-induced menstrual pain, increased ECP expression, and HIS level were abolished by CCL11. *Conclusion*. TN alleviated menstrual pain by improving the uterine inflammatory environment via suppressing eotaxin/CCR3 axis to weak EOS-MC activation in CCD rats. The study findings support the acupuncture as a promising approach for dysmenorrhea, meanwhile, indicating the importance of performing appropriate needling technique.

1. Introduction

As a common gynecological disorder, primary dysmenorrhea (PD) refers to spasmodic pain in the lower abdomen without obvious pelvic organic lesions before, during, or after menstruation [1]. About 45% to 90% of women in the world suffer from dysmenorrhea, among which 10% to 25% are severe dysmenorrhea [2]. PD leads to not only physical pain to patients but also huge economic loss to the society [3]. At present, PD is commonly treated with nonsteroidal anti-inflammatory medications and oral contraceptives. However, these drugs are accompanied by some side effects, such as headache, dizziness, fatigue, loss of appetite, nausea, vomiting, and drowsiness [4], and approximately 18% of women do not respond these [5]. Therefore, prevention and treatment of PD are important.

The menstruation could be regarded as an inflammatory event [6]. Previous researches suggested that eosinophils (EOS) could be seen in endometrium before and during menstruation [7, 8]. EOS and MCs played essential roles in

morphological changes associated with the menstrual cycle and were believed to be important inflammatory cells involved in uterine inflammatory microenvironment [7, 9]. Recent evidence showed that chemokine-mediated inflammation involved in the introduction and maintenance of pain, and chemokines revealed as new players in pain control [10, 11]. Eotaxin, the most selective chemoattractant protein for EOS [12], can trigger the EOS recruitment into the inflammatory tissues via CC chemokine receptor 3 (CCR3), which is expressed in EOS [13, 14]. A recent study has showed that the serum level of eotaxin is increased in patients with primary dysmenorrhea [15]. Eosinophil cationic protein (ECP) has been shown to stimulate mast cells (MCs) to release histamine (HIS), which promotes uterine contractions and exacerbates pain [16]. Therefore, inactivation of eotaxin/CCR3 axis weakening EOS-MC activation could be involved in anti-inflammatory and analgesic mechanism subsequently modulating menstrual pain.

As a nonpharmacologic intervention, acupuncture has been increasingly used for treatment of pain [17], including PD [18, 19], and is recognized as an effective and safe therapy with little risk of serious adverse effects [20]. Recently, it has been pyramidally recognized that the anti-inflammatory effect of acupuncture contributes to its analgesic mechanism [21–23]. Moreover, acupuncture showed positive results of relieving asthma and allergic rhinitis via reducing eotaxin secretion [24, 25]. Sanyinjiao (SP6), located on the tibial aspect of the leg, posterior to the medial border of the tibia and 3 B-cun (proportional bone cun), above the prominence of the medial malleolus [26], is the intersection point of liver, spleen, and kidney channels, which is closely related to the uterus with the effects of moving Qi and regulating blood. A variety of studies affirmed that SP6 is one of the most common used points for PD, with satisfactory analgesic effect [27, 28]. Clinically, needling techniques, such as the depth, angle, and direction of needling, play an essential role for acupuncture efficacy. However, the mechanism induced by different needling techniques at the same acupuncture point is often overlooked and worthy of further study. Our previous studies provided evidence that needling techniques could affect the pain-relief effect of SP6 for PD via different pathways [29–31].

Considering that there are data showing the modulation of eotaxin on menstrual pain, we hypothesized that acupuncture might reduce eotaxin and CCR3, thus weakening the EOS-MC activation to alleviate the inflammatory environment and, subsequently, relieving menstrual pain.

2. Materials and Methods

2.1. Animals. Three-month-aged female Sprague-Dawley (SD) rats of sexually mature and nonmating weighing 230 ± 20 g were obtained from Beijing Vital River Laboratory Animal Technology. All rats were caged individually and handled in a constant environment with food and water freely available throughout the experiment. The constant environment was maintained at 23 ± 2°C with humidity of 45% ± 5% and a standard 12-hour light-and-dark cycle (lights on at 08:00 am). Animal care and experiments were performed strictly in conformity with the Guidelines for the Care and Use of Laboratory Animals by the National Institutes of Health and approved by the Animal Ethics Committee of Beijing University of Chinese Medicine (Approval No. BUCM-4-2019070502-3015), and all efforts were made to minimize animal suffering.

2.2. Establishment of Cold Congealing Dysmenorrhea (CCD) Rat Model. The CCD rat model was established as described previously [5, 32, 33]. The rats were frozen and subcutaneously injected with Estradiol Benzoate (Ningbo Sansheng Biotechnology Co., Ltd., Ningbo, China) in the abdomen [34, 35]. First, female rats were selected based on the estrous cycle for the induction of dysmenorrhea. The vaginal smear was collected and analyzed with a light microscope, and the selected rats during diestrus were subcutaneously injected Estradiol Benzoate for 10 days (0.5 mg/rat on the 1st and 10th days, and 0.2 mg/rat for the 2nd to 9th days). Second, on the 1st to 5th days after administration, the rats were placed in a −25°C freezer for continuous freezing for 4 h and ventilated for 5 s at 2 h. Third, 1 h after the last administration on the 10th day, each rat was injected 2U of oxytocin (Chifeng Bone Pharmaceutical Co., Ltd., Chifeng, China) intraperitoneally. The blank group was treated with the same dose of saline solution (Figure 1(a)).

2.3. Experimental Design

2.3.1. Experiment 1. To observe the therapeutic effect of acupuncture and to find the most effective needling method, we performed the writhing response and the uterine contraction test to evaluate menstrual pain. The rats were divided randomly into 4 groups: the blank group, the CCD model group, the Model + perpendicular needling (PN) group, and the Model + transverse needling (TN) group ($n = 12$/group). The writhing response ($n = 6$/group) and the uterine contraction test ($n = 6$/group) were evaluated.

2.3.2. Experiment 2. To investigate whether TN-induced analgesia associated with the eotaxin/CCR3 pathway, we detected the level of the key factors and the expression of inflammatory cells. The rats were divided randomly into 3 groups: Blank, Model, and Model + TN ($n = 6$/group).

2.3.3. Experiment 3. To explore the role of CCR3 played in the mechanism of TN, intraperitoneal injection of CCR3 selective antagonist SB328437 and agonist CCL11 was performed. The rats were randomly divided into the following 6 groups: Model, Model + SB328437, Model + Vehicle1, Model + CCL11 + TN, Model + Vehicle2 + TN, and Model + Vehicle1 + TN ($n = 6$/group). SB328537 and CCL11 were dissolved in Vehicle 1 or Vehicle 2, respectively.

2.4. Acupuncture Interventions. After oxytocin injection on the 10th day, Sanyinjiao (SP6), located 10 mm proximal to the prominence of the medial malleolus [34, 35], was needled bilaterally by an ameliorated filiform needle

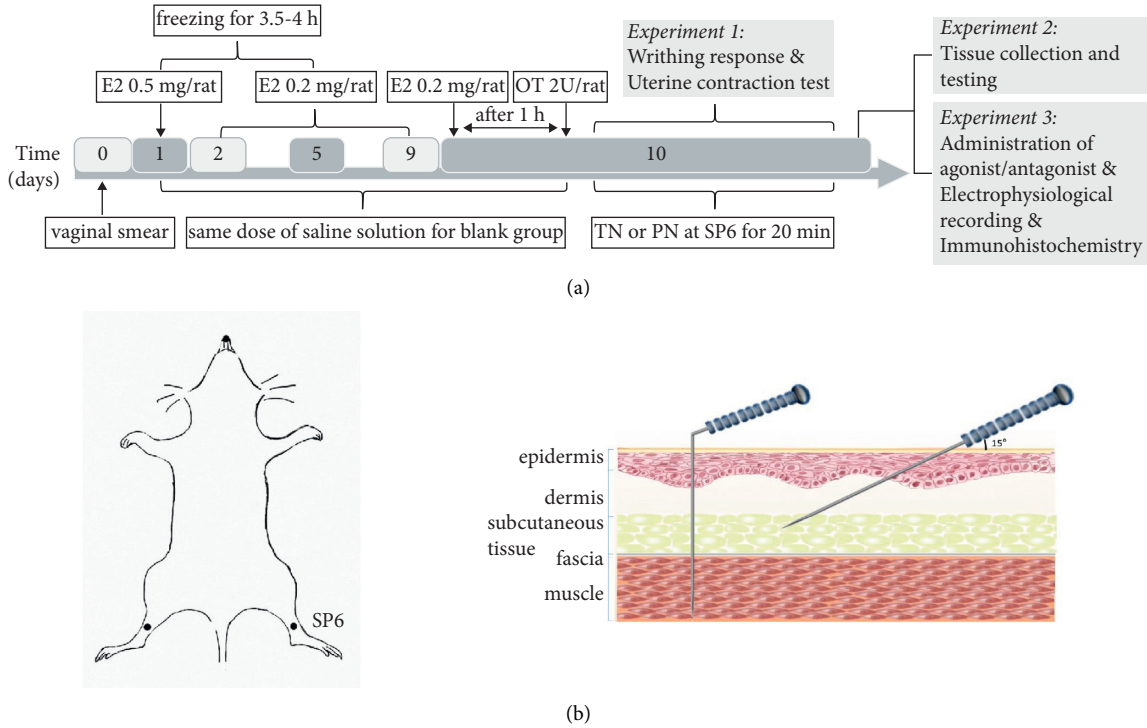


Figure 1: (a) Experimental design. (b) The location of SP6 and acupuncture interventions. Perpendicular needling method, PN; transverse needling, TN.

(Φ0.18 mm × 5 mm) in 2 needling groups. In the Model + PN group, needles were inserted vertically at a depth of 4-5 mm. In the Model + TN group, needles were inserted transversely 4-5 mm toward the abdomen, at an angle of about 15° between the needle and the skin surface (Figure 1(b)). In order to observe the writhing response effectively, we ameliorated the needle as shown in Figure 1(b). The needle handle was bent and then fixed with an adhesive tape after a needle insertion; therefore, the rats could move freely during needle retention. Needles were retained for 20 min. Both the blank group and the model group used the same method for grasping fixation, but no treatment was performed.

2.5. Drug Administration. The CCR3 receptor antagonist SB328437 (ab120648, Abcam, Cambridge, UK), dissolved in DMSO (Vehicle1), was administered intraperitoneally at a dose of 5 mg/kg 30 min prior to the acupuncture. The specific CCR3 agonist CCL11 (420-ME-100/CF, R&D Systems, MN, USA), which was reconstituted at 100 μg/mL in sterile PBS (Vehicle2), was injected intraperitoneally at a dose of 10 μg/per animal 30 min before the acupuncture. The dosage and route of drug administration were based on the previous study with certain minor modifications according to the experimental condition [36].

2.6. Behavioral Test

2.6.1. Writhing Response. The writhing response of rats was observed within 3 or 20 min after injection of oxytocin on the 10th day. The latency and score of the writhing response, which were proposed by Schmauss C, were recorded [37].

2.7. Electrophysiological Recordings In Vivo. The uterine contraction test was recorded by the BL420S Biological Function Experimental System Recorder (Chengdu Taimeng Technology Co., Ltd., Chengdu, China). After injection of Estradiol Benzoate on the 10th day, intramuscular injection of 1% pentobarbital sodium (40 mg/kg) was performed in the rats. And a 2-3 cm longitudinal incision was performed at about 0.5 cm apart from the midline of the abdomen. The line was threaded at 1 cm on the bifurcation of uterine horn, and the free end of the line was connected to the tension sensor. After 0.1 g of afterload was settled, the uterine contraction wave was recorded. 2U oxytocin was injected directly into the uterus in the model group and 2 needling groups, and saline in the same dose injected in the blank group. Results were recorded 20 min after acupuncture intervention in 2 needling groups: uterine activity = number of uterine contraction wave × peak-to-peak wave value.

2.8. ELISA. According to the manufacturer's instructions, Rat Eotaxin/CCL11 ELISA Kit (RGB-60563R, RGB & CHN, Beijing, China), Rat HIS ELISA Kit (RGB-60351R, RGB & CHN), and Rat IL6 ELISA Kit (RGB-60302R, RGB & CHN) were used for the measurement of eotaxin, histamine, and IL6. After anesthesia, the blood and uterus were obtained. Blood was immediately placed in a biopsy tube with EDTA to prevent clotting. Then the whole blood was centrifuged to separate the plasma. The plasma samples were stored in the −80°C until assays were performed. An appropriate amount of uterine tissue was cut and weighed, and a certain amount of PBS was added (tissue mass : PBS = 1 : 20). After homogenate with a homogenizer (Lanyi-GTM, Shanghai,

China), centrifugate for about 10 minutes (4000 r/min), collect the supernatant, and then detect the total protein. Protein concentration in tissue extract = detected protein concentration in the extract/TP value of the extract.

2.9. Real-Time Quantitative Polymerase Chain Reaction (RT-qPCR). Total RNA of the cells was isolated with TRIzol (Invitrogen) and then was reversely transcribed into cDNA using a Prime-Script RT reagent kit (CW2569, Kangwei Century, Beijing, China). The gene expression levels of CCR3 and histamine H1 receptor (H1R) were quantified by SYBR FAST qPCR Kit Master Mix (2×) (KK4601, KAPA Biosystems, MA, USA) and detected by the StepOne Plus Real-Time PCR Detection System (ABI, CA, USA). The PCR reaction conditions were as follows: predenaturation at 95°C for 3 minutes, denaturation at 95°C for 10 s, annealing/extension at 59°C for 60 s, and a total of 40 cycles of denaturation to extension. The relative expression levels of CCR3 mRNA and H1R mRNA were calculated by the $2^{-\triangle\triangle Ct}$ method as normalized by β-actin (an endogenous housekeeping gene). Primer sequences and amplification sizes are presented in Table 1.

2.10. Western Blot. The uterine tissue was homogenized in radioimmunoprecipitation assay buffer. And then the homogenate was centrifuged at 13,000 rpm for 20 min at 4°C and the supernatant was collected. The protein concentration was determined using the bicinchoninic acid method (BCA) according to the kit's instruction (02912E, Kangwei Century, Beijing, China), and 20 mg of protein was loaded in each lane. Protein samples were separated on 5 to 10% sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) gels and electrophoretically transferred to polyvinyl difluoride (PVDF) membranes (Millipore, MO, USA). The membranes were blocked with 5% nonfat milk at room temperature for 1 hour, followed by overnight incubation at 4°C with the following primary antibodies diluted in blocking buffer : anti-CCR3 (1 : 1000, ab32512, Abcam) and anti-HR1R (1 : 500, BS-6663R, Bioss, Beijing, China). Subsequently, the immunoblots were incubated with second antibodies (1 : 10000, 111-035-003, Jackson, PA, USA) for 1 hour at room temperature. GAPDH (1 : 1000, 5174, CST, MA, USA) was used as an internal control. The immunoreactivity was detected by an ECL chemiluminescence detection system (WBKLS0500, Millipore) and analyzed by an image analysis system (Tanon 4200, Shanghai, China).

2.11. Histological Analysis. The uterine tissue was immersed in 4% paraformaldehyde for more than 24 h. After dehydration, tissues were imbedded in paraffin and sliced to a thickness of 4–6 μm. Then, hematoxylin-eosin staining (HE) or Toluidine Blue Staining (TB) were, respectively, used to analyze the EOS and MCs. The sliced tissues were stained with hematoxylin and eosin or toluidine blue. The slides were examined at 800x or 400x or 200x magnification with a microscope.

Table 1: The primers used for RT-qPCR analysis.

Name		Sequences (5′-3′)
β-Actin	Forward primer	5′-GCACCATGAAGATCAAGATCAT
	Reverse primer	3′-TAACAGTCCGCCTAGAAGCATT
CCR3	Forward primer	5′- GTCTTGCAGTATTGGCAGCAT
	Reverse primer	3′-TTCTTCGCCCTCTGGATAGC
H1R	Forward primer	5′- TGTGTGAGGGGAACAGGACA
	Reverse primer	3′- ACAGCACCAGCAGGTTGAGG

2.12. Immunohistochemistry. The embedded uterine tissue slides (4–6 μm) were initially treated for deparaffinization, rehydration, and antigen retrieval, followed by 3% H_2O_2 incubation for 30 minutes. Sections were incubated with anti-EPX (1 : 200, BS-3881R, Bioss) and anti-ECP (1 : 200, BS-1754R, Bioss) and then incubated with peroxidase-labeled IgG secondary antibody (PV-6000, Beijing ZSGB Biotechnology Co., Ltd., Beijing, China). Fields from each slide were examined and photographed under a light microscopy (×400). Image-Pro Plus 6.0 image analysis software (media controls, Silver Spring, MD, USA) was used for analyzing the values of total cross-sectional integrated optical density (IOD) of each image.

2.13. Statistical Management. Data were expressed as mean ± SEM. All data were normally distributed and one-way ANOVA was carried out. Homogeneity of group variances was checked with a Levene's test. The Bonferroni test was used to evaluate homogeneously distributed data, and the Tamhane T2 test was used to evaluate nonhomogeneous data. The value of $P < 0.05$ was considered statistically significant. SPSS 23.0 software was used for the statistical analysis.

3. Results

3.1. Acupuncture Alleviated Menstrual Pain in CCD Rats. As shown in Figure 2, the writhing latencies (Figure 2(a)) and writhing scores in 3 min (Figure 2(b)) of the rats in each model group were significantly different from those of the rats in the Blank group ($P < 0.01$), which indicated that the dysmenorrhea model was successfully established.

After acupuncture, compared with model group, a writhing score in 20 min significantly decreased in the two acupuncture groups ($P < 0.05$, $P < 0.01$, Figure 2(c)). Interestingly, a writhing score in 20 min significantly decreased in the Model + TN group compared with the Model + PN group ($P < 0.05$, Figure 2(c)), indicating that TN performs better in dysmenorrhea.

3.2. Acupuncture Attenuated Uterine Contraction in CCD Rats. As presented in Figure 3, electrophysiological results showed that acupuncture attenuated uterine contractions in CCD rats (Figures 3(a)–3(d)). After model preparation, the

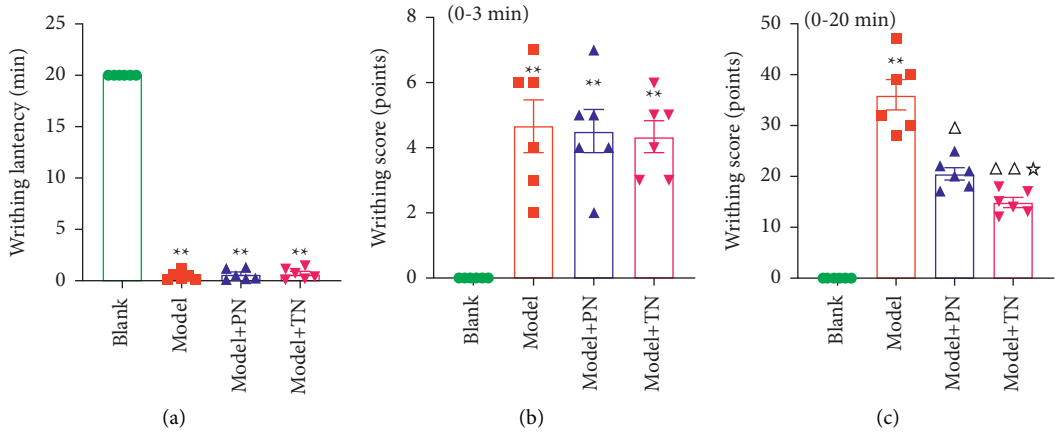


Figure 2: Acupuncture alleviated menstrual pain in cold congealing dysmenorrhea (CCD) rats. (a) The writhing latency. (b) The writhing scores in 3 min. (c) The writhing scores in 20 min. Data were analyzed by the Tamhane T2 post hoc test and expressed as means ± SEM ($n = 6$ rats/group). $^{**}P < 0.01$ vs. Blank group; $^{\triangle\triangle}P < 0.01$ vs. Model group; $^{\triangle}P < 0.05$ vs. Model group; $^{\star}P < 0.05$ vs. Model + PN group.

number of uterine contraction wave, uterine peak-to-peak value, and uterine activity of model group were significantly increased ($P < 0.01$; Figures 3(b)–3(d)) compared with the blank group. After acupuncture, compared with the model group, the number of uterine contraction wave, the uterine peak-to-peak value, and uterine activity was significantly decreased ($P < 0.05$, $P < 0.01$; Figures 3(b)–3(d)) in two needling groups. Compared with the Model + PN group, uterine activity in the Model + TN group was significantly decreased ($P < 0.01$, Figure 3(d)).

The overall therapeutic effects of acupuncture with TN were superior to PN. Thus, we used TN at SP6 throughout the later experiments.

3.3. TN Reduced Eotaxin Levels and Attenuated the Expression of CCR3 in the Uterus. To determine whether TN could modulate eotaxin/CCR3 axis, we applied ELISA to detect the eotaxin level, RT-qPCR, and Western blot to analyze the expression of CCR3. Compared with the blank group, the eotaxin levels in uterus and plasma were significantly increased in the model group ($P < 0.01$; Figures 4(a) and 4(b)). In addition, we found that TN at SP6 attenuated this change ($P < 0.01$; Figures 4(a) and 4(b)). This attenuation was accompanied by decreased mRNA and protein expression of CCR3 ($P < 0.01$; Figures 4(g) and 4(j)).

3.4. TN Decreased Histamine Levels and the Expression of H1R in the Uterus. To investigate whether the HIS expression in uterus is associated with the eotaxin/CCR3 axis, we applied ELISA to detect the HIS level, RT-qPCR, and Western blot to analyze the expression of its receptor, H1R. The HIS level of model group was significantly increased compared with that of blank group in uterus and plasma ($P < 0.01$; Figures 4(c) and 4(d)). Furthermore, we found that TN at SP6 attenuated this change ($P < 0.01$; Figures 4(c) and 4(d)). This attenuation was accompanied by decreased mRNA and protein expression of H1R ($P < 0.01$, $P < 0.05$; Figures 4(h) and 4(k)).

3.5. TN Decreased IL-6 Levels in the Uterus and Plasma. To detect whether TN could modulate IL-6, we applied ELISA to identify IL-6 levels in uterus and plasma. The IL-6 levels of model group were significantly increased compared with those of blank group in both uterus and plasma ($P < 0.01$; Figures 4(e) and 4(f)). TN at SP6 decreased IL-6 levels in uterus and plasma ($P < 0.01$, $P < 0.05$; Figures 4(e) and 4(f)).

3.6. TN Attenuated the Activation of EOS and MCs in the Uterus. To observe the association between eotaxin/CCR3 axis and HIS, we applied HE or TB, respectively, to analyze the main inflammatory cells, EOS and MC. As shown in Figure 5, compared with the model group, the Model + TN group attenuated the EOS and MC migration to uterus and their activation (Figures 5(a) and 5(c)). The number of EOS and MC in the Model + TN group decreased compared with the model group ($P < 0.05$; Figures 5(b) and 5(d)). Immunohistochemical staining results showed the positive expression of EPO (Figures 6(a)) and ECP (Figure 6(b)) of all groups in rat uterus. The IOD values of EPO and ECP in the Model + TN group decreased significantly compared with the model group ($P < 0.01$; Figures 6(c) and 6(d)).

3.7. CCR3 Agonist Blocked TN-Induced Analgesic Effect. TN at SP6 can relieve pain by attenuating the expression of eotaxin/CCR3 axis and EOS-MC activation. To further address whether pain-relief induced by TN was dependent on the activation of the eotaxin/CCR3 axis, we injected CCR3 antagonist SB328437 and agonist CCL11. Administration of SB328437 could mimic the effects of TN on uterine activity, and CCL11 blocked the effects of TN on uterine contractions in CCD rats (Figures 7(a)–7(d)). The number of contraction wave, uterine peak-to-peak value, and degree of contraction was significantly decreased compared with the model group after SB328437 application in the Model + SB328437 group ($P < 0.05$; Figures 7(b)–7(d)). However, after the application of CCL11 in the TN group, it markedly

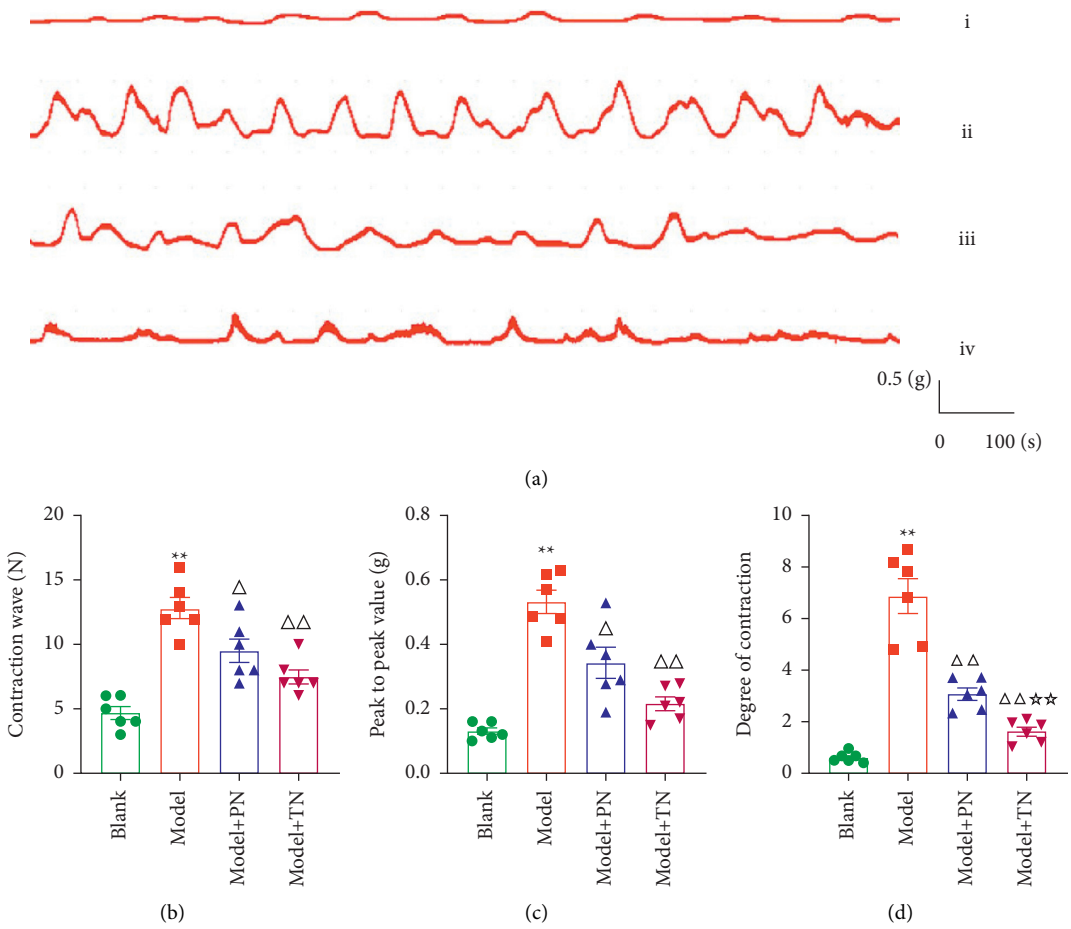


Figure 3: Acupuncture attenuated uterine contraction in CCD rats. (a) The representative uterine contraction curves of all groups. (i–iv) Blank group, Model group, Model + PN group, and Model + TN group, respectively. (b) The number of contraction wave. (c) The peak-to-peak value. (d) The degree of contraction. All data are expressed as the means ± SEM ($n = 6$ rats/group). (b) One-way ANOVA followed by the Bonferroni post hoc test was used. (c, d) The Tamhane T2 post hoc test was used. $^{**}P < 0.01$ vs. Blank group; $^{\triangle\triangle}P < 0.01$ vs. Model group; $^{\triangle}P < 0.05$ vs. Model group; $^{\star\star}P < 0.01$ vs. Model + PN group.

weakened TN-induced pain relief compared with the Model + Vehicle2 + TN group as manifested by the number of contraction wave, uterine peak-to-peak value, and degree of contraction ($P < 0.01, P < 0.05$; Figures 7(b)–7(d)). Compared with the Model + Vehicle1 + TN group, the number of contraction wave, uterine peak-to-peak value, and degree of contraction increased in the Model + Vehicle1 group ($P < 0.01, P < 0.05$; Figures 7(b)–7(d)). There was no significant difference in the number of contraction wave, peak-to-peak value, and degree of contraction between Model + Vehicle2 + TN and Model + Vehicle1 + TN groups ($P > 0.05$; Figures 7(b)–7(d)).

3.8. CCR3 Agonist Reversed the Decreased ECP Expression and HIS Level in the Uterus Induced by TN. After administration of SB328437, compared to the model group, the ECP expression and the HIS level were significantly decreased in the Model + SB328437 group ($P < 0.01$; Figures 8(a)–8(c)), which indicated that the antagonist of CCR3 could mimic the effects of TN. Compared to the Model + vehicle2 + TN group, the ECP expression and the HIS level were significantly increased in the Model + CCL11 + TN group ($P < 0.01$; Figures 8(a)–8(c)). Compared with the Model + Vehicle1 + TN group, the ECP expression and the HIS level increased in the Model + Vehicle1 group ($P < 0.01, P < 0.05$; Figures 8(a)–8(c)). There was no significant difference in the ECP expression and the HIS level between Model + Vehicle2 + TN and Model + Vehicle1 + TN groups ($P > 0.05$; Figures 8(a)–8(c)).

4. Discussion

In this study, acupuncture significantly relieved menstrual pain in CCD rats. These positive therapeutic effects may be achieved through the eotaxin/CCR3 axis, as manifested by

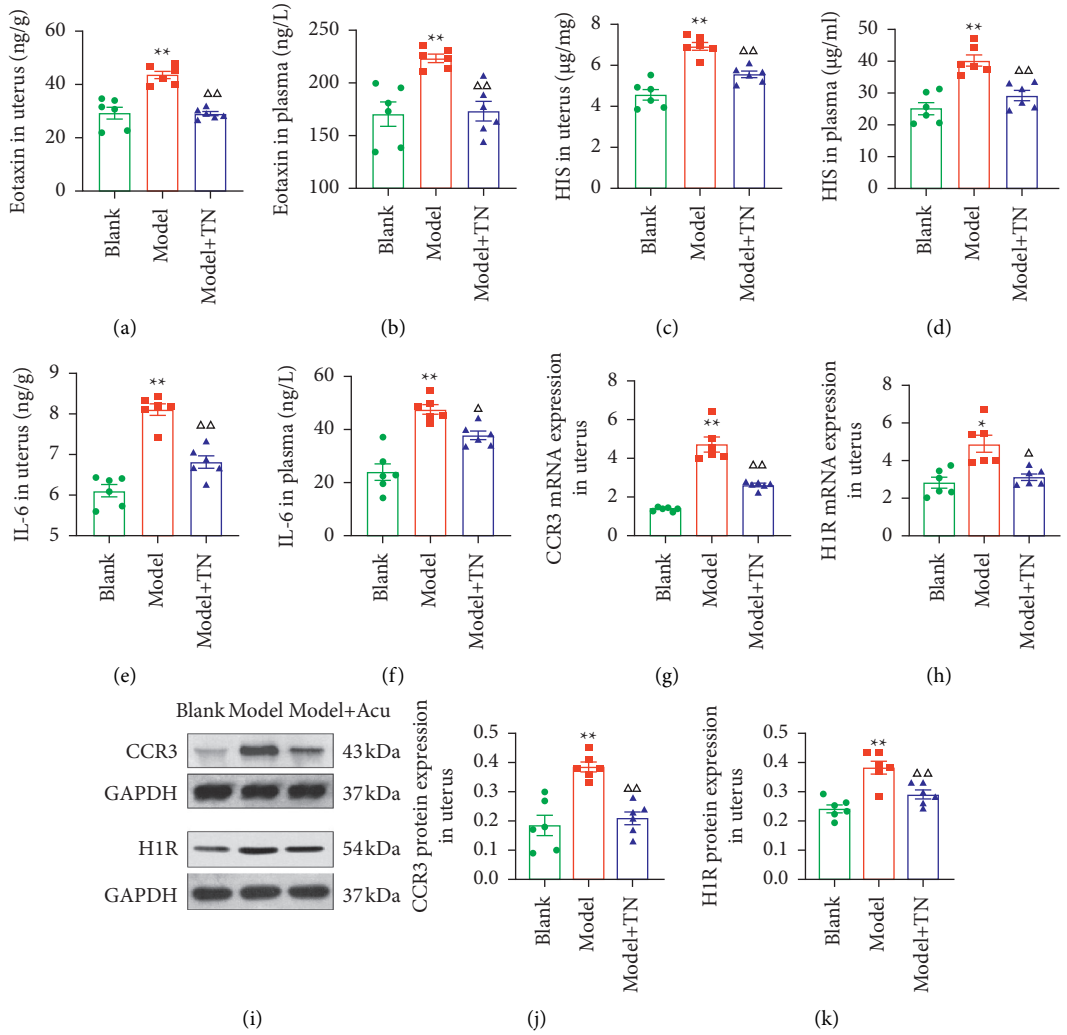


FIGURE 4: TN reduced eotaxin and histamine (HIS) levels and decreased the expression of CC chemokine receptor 3 (CCR3) and histamine H1 receptor (H1R) in CCD rats. The levels of eotaxin (a, b), HIS (c, d), and interleukin-6 (IL-6) (e, f) in uterus and plasma were detected by ELISA. The mRNA expression of CCR3 (g) and H1R (h) in the uterus was analyzed by RT-qPCR. (i–k) Representative blots (i), and protein expression levels of CCR3 (j) and H1R (k) in uterus. All data are expressed as the means ± SEM ($n = 6$ rats/group). (b–f, j, k) One-way ANOVA followed by the Bonferroni post hoc test was used. (a, g, h) The Tamhane T2 post hoc test was used. $^{**}P < 0.01$ vs. Blank group; $^{*}P < 0.05$ vs. Blank group; $^{\triangle\triangle}P < 0.01$ vs. Model group; $^{\triangle}P < 0.05$ vs. Model group.

weakening EOS-MC activation and restraining release of HIS. Moreover, the CCR3 agonist CCL11 significantly abolished a TN-induced analgesic effect and related benefits, while CCR3 antagonist SB328437 could mimic the analgesic effect, supporting this contention.

PD is a common gynecological disorder and the pain was attributed to increased uterine contractility [38, 39]. As an economical and practical therapy, acupuncture can be applied in relieving menstrual pain [18–20]. PD with cold congealing syndrome in traditional Chinese medicine (TCM) is the most common pattern [29]. Clinical and experimental studies indicated that alterations in a needling technique have an impact on therapeutic outcomes [40–42]. Although SP6 has been affirmed as one of the most commonly used acupoints for PD [27–29], which needling technique is the most effective method remains unclear. In order to reduce the interference of other acupoints and better observe the influence of different needling techniques on acupoints, a solo acupoint SP6 was used in the present study. To investigate the influence of needling techniques on SP6's analgesic effect is crucial for acupuncture clinical practice. TN, almost subcutaneously inserting the needle, is believed to be an adaptive needling technique for cold congealing syndrome according to the classic TCM theory. Our results suggested that increased uterine contraction was ameliorated by acupuncture treatment. Moreover, the overall therapeutic effect of TN was superior over PN.

The leukocytic invasion and subsequent production of inflammatory mediators are observed during menstruation; hence, the menstruation could be considered as an inflammatory event [6]. When dysmenorrhea occurs, the inflammatory response of the uterus increases [43]. As an

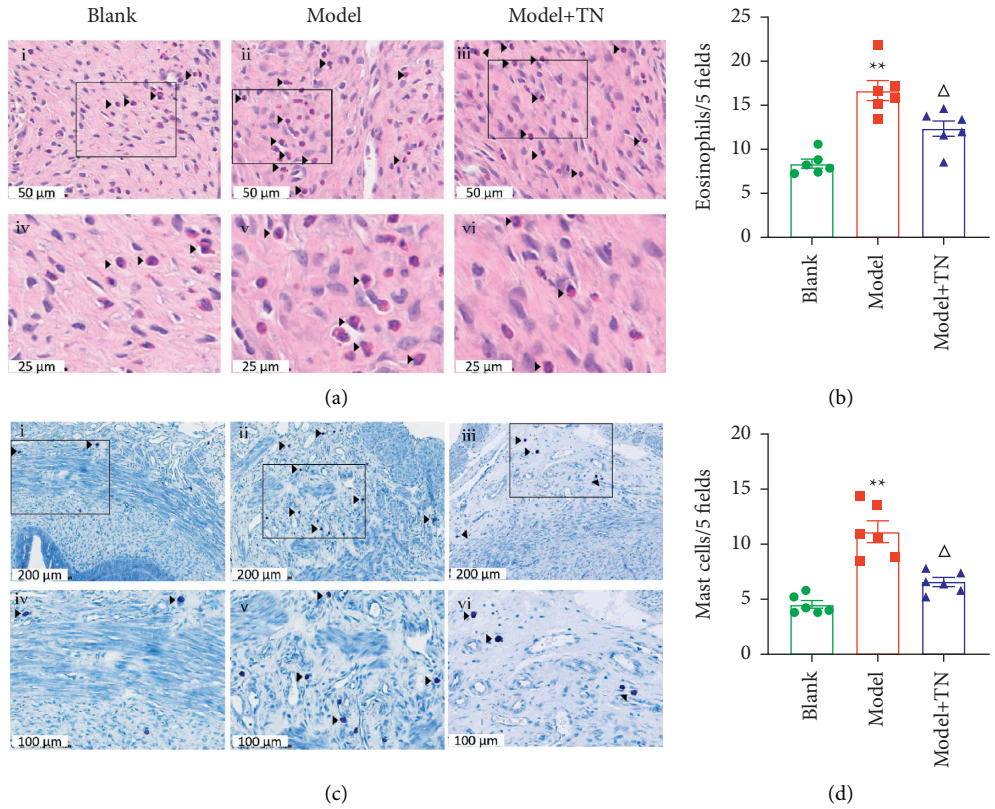


Figure 5: TN attenuated the expression of eosinophils (EOS) and mast cells (MCs) in the uterus of CCD rats. (a) The representative photomicrographs of EOS hematoxylin-eosin staining (HE) of all groups in the rat uterus. The regions represented by (i–iii) or (iv–vi) in (a) are photographs at 400x and 800x magnifications, respectively. (c) The representative photomicrographs of MCs' Toluidine Blue Staining (TB) of all groups in the rat uterus. The regions represented by (i–iii) or (iv–vi) in (c) are photographs at 200x and 400x magnifications, respectively. (b, d) A statistical histogram of the number of positive expression cells in each group in CCD rats. The arrows indicate EOS and MCs. All data are expressed as the means ± SEM ($n = 6$ rats/group). (b) One-way ANOVA followed by the Bonferroni post hoc test was used. (d) The Tamhane T2 post hoc test was used. $^{**}P < 0.01$ vs. Blank group; $^{\triangle}P < 0.05$ vs. Model group.

important aspect of promoting inflammation and causing tissue damage, EOS plays a major role in the pathophysiology of PD. The involvement of eosinophils in the female reproductive tract has been reported since the 1960s [44]. Also, in recent years, the role of EOS in the reproductive system has attracted a lot of attention [7, 8, 15, 36]. Studies found that EOS became evident prior to and during menstruation [7–9]. Chemokines and their cognate receptors play an important role in the control of leukocyte chemotaxis. Eotaxin, a member of the C-C chemokine family, is a specific chemotactic agent for EOS into the inflammatory uterus [45]. Eotaxin chemokines are grouped into 3 subtypes of eotaxin-1/CCL11, eotaxin-2/CCL24, and eotaxin-3/CCL26 [46], which are structurally different but functionally similar, selectively activating EOS by binding to a common receptor, CC chemokine receptor 3 (CCR3) [45]. And it is reported that CCL11 is the most closely associated with the uterus [47]. Eosinophil peroxidase (EPO) is utilized to assess the migration and activation of EOS. In this study, the expression of eotaxin, EOS, and EPO was increased in CCD rats, and acupuncture significantly reversed this change. Interleukin 6(IL-6) is a multifunctional proinflammatory cytokine and has multidirectional effects on both innate and acquired immune system cells. Studies have shown that plasma IL-6 concentration is related to the menstrual cycle [48], and IL-6 can promote the synthesis or release of prostaglandins [49]. The plasma IL-6 concentration increased on the first day of menstruation in women with PD [50], leading to excessive contraction of uterine muscles and reduced blood flow, ultimately ischemic pain. Currently, studies have proved that it is an effective way to treat dysmenorrhea by regulating IL-6 concentration [51]. In our study, we detected the levels of IL-6 in plasma and uterus and found that acupuncture could reduce the IL-6 levels, further verifying the anti-inflammatory effect of acupuncture.

It was reported that MCs and EOS were found at close range [52]. EOS have the capacity to modulate MC functions, such as producing ECP to stimulate HIS release from MCs [53]. In this study, the expression of ECP, MC, and HIS was increased in CCD rats, and the increase was reversed by acupuncture. The activation of inflammatory cells leads to the release of HIS, which is an important cause of dysmenorrhea. It was reported that HIS can promote the production of estradiol and PGF2α when combined with H1R, which in turn promotes uterine contractility and exacerbates pain [16]. In addition, the contraction of rat

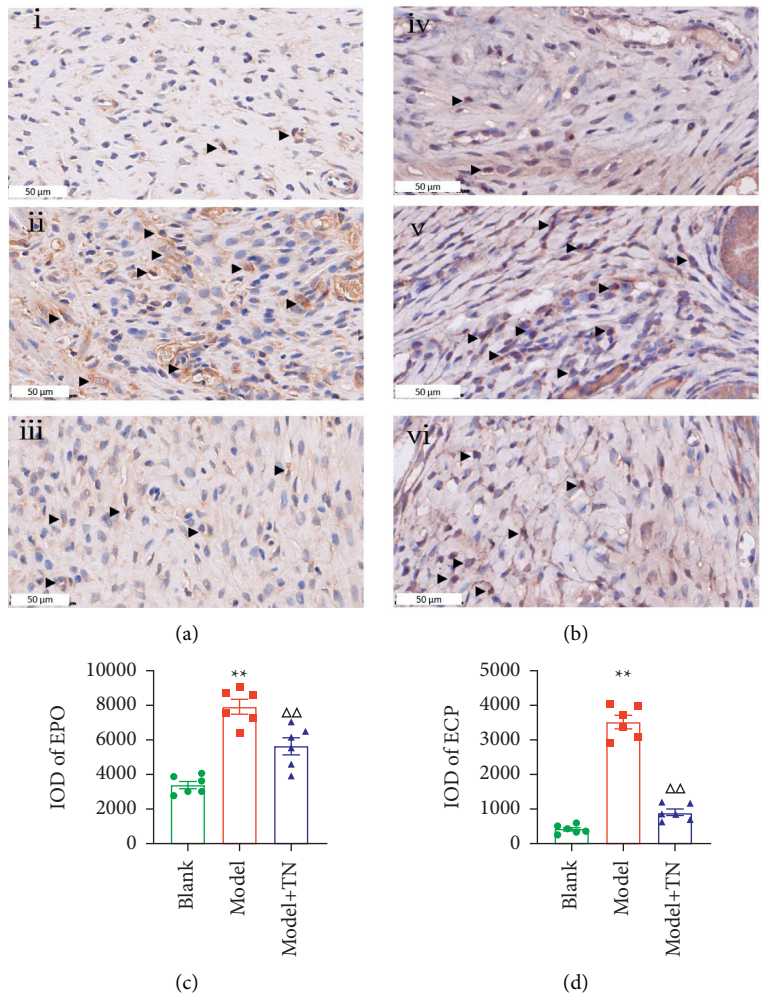
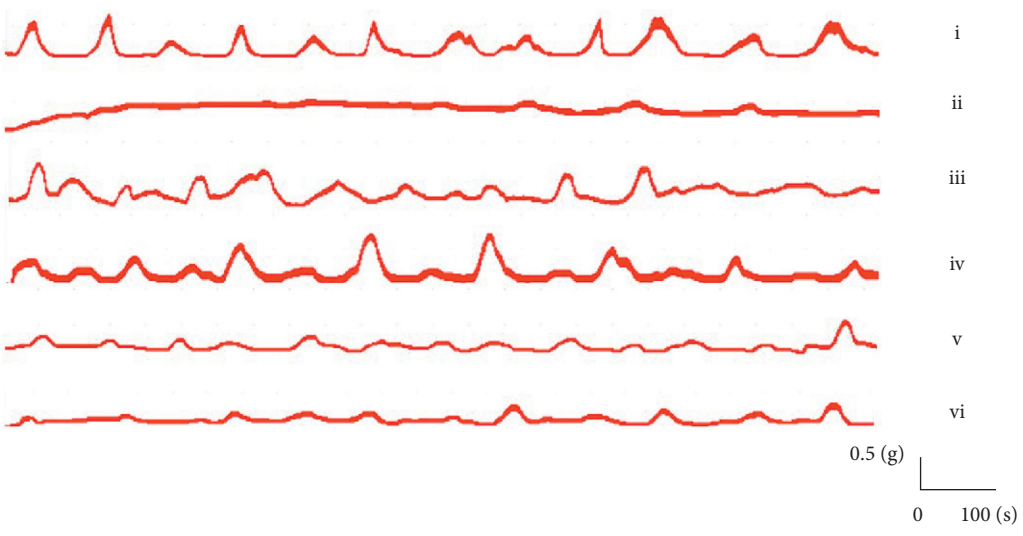


Figure 6: TN reduced the expression of eosinophil peroxidase (EPO) and eosinophil cationic protein (ECP) in the uterus of CCD rats. (a, b) The representative photomicrographs of EPO and ECP immunohistochemical staining of all groups in rat uterus. (c, d) Histogram of IOD value of EPO and ECP in the uterus of CCD rats in each group. (i and iv) Blank group; (ii and v) Model group; and (iii and vi) Model + TN group. The regions are photographs at 400x magnification. The arrows indicate a positive expression of EPO and ECP. All data are expressed as the means ± SEM ($n = 6$ rats/group). (c) One-way ANOVA followed by the Bonferroni post hoc test was used. (d) The Tamhane T2 post hoc test was used. $^{**}P < 0.01$ vs. Blank group; $^{\Delta\Delta}P < 0.01$ vs. Model group.



(a)

Figure 7: Continued.

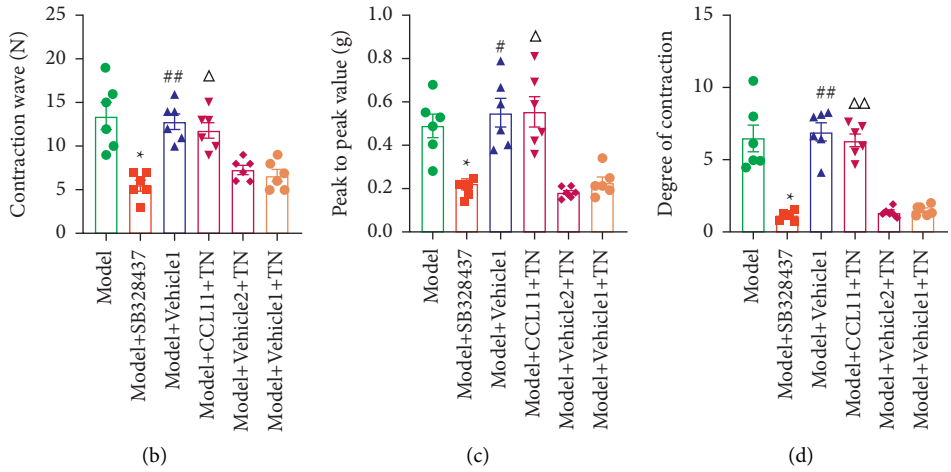


Figure 7: CCR3 agonist blocked the effect of TN against CCD-induced menstrual pain. (a) The representative uterine contraction curves of all groups. The representative images of the rat uterine activities. (i–vi) Model, Model + SB328437, Model + Vehicle1, Model + CCL11 + TN, Model + Vehicle2 + TN, and Model + Vehicle1 + TN. (b) The number of contraction wave. (c) The peak-to-peak value. (d) The degree of contraction. Data were analyzed by the Tamhane T2 post hoc test and expressed as means ± SEM ($n = 6$ rats/group). $^{*}P < 0.05$ vs. Model group; $^{\triangle\triangle}P < 0.01$ vs. Model + Vehicle2 + TN; $^{\triangle}P < 0.05$ vs. Model + Vehicle2 + TN; $^{\#\#}P < 0.01$ vs. Model + Vehicle1 + TN; $^{\#}P < 0.05$ vs. Model + Vehicle1 + TN.

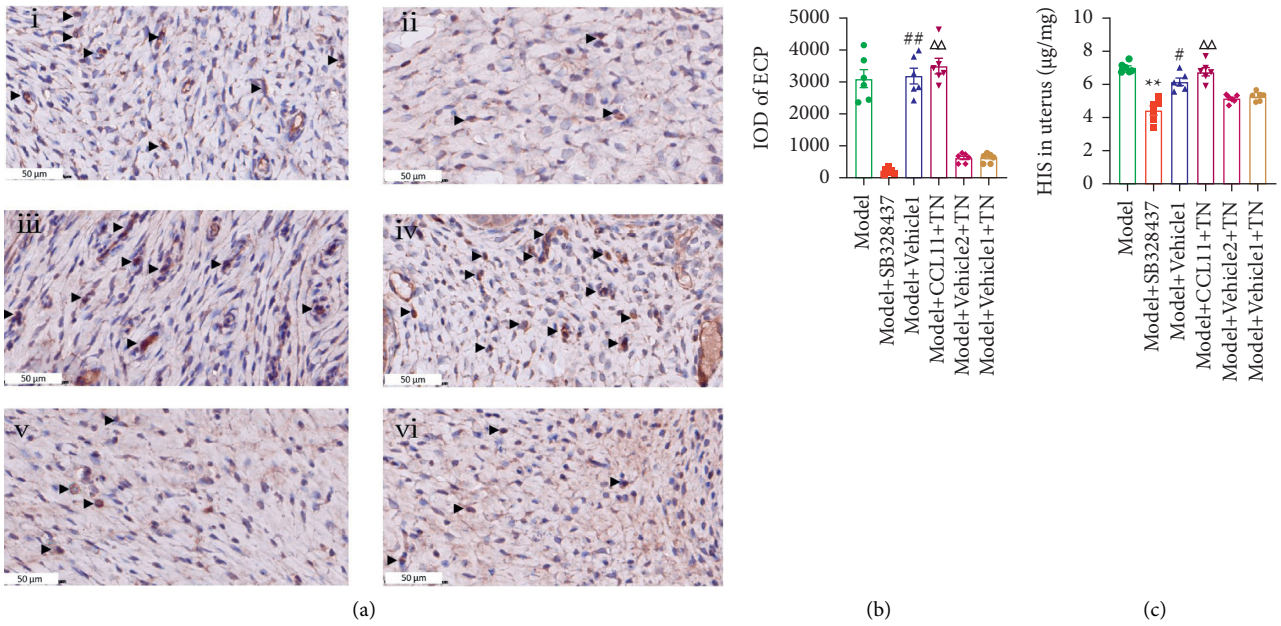


Figure 8: CCR3 agonist abolished the effect of TN against CCD-induced increased ECP expression and HIS level. (a) The representative photomicrographs of ECP immunohistochemical staining of all groups in the rat uterus. (i–vi) Model, Model + SB328437, Model + Vehicle1, Model + CCL11 + TN, Model + Vehicle2 + TN, and Model + Vehicle1 + TN. (b) Histogram of IOD value of ECP in the uterus of CCD rats in each group. (c) The level of HIS in uterus was detected by ELISA. The regions are photographs at 400x magnification. The arrows indicate a positive expression of ECP. All data are expressed as the means ± SEM ($n = 6$ rats/group). (b) The Tamhane T2 post hoc test was used. (c) One-way ANOVA followed by the Bonferroni post hoc test was used. $^{**}P < 0.01$ vs. Model group; $^{\triangle\triangle}P < 0.01$ vs. Model + Viecle2 + TN; $^{\#\#}P < 0.01$ vs. Model + Viecle1 + TN; $^{\#}P < 0.05$ vs. Model + Viecle1 + TN.

vascular smooth muscle was induced by HIS through H1R [54]. A study showed that both MCs' stabilizer group and H1R antagonist group could inhibit uterine contraction [55]. Furthermore, H1R gene knockout mice had lower pain sensitivity and higher pain threshold than normal mice [56]. Similarly, our research indicated that rats showed obvious writhing response and increased uterine contractility after modeling along with the increased HIS level and H1R

expression, while pain behaviors and uterine contractility were relieved accompanied by decreased HIS level and H1R expression after acupuncture.

Although eotaxin is important in dysmenorrhea, the mechanism remains unclear. CC chemokine receptor 3 (CCR3), a 7-transmembrane-domain G protein-coupled receptor, is expressed selectively in EOS, making it an excellent candidate for an eotaxin receptor [45, 57]. Previous researches showed that the eotaxin/CCR3 axis is a key regulatory pathway for EOS recruitment and migration to inflammatory tissues [45, 58, 59]. Allergen-induced airway EOS recruitment was almost eliminated in CCR3-deficient and eotaxin knockout mice (70%) [60]. Simultaneously, another research indicated that a CCR3 antagonist impaired EOS migration in the rat uterus [36]. What's more, acupuncture showed positive results of relieving asthma and allergic rhinitis via reducing eotaxin secretion [24, 25]. In our study, the expression of CCR3 was significantly increased in CCD rats, but TN reversed the change. Moreover, our present study also found that the effects of TN against CCD-induced menstrual pain, increased ECP expression, and HIS level could be blocked by CCR3 agonist CCL11, which further provided a strong support for our conclusion that eotaxin exerted its effect in menstrual pain at least partly through the activation of CCR3. To prove it further, CCR3 antagonist SB328437 could simulate the effect of TN, significantly ameliorating the uterine contraction and reducing ECP expression and HIS level in CCD rats.

There are some limitations in our study. First, the local signal amplification and transmission caused by TN and PN at SP6 as well as the regulation of central neuroendocrine system on target organ and related molecules should be further improved. Second, changes of classical inflammatory factors in uterine inflammatory microenvironment induced by acupuncture, especially those related to EOS and MC, deserve further observation.

5. Conclusion

This study reported a preferable effect of acupuncture at SP6 with TN on pain relief in CCD rats, which was partly achieved by weakening EOS-MC activation and HIS release via inactivation of eotaxin and CCR3. Accordingly, our data support that acupuncture as a potentially promising therapeutic approach for the treatment of primary dysmenorrhea, meanwhile, indicating the importance of performing appropriate needling technique.

Data Availability

All data in the current study are available from the corresponding authors on reasonable request.

Conflicts of Interest

The authors declare no conflicts of interest in this work.

Authors' Contributions

Liangxiao Ma conceived the study design and supervised the paper. Wenyan Yu drafted the manuscript. Wenyan Yu and Yuan Tian conducted the experiments. Jiedan Mu and Zhou Zhang contributed to the analysis and interpretation of data. Tianyi Sun, Xu Qian, and Junxiang Wang assisted during the experimentation and manuscript preparation. All authors have read and approved the final version of the manuscript.

Acknowledgments

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Review Article

Progress on the Experimental Research of Sciatic Nerve Injury with Acupuncture

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Objective. To collect and summarize relevant literatures on the experimental researches of sciatic nerve injury (SNI) with acupuncture during the last decade providing a guideline for effectively treating SNI with acupuncture in the future. *Methods*. The Chinese and English databases including China National Knowledge Infrastructure (CNKI), Wanfang Data Knowledge Service Platform (WanFang Data), VIP Information Chinese Journal Service Platform (VIP Date), and PubMed were searched from 2009 to 2020 with keywords of "acupuncture and moxibustion OR acupuncture OR electroacupuncture OR scalp acupuncture OR wrist-ankle acupuncture OR acupoint injection OR ear acupuncture" AND "sciatic nerve OR sciatic nerve injury OR sciatic injury OR SNI." The collected data were mainly evaluated in the items of animal model of SNI, type of interventions, selection of acupuncture points (acupoints), course of treatment and its frequency, and approaches of assessment. *Results*. A total of 89 studies were included in this analysis. Among them, the most commonly used animal models of SNI were produced by the clamp or transverse injury in the rats; the most frequently used intervention was electroacupuncture with dilatational wave of 2/100 Hz; the frequency of acupuncture was mainly performed once per day lasting for more than 2 weeks; the mainly selected acupoints were Huantiao (GB30), Zusanli (ST36), and Yanglingquan (GB34); and the approaches of assessment were contained with behavioral, functional, morphological, histological, cellular, and molecular measurements. *Conclusion*. The results indicated that the experimental researches of SNI with acupuncture has made marked progress in recent years, which may provide important clues for further investigating the underlying mechanisms of acupuncture for the treatment of SNI in the future.

1. Introduction

Sciatic nerve injury (SNI), as a typical type of peripheral nerve injury, is a common disorder to meet the acupuncture treatment [1–4]. Usually, SNI might be caused by incidents such as trauma, crush injury, sharp instrument attacking, drug injection, pelvic fracture, and hip dislocation, manifesting with sensory dysfunction such as pain, numbness, and loss of sensation, as well as locomotor dysfunction such as muscular atrophy, muscular tone decreasing, and limb paralysis [1, 5–7]. Although SNI is not lethal, it can cause long-term functional deficits in patients [8, 9].

Up to now, in order to treat SNI, many efforts have been made in clinical practices, including the transplantation of neural tissues and stem cells, the administration of neurotrophic medicine and nerve growth factor, as well as physical and laser therapies [10, 11]. Although all of them had certain curative effects on the SNI, considering their side effects and relatively high costs, some of the patients also accepted acupuncture as a complementary or alternative choice and obtained satisfied effects from treatment [12–14]. However, the underlying mechanism of acupuncture for meliorating SNI still remains unclear.

In order to tackle this problem, a large number of experimental researches on SNI with acupuncture has been carried out and reported separately in the last decade [15–17]. In this study, we collected the relevant literatures in this field from the Chinese and English databases with correlated keywords and evaluated them in the items of the animal model of SNI, type of interventions, selection of acupuncture points (acupoints), course of treatment and its frequency, and approaches of assessment, respectively. By analyzing these data, we expect to summarize the progress on the experimental research of SNI with acupuncture and provide a clue for further investigating its underlying mechanism associated with the clinical application.

2. Materials and Methods

2.1. Criteria for Study Inclusion and Exclusion

(1) Type of studies: the original studies related to the experimental research of acupuncture for SNI were included. However, the clinical trials, experience introductions, reviews, and other relevant literatures would be excluded.

(2) Type of research object: rat, rabbit, mouse, cat, monkey, dog, etc., no limitation on animal species and gender.

(3) Type of intervention: any type of acupuncture was included, including acupuncture, electroacupuncture, scalp acupuncture, ear acupuncture, wrist-ankle acupuncture, and acupoint injections.

(4) Types of outcomes: the animal model of SNI, type of interventions, selection of acupoints, course of treatment and its frequency, and approaches of assessment were mainly analyzed.

2.2. Search Strategy. Taking "acupuncture and moxibustion OR acupuncture OR electroacupuncture OR scalp acupuncture OR wrist-ankle acupuncture OR ear acupuncture OR acupoint injection" AND "sciatic nerve OR sciatic nerve injury OR sciatic injury OR SNI" as the keywords searched for the literature related to acupuncture intervention for SNI on the Chinese and English databases, including China National Knowledge Infrastructure (CNKI, 2009–2020), Wanfang Data Knowledge Service Platform (WanFang Data, 2009–2020), VIP Information Chinese Journal Service Platform (VIP Date, 2009–2020), and PubMed (2009–2020).

2.3. Data Extraction and Analysis. Two reviewers (Hui Wang and Jia Wang) independently used a predesigned data extraction form for rigorous data collection, including general information such as authors of studies, year of publication, and animal model of SNI, as well as treatment information including type of intervention, selection of acupoints, course of treatment and its frequency, and approaches of assessment. Finally, it would be systematically summarized and analyzed.

3. Results

3.1. Study Selection. Through electronic searching, 10795, 13746, and 8562 records in Chinese were identified from CNKI, Wanfang, and VIP Date databases, respectively, and 166 records in English were found from PubMed. By eliminating studies that were irrelevant, repetitive, and unable to obtain the full text, there were totally 89 studies included in accordance with the inclusion and exclusion criteria. Among these, there were 83 studies published in Chinese and 6 studies in English.

3.2. Characteristics of Included Studies

3.2.1. Annual Publication. During the past twelve years (2009–2020), there has been an overall upward trend in the number of annual publications on acupuncture treatment for SNI. Counting by the number of publications in a two-year interval, it was observed that the highest number of publications appeared in 2019–2020 with 29 studies, followed by 2015–2016 with 22 studies (Figure 1).

3.2.2. Animal Species. In the 89 included studies, the animals used for preparing the SNI model included rats, mice, and rabbits. Among them, the rat was by far the most commonly employed animal model in SNI experimental research, with the rabbit being the second most popular model, accounting for 84.3% (75/89) and 14.6% (13/89), respectively. There was only one study that applied to the mouse (Figure 2).

3.2.3. Animal Model of SNI. By summarizing the included studies, the animal models of SNI were established by many methods, including transverse, clamp, ligation, or injection injury to sciatic nerve [18–21]. Among them, the model with clamp injury was the most frequently used, and the model with transverse injury was the second most applied, with 61 (61/89, 68.5%) and 19 studies (19/89, 21.3%), respectively.

3.3. Application Characteristics of Acupuncture on SNI

3.3.1. Type of Interventions. Among the 89 included studies, the types of interventions mainly included electroacupuncture, manual acupuncture, ankle acupuncture, and scalp acupuncture, accounting for 93.3% (83/89), 4.5% (4/89), 1.1% (1/89), and 1.1% (1/89), respectively (Figure 3). In some of the included studies, multiple interventions of acupuncture were simultaneously used.

In the 83 studies of electroacupuncture, besides 7 studies that did not clearly indicate the stimulated parameters, the other 76 studies marked the definite stimulated styles, in which the waveforms of electroacupuncture were used orderly with dilatational wave (64.5%, 49/76), sparse wave (19.7%, 15/76), continuous wave (11.8%, 9/76), and intermittent wave (3.9%, 3/76). The frequencies of electroacupuncture were applied orderly with 2/100 Hz (64.5%, 49/76), < 10 Hz (19.7%, 15/76), 15 Hz (9.2%, 7/76), and

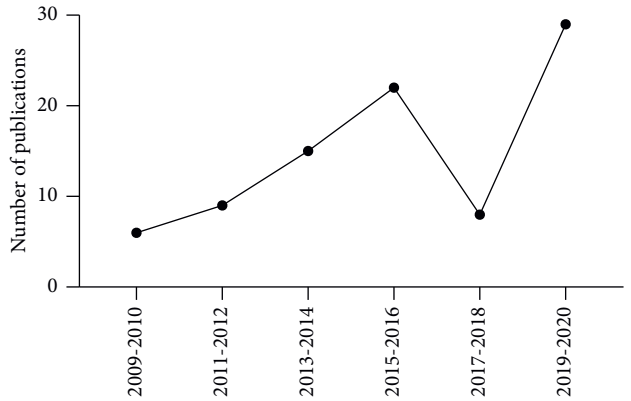

Figure 1: Publication status of experimental research of SNI with acupuncture during 2009–2020.

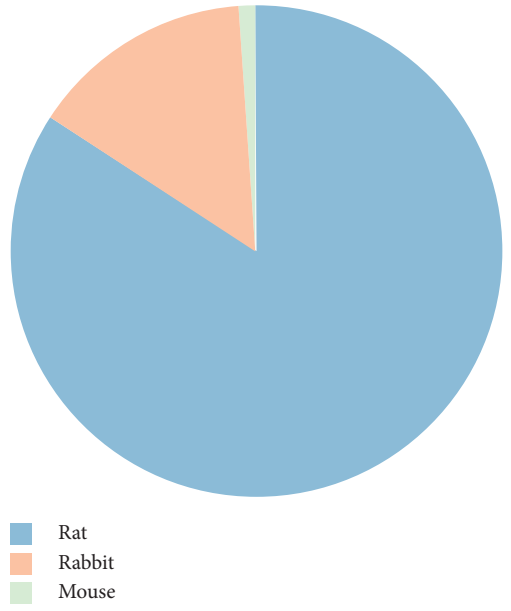

Figure 2: Animal application status in experimental research of SNI with acupuncture.

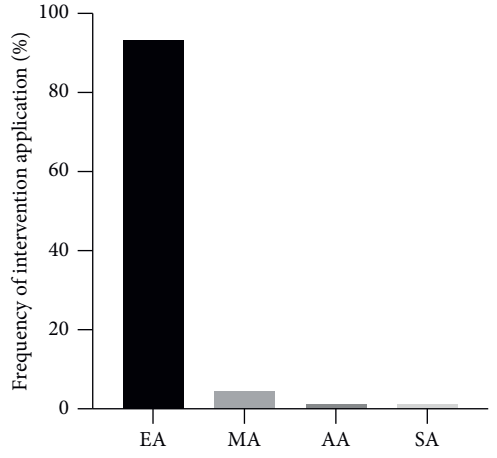

Figure 3: The intervention application status in experimental research of SNI with acupuncture. Note. EA: electroacupuncture; MA: manual acupuncture; AA: ankle acupuncture; SA: scalp acupuncture.

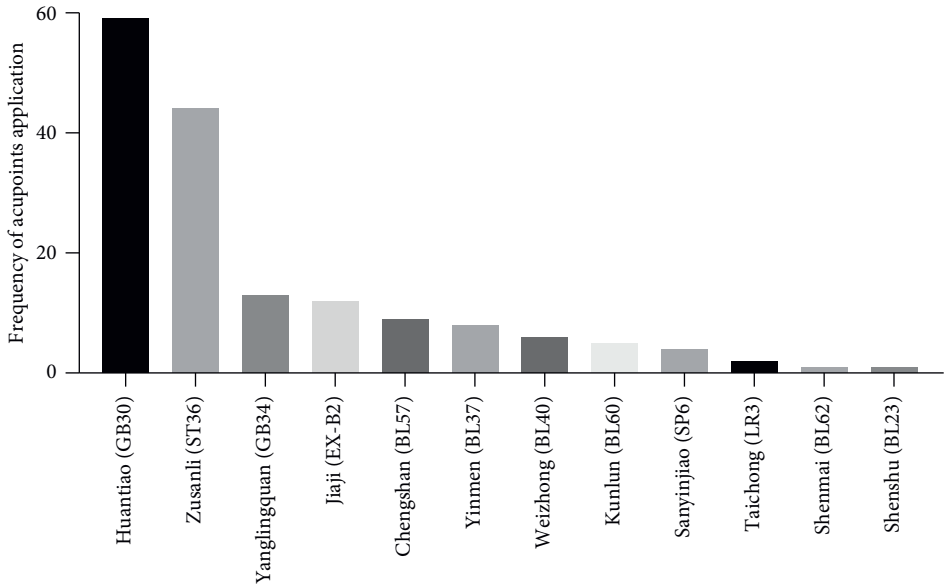

Figure 4: The acupoint selection in experimental research of SNI with acupuncture.

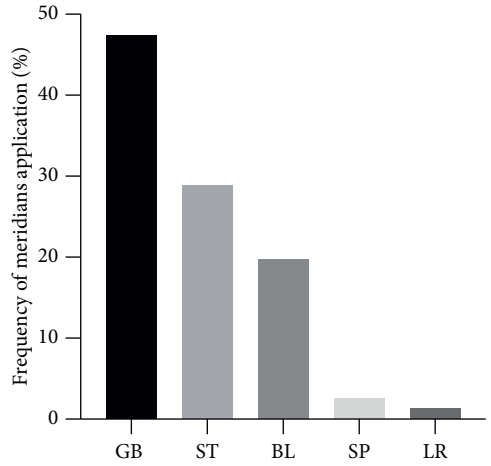

Figure 5: The meridians selection in experimental research of SNI with acupuncture. Note. GB: gallbladder meridian; ST: stomach meridian; BL: bladder meridian; SP: spleen meridian; LR: liver meridian.

20 Hz (6.6%, 5/76), and the electric current and voltage were used to cause mild muscle tremors (84.2%, 64/76), 1.5 mA (3.9%, 3/76), 2 mA (3.9%, 3/76), 20 mV (3.9%, 3/76), and 1.5 V (3.9%, 3/76). In addition, the time of treatment was varied from 9 min to 30 min in 80 studies in order of 20 min (36.25%, 29/80), 10 min (31.25%, 25/80), 15 min (25.0%, 20/80), 30 min (3.75%, 3/80), and 9 min (3.75%, 3/80).

3.3.2. Selection of Acupoints. A total of 13 acupoints appeared in the 89 studies, in which Huantiao (GB30, 59/89, 66.3%) and Zusanli (ST36, 44/89, 49.4%) appeared most frequently, followed with Yanglingquan (GB34, 13/89, 14.6%) and Jiaji (EX-B2, 12/89, 13.5%) (Figure 4).

Except Jiaji, the other acupoints mainly belong to the 5 meridians of the gallbladder (GB), stomach (ST), bladder (BL), spleen (SP), and liver (LR) meridians (Figure 5).

3.3.3. Treatment Frequency and Course. In the included studies, only one study did not mention the frequency of acupuncture. Among the other 88 studies, most studies applied the frequency of once per day (86/88, 97.7%). Only 2 studies applied once in every 2 days (2/88, 2.3%).

Regarding the treatment course, many studies performed acupuncture for more than 2 weeks (56.2%, 50/89), while another 33 studies performed for 1–2 weeks (37.1%, 33/83), and 6 studies performed for less than 1 week (6.7%, 6/89).

3.4. Analysis Methods Related to SNI with Acupuncture

3.4.1. Behavioral Measurement. Among the total included studies, 52 studies applied the behavioral measurements, including sciatic nerve function index (SFI), pain threshold,

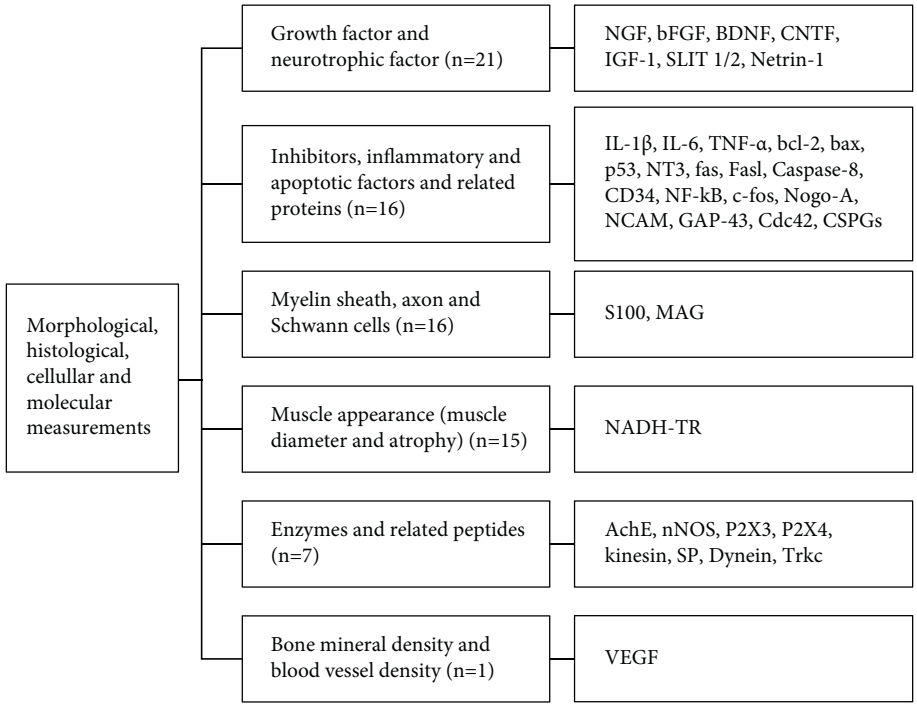

Figure 6: The detailed measurements and indicators in the experimental research of SNI with acupuncture. Note: AchE: acetylcholinesterase; bcl-2: b cell lymphoma-2; BDNF: brain-derived neurotrophic factor; bFGF: basic fibroblast growth factor; Cdc42: cell division cycle 42; CNTF: ciliary neurotrophic factor; CSPGs: chondroitin sulfate proteoglycans; GAP-43: growth-associated protein-43; IGF-1: insulin-like growth factor 1; IL-1β: interleukin-1 beta; IL-6: interleukin 6; MAG: myelin-associated glycoprotein; NADH-TR: nicotinomide adenine dinucleotide dehydrogenase-tetrazolium reductase; NCAM: neural cell adhesion molecule; NF-kB: nuclear factor kB; NGF: nerve growth factor; NT3: neurotrophin-3; nNOS: neuronal nitric oxide synthase; SLIT 1/2: slit guidance ligand 1/2; SP: substance P; TNF-α: tumor necrosis factor-α; Trkc: tropomyosin receptor kinase C; VEGF: vascular endothelial growth factor.

toe abduction reflex, and inclined pulling test. Among them, SFI was the most widely used (57.7%, 30/52), followed by the measurement of thermal pain threshold (30.8%, 16/52). In addition, 15.6% (5/52) and 1.9% (1/52) of studies applied the toe abduction reflex and inclined pulling test, respectively.

3.4.2. Functional Assessment. There were 33 studies that applied the functional assessment, including nerve and muscle function assessments. Among them, 30 studies mainly measured the sciatic nerve function as well as sensory and motor nerve conduction velocity. Another 3 studies measured electromyography and muscle contractility.

3.4.3. Morphological, Histological, Cellular, and Molecular Measurements. The approaches of assessments related to the effects and mechanisms of SNI with acupuncture mainly included morphological, histological, cellular, and molecular measurements. There were 58 studies that applied morphological and histological testing, and 44 studies used cellular and molecular testing. The spinal cord, dorsal root ganglia (DRG), sciatic nerve and its related muscles were the main tissues to be examined. The growth factor and neurotrophic factor were the most frequently tested indicators, with 21 studies applied. Besides, inhibitors inflammatory and apoptotic factors, and related proteins were commonly detected in 16 studies. Besides, there were 16 studies focused on the detection of the myelin sheath, axon, and Schwann cells of the sciatic nerve, and 15 studies focused on the muscle appearance (measurement of muscle diameter and atrophy). Additionally, there were 7 studies that paid attention to the testing of enzymes and related peptides, but only one study had done the observation on the bone mineral density and blood vessel density tests. The detailed description of the molecules or factors involved are displayed in Figure 6.

3.5. Observation on the Mechanism of SNI with Acupuncture. According to the summary of the research in the past twelve years, the current speculated relevant mechanisms of SNI with acupuncture may include the following: ① acupuncture could promote nerve regeneration and inhibit cell apoptosis by promoting the expression of nerve growth factor in SNI [22–24]; ② acupuncture could reduce the expression of inflammatory factors and increase the content of related proteins to promote the recovery of SNI [25, 26]; and ③ acupuncture could promote the proliferation of Schwann cells to the injured sciatic nerve to protect the nerve [27–29]. However, the research on the mechanism was mostly scattered, and they still could not represent the exact mechanism of acupuncture for SNI.

Table 1: Summary table showing the application characteristics of acupuncture on SNI.

Treatment strategy	Selected acupoints	Treatment frequency	Treatment course	Stimulation parameters of EA				Analysis methods	Detailed molecules or factors
				Waveform	Frequency	Current or voltage	Time of treatment		
EA (93.3%) MA (4.5%) AA (1.1%) SA (1.1%)	Huantiao (GB30, 66.3%) Zusanli (ST36, 49.4%) Yanglingquan (GB34, 14.6%) Jiaji (EX-B2, 13.5%), Chengshan (BL57, 10.1%), Yinmen (BL37, 9.0%), Weizhong (BL40, 6.7%), Kunlun (BL60, 5.6%), Sanyinjiao (SP6, 4.5%), Taichong (LR3, 2.2%), Shenmai (BL62, 1.1%), and Shenshu (BL23, 1.1%)	Once per day (97.7%); once every 2 days (2.3%)	> 2 W (56.2%); 1–2 W (37.1%); < 1 W (6.7%)	Dilatational wave (64.5%) Sparse wave (19.7%) Continuous wave (11.8%) Intermittent wave (3.9%)	2/100 Hz (64.5%); < 10 Hz (19.7%); 15 Hz (9.2%); 20 Hz (6.6%)	Cause mild muscle tremor (84.2%); 1.5 mA (3.9%) 2 mA (3.9%); 20 mV (3.9%); 1.5 V (3.9%)	20 min (36.25%); 10 min (31.25%); 15 min (25.0%); 30 min (3.75%); 9 min (3.75%)	① Behavioral measurement (SFI, pain threshold, toe abduction reflex, and inclined pulling test); ② Functional assessment (sensory and motor nerve conduction velocity, electromyography, and muscle contractility); ③ Morphological, histological, cellular, and molecular measurements	① Growth factor and neurotrophic factors (NGF, bFGF, BDNF, CNTF, IGF-1, SLIT 1/2, Netrin-1) ② Inhibitors, inflammatory, and apoptotic factors and related proteins (IL-1β, IL-6, TNF-α, bcl-2, bax, p53, NT3, fas, fasL, caspase-8, CD34, NF-kB, c-fos, nogo-a, NCAM, GAP-43, Cdc42, and CSPGs) ③ Muscle appearance (NADH-TR) ④ Enzymes and related peptides (AchE, nNOS, P2X3, P2X4, kinesin, SP, dynein, andTrkc) ⑤ Myelin sheath, axon, and Schwann cells (S100, MAG) ⑥ Bone mineral density and blood vessel density (VEGF)

Note. EA: electroacupuncture; MA: manual acupuncture; AA: ankle acupuncture; SA: scalp acupuncture; W: week; AchE: acetylcholinesterase; bcl-2: b cell lymphoma-2; BDNF: brain-derived neurotrophic factor; bFGF: basic fibroblast growth factor; Cdc42: cell division cycle 42; CNTF: ciliary neurotrophic factor; CSPGs: chondroitin sulfate proteoglycans; GAP-43: growth-associated protein-43; IGF-1: insulin-like growth factor 1; IL-1β: interleukin-1 beta; IL-6: interleukin 6; MAG: myelin-associated glycoprotein; NADH-TR: nicotinomide adenine dinucleotide dehydrogenase-tetrazolium reductase; NCAM: neural cell adhesion molecule; NF-kB: nuclear factor kB; NGF: nerve growth factor; NT3: neurotrophin-3; nNOS: neuronal nitric oxide synthase; SLIT 1/2: slit guidance ligand 1/2; SP: substance P; TNF-α: tumor necrosis factor-α; Trkc: tropomyosin receptor kinase C; VEGF: vascular endothelial growth factor.

4. Discussion

In this study, the data about the experimental research of SNI with acupuncture has been collected from 2009 to 2020 and summarized in the items of animal model of SNI, type of interventions, selection of acupoints, course of treatment and its frequency, as well as approaches of assessment (Table 1). During the past twelve years, research in this field has made marked progress, step by step, to understand the underlying mechanisms of why acupuncture can play an effective role in alleviating SNI.

It is well known that, from bench to bed, experimental research is an indispensable way to promote the development of clinical progress. Although animal models with different types of SNI have been introduced to experimental research, such as transverse, ligation, clamp, traction, freezing, or drug injuries to the sciatic nerve, it is still difficult to select an appropriate one for mimicking the clinical patients. Among these models, the model with clamp injury is widely used to evaluate the effect of acupuncture on neural recovery [15, 17, 19, 30], and the model with transverse injury is mainly applied to assess the effect of acupuncture on neural regeneration [31]. Besides these two types, the model with ligation injury is also used; however, it seems difficult to control the tightness of the ligation in unity [20, 32]. For the model with traction injury, it is also difficult to control the elongation of the sciatic nerve with two forceps between both ends of the nerve [33]. Additionally, the model with freezing injury is also applied. The problem is that the cryocooling probe not only damages the nerve, but also involves its surrounding tissues [33]. Therefore, considering the advantages and disadvantages of these animal models, the model with clamp injury is more suitable for investigating the underlying mechanism of acupuncture for meliorating the SNI.

In terms of the interventions for the SNI, electroacupuncture was recommended according to the collected data, which is consistent with the situation in clinical treatment. The possible reason might be that the parameters of stimulation with electroacupuncture can be controlled as demands on its strength and frequency [34]. Indeed, numerous studies have demonstrated that electroacupuncture has a good analgesic effect on various kinds of painful diseases [35, 36]. Here, it should be noted that although the 2/100 Hz dilatational wave is an appropriate choice for the animal models [37, 38] since the body size is different between small animals and humans, whether this parameter is suitable for the patients remains unanswered.

For the selection of acupoints, it is clear that, besides Jiaji (EX-B2) on the back, the other twelve acupoints are located closely or along the pathway of the sciatic nerve including its main trunk and its branches of the tibial, common peroneal, and sural nerves [39]. Traditionally, these acupoints belong to the meridians of the gallbladder (GB), stomach (ST), bladder (BL), spleen (SP), and liver (LR), respectively, and are orderly arranged along the longitudinal axis of the hind limb [39]. Although GB30 and ST36 are used most frequently, most acupoints belong to the bladder meridian including BL23, BL37, BL40, BL57, BL60, and BL62. Coincidently, the course of the bladder meridian as the traditional description is similar to the pathway of the sciatic nerve [39]. According to the spatial correlation between these acupoints and the sciatic nerve, they are the adjacent points to the sciatic nerve or its branches. In the clinical treatment of SNI, these acupoints can be used individually or together.

Although acupuncture treatment has obtained satisfactory effects on patients with SNI, due to the limitation of clinical studies, these effects are mainly evaluated on the variation of clinical symptoms and are seldom involved in their underlying mechanism [12–14]. As a comparison, experimental research has its own advantages in obtaining much more biological information from the perspective of pathologic histology. According to our collected data, most studies on the SNI with acupuncture were concentrated on neural protection and neural regeneration by way of anti-inflammation and antiapoptosis [22–27]. These studies suggested that acupuncture could promote nerve regeneration, inhibit cell apoptosis, reduce the expression of inflammatory factors, improve the microenvironment of the injured area, and promote the proliferation of Schwann cells to repair the injured sciatic nerve [22–27]. However, there is still an absence of high-quality studies in this field. Therefore, the definite mechanism associated with acupuncture treatment on the SNI still needs further investigation in the future. Recently, there has been a new trend in SNI studies. Some researchers have paid attention to the SNI from the peripheral nervous system to the central nervous system, including the sensory input to the spinal dorsal horns and motor output from the spinal ventral horns [25, 26, 40]. It might be a new direction for acupuncture research on the SNI in the future.

5. Conclusion

In summary, the experimental research on the SNI with acupuncture has made marked progress in recent years, providing rich evidence for insight into the underlying mechanisms of acupuncture and how to alleviate the SNI. These experimental data are not only beneficial to future studies in this field but also for selecting appropriate acupoints and stimulating styles as clinical demands with SNI.

Data Availability

The datasets used for the current study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

The article presented here was carried out in collaboration between all the authors. Hui Wang, Jia Wang, Jingjing Cui, Shitong Zhao, Dongsheng Xu, and Shuang Wu searched the literature and analyzed the data. Jia Wang and Waizhu Bai designed the study. Hui Wang, Jia Wang, and Wanzhu Bai wrote the article. All authors read and approved the final version of the manuscript.

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Research Article

Electroacupuncture Alleviates Hyperalgesia by Regulating CB1 Receptor of Spinal Cord in Incisional Neck Pain Rats

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Acupuncture therapy is effective in relieving postoperative pain of neck surgery, but its underlying mechanisms remain largely unknown. This study, in the incisional neck pain rat model, was designed to explore whether the endocannabinoid receptor 1 (CB1) in the cervical spinal cord is involved in the analgesic effect of electroacupuncture (EA) or not.The incisional neck pain model was established by making a longitudinal incision and applied EA treatment of Futu (LI18), Hegu-Neiguan (LI4-PC6), or Zusanli-Yanglingquan (ST36-GB34) for pain relief. The results showed that EA LI18 and EA LI4-PC6 effectively relieve allodynia caused by neck incision, which was obviously better than EA ST34-GB34 ($P < 0.05$). After EA, the expression levels of CB1 mRNA at 4h in the EALI18 group, and 24 and 48h in both EALI18 and EALI4-PC6 groups, and those of CB1 protein at 4, 24, and 48h in the EALI18 group, and the immunoactivity of CB1 in both EALI18 and EALI4-PC6 groups at 4h were significantly upregulated in contrast to those of the model group ($P < 0.05$). EA of either acupoint group had no effect on the expression of CB2 protein ($P > 0.05$). Moreover, the antinociceptive effect of EA was reversed by AM251 (CB1 antagonist). Immunofluorescence dual staining showed that CB1 expressed in astrocytes in the superficial layer (laminae I and II) of dorsal horns of the cervical spinal cord. Therefore, the findings of this study revealed that upregulation of CB1 expression in the cervical spinal cord contributes to the analgesic effect of EA in incisional neck pain rats. The CB1 receptor expresses on astrocytes.

1. Introduction

Postsurgical pain is a common acute pain and frequently seen in many common operations, such as thyroid surgery, inguinal hernia repair, and breast and chest surgery. About 86% of the surgery patients suffer from moderate to severe postoperative pain [1], and more than 10–50% of them may experience persistent postoperative pain [2], seriously affecting their daily life quality. Therefore, more and more attention has been paid to effective postoperative management, but the existing anesthetics or analgesics have obvious limitations and adverse reactions, including tolerance, dependence, gastrointestinal dysfunction, cognitive impairment, narrow treatment window, and patients' low satisfaction with analgesics [3, 4]. As a result, more and more doctors and patients seek nonpharmaceutical treatment to relieve the pain. A large number of studies have shown that electroacupuncture (EA) can significantly relieve postsurgical pain, with fewer side effects [5–11], but its mechanism is still unclear.

The endocannabinoid system, consisting of two main ligands N-arachidonoylethanolamine (AEA) and 2-arachidonoylglycerol (2-AG), which are considered to be the main endogenous ligands of the two corresponding receptors: cannabinoid receptor 1 (CB1) and cannabinoid receptor 2 (CB2), is one of the key endogenous systems for processing pain sensation. The CB1 is the prominent subtype in the central nervous system (CNS), including spinal cord, thalamus, periaqueductal grey (PAG), amygdala, and rostroventromedial medulla [12, 13] and also exists in the dorsal root ganglion, at presynaptic sites throughout the peripheral system and CNS [14], whereas CB2 is found principally (but not exclusively) on immune cells. Both of them participate in

nociceptive processing [14]. In the spinal dorsal horns (DHs), CB1 is found to express on microglia, oligodendrocytes, astrocytes, and interneurons [15, 16]. Intrathecal injection of CB1 antagonist caused obvious hyperalgesia in mice [17] and enhanced electric stimulation c-fiber induced discharge of neurons in the spinal dorsal horns (DHs) [18]. In incisional paw pain rats, the spinal anandamide level was evidently decreased at the early time, being consistent with the maximum mechanical hypersensitivity, and restored to the baseline level as the noxious behavior subdued. Administration of CB1 and CB2 antagonists blocked the reduction of postsurgical allodynia and caused persistent overexpression of glial fibrillary acidic protein (GFAP) and p-p38 in astrocytes [19]. Recent studies have shown that astroglial CB1 receptors control synaptic transmission and plasticity [20]. CB1 receptors in astrocytes may be aslo involved in the anti-hyperalgesic action of exogenous cannabinoids [21].

It has been demonstrated that EA has both analgesic and anti-inflammation effects via regulating the endocannabinoid system, including activation of CB1 and CB2, and also can reduce the adverse reactions caused by exogenous administration of cannabinoid [22]. The expression levels of CB1 and CB2 genes were upregulated 6 hours after EA treatment in temporomandibular arthritis rats [23]. Application of CB1 selective antagonist AM251 significantly reversed the analgesic effect of EA, and application of CB2 selective antagonist AM630 significantly reversed the anti-inflammatory effect of EA [23]. Activation of spinal CB1 and upregulation of spinal CB1-ERK1/2 signaling could enhance the antinociceptive effect of EA in morphine-induced hyperalgesia rats [24]. Although these outcomes have detailed the functions of CB1 and CB2 in mediating the analgesic effect of EA, their association with the effect of EA in inducing the reduction of incisional neck pain (INP) has not been reported. Our past studies displayed that EA Futu (LI18) could suppress neck incision-induced thermal hyperalgesia which may be related to its functions in upregulating the expression levels of GABA and its receptors [6] and downregulating P2X7R/fractalkine/CX3CR1 signaling [25] SP/NK1, COX-1, and PGE2 levels [26] in the cervicospinal DHs. Immunofluorescence double staining showed that GABA was expressed on astrocytes and neurons, and GABA-B was expressed only on neurons [6]. However, it was reported that the activated CB1 and GABA have a role in dorsal horn pain-controlling circuits, and spinal antinociceptive effects of GABA(B) receptor agonists are likely through endocannabinoid modulation [27, 28].

Therefore, this study was designed to select the appropriate acupoint with the best analgesic efficacy for INP, then, to observe the dynamic effect of EA on the expression of CB1 mRNA and protein in DHs of the cervical spinal cord, and at last, to verify the effect of CB1 in mediating EA-induced analgesic effect by intrathecal and intraperitoneal administration of CB1 antagonist.

2. Method

2.1. Ethical Statement. All experimental protocols and animals' treatment were approved by the Animal Welfare and Use Ethics Committee of the Institute of Acupuncture and Moxibustion, China Academy of Medical Sciences (Approval Number: 20140014); all experimental methods were conducted in accordance with the welfare ethics and "3R" principles of laboratory animals and conformed to the Guidelines for the Care and Use of Laboratory Animals made by the National Institutes of Health (NIH Publication No.85-23, revised in 1985).

2.2. Animals and Grouping. A total of 316 male Sprague-Dawley (SD) rats (200–220 g in weight), purchased from the Experimental Animal Center of China Academy of Medical Sciences (License No.: SCXK (Beijing) 2014-0013), were housed in the standard animal room with $22 \pm 2^{\circ}$C and 12 h : 12 h of the light-dark cycle. The rats were given free access to food and water and acclimatized to the laboratory conditions for 7 days before the experiment.

These rats were randomly divided (by using a random digital table) into 5 groups, control, model, EA Futu (LI 18), EA Hegu (LI 4)-Neiguan (PC 6) group, and EA Zusanli (ST 36)-Yanglingquan (GB 34), which were further randomized into three subgroups: 4 (with 16 rats in each subgroup), 24(with 21 rats in each subgroup), and 48(with 16 rats in each subgroup). In order to verify the role of CB1 receptor in mediating EA analgesia, the rats were randomly divided into two groups, DMSO + EA and CB1 antagonist (AM251) + EA, which were further randomized into two subgroups: intraperitoneal injection(I.P.) and intrathecal injection (I.T.), with 12 rats in each subgroup. Three rats were excluded due to dyskinesia.

2.3. Establishment of Incisional Neck Pain Model. The day before the operation, the rat's neck hair was removed using appropriate amount of depilatory cream. Mild anesthesia was performed with isoflurane (0.5–1% oxygen) inhaled from the nasal cone using a desktop animal anesthesia ventilator system (VME, Matrix Company, USA). According to our previous study [26], the rat's baseline thermal pain threshold was detected first, and then, a 1.5 cm longitudinal incision was made along the midline of the neck, followed by repeated blunt dissection stimulation along the bilateral sternohyoideus around the thyroid gland regions for 30 min with a pair of forceps. The incision was then sutured in layers with a piece of 4.0 surgical catgut. After surgery, the rats were placed back to the cages for recovery. Rats in the sham operation (control) group were similarly anesthetized, but without incision and dissection stimulation. The neck incisional pain models were measured by the thermal pain threshold as our previous study [26].

2.4. Electroacupuncture Intervention. According to the graphical representation and word description of rat acupoints and human acupoints in book "*Experimental Acupuncture and Moxibustion*" [29], Futu (LI 18) is located on the lateral side of neck, between the sternal head and clavicular head of the sternocleidomastoideus, at the level of the 4th cervical vertebra, Hegu (LI 4) is located between the 1st and 2nd metacarpals of the forelimb; Neiguan (PC 6) is

located on the ventral side of the forelimb, about 3 mm from the transverse crease axis of wrist, between the ulnar and radius; Zusanli (ST 36) is located on the lateral side of the knee joint of the hind limb, 5 mm below the head of fibula; and Yanglingquan (GB 34) is located 5 mm superior lateral to Zusanli (ST 36).

Under anesthesia with 1.5% isoflurane, the rats of the three EA groups received the insertion of acupuncture needles (No. 32, Suzhou Acupuncture Products Co., Ltd, China) into bilateral LI18, LI4, PC6, or bilateral ST36 and GB34, to a depth of about 2–4 mm. After needle insertion, the needle handles were connected to the output terminals of the HANS apparatus (HANS-200A, Jisheng Medical Technology, Co., Ltd., China) for stimulating the acupoints with a frequency of 2/100 Hz and intensity of 1 mA for 30 min during neck incision and 20 h and 44 h after incision.

2.5. Measurement of Thermal Pain Threshold. The rats in each group were wrapped in special cloth bags, with their necks exposed. The thermal pain threshold of the neck incision area was measured with Tail Flick (37360, UGO Basile, Italy) before operation, and 4 and 24 h after surgery. A radiant heat lamp was focused on the center of the neck incision area; when the rat rapidly removed its neck from the heat source, it was recorded as the thermal pain threshold (TPT, i.e., neck withdrawal latency, NWL). Each rat was tested 3 times with an interval of about 5–10 min, and the average value was taken. The researcher who analyzed the TPT results was unaware of the grouping and not involved in the acupuncture process either.

2.6. Intrathecal Tube Implantation. Intrathecal tube surgery was performed under inhalational isoflurane (1–2% oxygen) anesthesia. The lumbar vertebrae (L5-L6) were exposed. A polyethylene (PE) 10 catheter (OD 0.61 mm, ID 0.28 mm, Smiths Medical, UK), prefilled with 0.9% sterile saline, was inserted into the narrow space between L5 and L6. According to the improved catheterization without laminectomy described previously [30], the PE catheter was advanced cephalically about 7.5 cm into the subarachnoid space of cervical vertebrae C2-C5. The local muscle and skin were sutured in layers with a piece of surgical catgut. The outside end of the catheter, exposed for about 2-3 cm and closed with a cautery pen, was embedded into the muscle layer and fixed. The rats were allowed to have a recovery period of 5–7 days before the next experimental procedure. At the end of the experiment, the position of the catheter was verified by lidocaine injection. Only those rats with transient forelimb paresis after lidocaine injection were included. Five days after surgery, the Salzman scale [31] was used to detect the hind limb motor function in each group, and 3 rats with a score lower than 6 points were excluded due to motor function defect.

2.7. Drug Administration. One week after intrathecal tube implantation, the rats were anesthetized with isoflurane and received an intrathecal injection of 10 μL CB1 antagonist AM251 solution (10 mg/mL, dissolved in DMSO) or 10 μL DMSO ($n = 12$/group), once a day for 5 days before the incision operation. Other 24 rats (without i.t. catheter) received an intraperitoneal injection of 200 μL CB1 antagonist AM251 at doses of 3 mg kg^{-1} of body weight (dissolved in DMSO) or 200 μL DMSO ($n = 12$/group), once daily for 5 days. All rats that underwent neck incision and bilateral Futu (LI 18) were stimulated by EA. The thermal hyperalgesia in the neck incision area was measured before and 4, 24, 48, and 72 hours after the operation.

2.8. Western Blot. Rats in each group were anesthetized by the administration of pentobarbital sodium (35 mg/kg weight, i.p.). The tissue sample of the cervical spinal cord (C2-C5) was taken out on ice and placed into a 1.5 m cryogenic microtube to be rapidly frozen in liquid nitrogen after semitransection and stored at −80°C till use. The frozen tissue sample (100 mg) was placed in the RIPAlysis buffer with protease inhibitor cocktail (Roche, Mannheim, Germany), and the target protein was extracted through proteolysis by using a tissue homogenizer. The concentration of the protein was detected using the bicinchoninic acid (BCA) method with a bovine serum albumin standard. The same amount of protein samples were separated in 5%, 12%, and 15% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a polyvinylidene difluoride (PVDF) membrane (0.2 or 0.45 μm, Millipore Corporation, Billerica, MA, USA). The membrane was blocked in 5% bovine serum albumin tris-buffered saline plus Twine (BSA-TBST) at room temperature (RT) for 1 h and then incubated with primary antibody: rabbit anti-CB1 (1 : 2000, ab137410, Abcam, UK), rabbit anti-CB2 (1 : 2000, ab3561, Abcam, UK), and mouse anti-β-actin (1 : 5000, YM3028, Immunoway, USA) at 4°C overnight. After washing with TBST 3 times, the membrane was incubated with horseradish peroxidase-conjugated goat anti-rabbit antibody (1 : 500, ab6721, Abcam, UK) and goat anti-mouse IgG (1 : 10,000, Jackson immunoresearch Laboratories, West Grove, PA, USA) at room temperature for 2 h. Standard X-ray film (Eastern Kodak Co., Rochester, NY, USA) was then used to show the immunoreactive bands through the enhanced chemiluminescence (Amersham Biosciences UK Limited, Buckinghamshire, England). The level of band intensity was quantified by using Image-Pro Plus software (Media Cybernetics, Silver Spring, MD). Band densities were normalized to individual β-actin internal controls.

2.9. Quantitative Real-Time PCR. Total RNA was extracted from the sample tissue of spinal dorsal portion (C2-C5) by using Trizol (Invitrogen, Carlsbad, CA). The reverse transcription of cDNA was obtained using the PrimeScript-TM Reagent Kit (Takara Bio, Shiga, Japan). The primers were designed by using primer 3.0 and synthesized by Sangon (Shanghai, China). The primer sequences are shown in Table 1. A fluorescence quantitative PCR reaction system was configured, and the tissue sample was added to each well of the 96-well plate and then placed onto the real-time quantitative fluorescence PCR system (ABI7500, Applied

Table 1: The primer sequences.

The primer	Sequences	Length (bp)
CB1	Forward, 5′-TCCACCGTGAACCCCATCATCTA-3′ reverse, 5′-GCTGTGTTGTTGGCGTGCTTGT-3′	194
GAPDH	Forward, 5′-TTCCTACCCCCAATGTATCCG-3′ reverse, 5′-CCACCCTGTTGCTGTAGCCATA-3′	270

Biosystems, USA), to construct the real-time PCR reaction system by using cDNA samples. PCR was performed under the following conditions: 95°C, 30 seconds; 40 PCR cycles (95°C, 5 seconds; 60°C, 40 seconds (to collect fluorescence), followed by 95°C, 10 seconds; 60°C, 60 seconds; 95°C, 15 seconds to establish the fusion curve of PCR products. The samples with target gene and reference gene were run on the same plate to avoid between-run variations, and each sample was tested for three complex holes. The relative expression of genes was calculated in accordance with the $2^{-\Delta\Delta CT}$ method.

2.10. Immunofluorescence Multiple Labeling. Under deep anesthesia with pentobarbital sodium (35 mg/kg weight, i.p.), the rats received a transcardiac perfusion with normal saline (250 mL) and then with 4% paraformaldehyde solution (200 mL) containing 1.5% picric acid in 0.1 M PB (pH 7.4). The cervical spinal cord segment (C2-C5) was removed and postfixed in the same fixative solution overnight and then dehydrated with 30% sucrose solution overnight. The spinal cord sample tissue was cut into sections (40 μm) by using a cryostat microtome (Thermo, Microm International GmbH, Germany). Free-floating tissue slices were cultured in primary antibody, mouse anti-GFAP (1 : 1000, 3670s, Cell Signaling Technology, USA) and rabbit anti-CB1 (1 : 1000, ab23703, Abcam, UK) at 4°C overnight and then cultured in secondary antibodies, donkey anti-mouse IgG antibody conjugated Alexa Fluor 488 (1 : 500, A21202, Life Technologies, USA) and donkey anti-rabbit IgG antibody conjugated Alexa Fluor 594 (1 : 500, A32754, Life Technologies, USA) at room temperature for 2 h. Finally, the slices were mounted onto glass slides and sealed with cover glasses. Three slices (within the dorsal horn) of each rat were photographed by using a multifunctional confocal microscope (Olympus, Tokyo, Japan). Five regions in the same part of the spinal DHs were randomly selected from a slice at the same magnification, and the immunofluorescence intensity of CB1 was measured by using NIS Elements software (Nikon, Tokyo, Japan). The mean intensity of five regions in each tissue slice (3 slices per rat) was calculated to be used as the fluorescence intensity value.

2.11. Statistical Analysis. All data were expressed as mean ± standard deviation (mean ± SD) and analyzed with SPSS20.0 statistical software. All data were subjected into the homogeneity test of variances first. Data of TPT were analyzed by two-way repeated measures analysis of variance (ANOVA), and data of quantitative real-time PCR and WB and immunofluorescence intensity were analyzed by one-way ANOVA, followed by LSD tests for comparison between two groups, or Student's *t*-tests. Statistical significance was defined as $P < 0.05$.

3. Result

3.1. Electroacupuncture Alleviates the Neck Incisional Pain. In order to observe the effect of EA on incisional neck pain, the TPT was measured before the neck incision and at 4, 24, and 48 h after the incision. The change rates of TPT = (TPT after neck incision − TPT before neck incision)/TPT before neck incision × 100%. Compared with the normal control group, the change rates of TPT in the model group were significantly reduced at 4, 24, and 48h ($P < 0.05$, Figure 1(b)), indicating an occurrence of hyperalgesia in the local incisional region. Compared with the level of 4h after neck incision, the change rates of TPT were increased gradually at 24 and 48 h. Compared with the model group, the change rates of TPT in the EA LI18 and EA LI4-PC6 groups were significantly increased at 24 and 48 h after incision ($P < 0.05$, Figure 1(b)), indicating that EA at LI18 and LI4-PC6 could effectively relieve the incisional neck pain. There was no significant difference between the model and EA ST36-GB34 groups in the TPT levels at the 3 time points ($P > 0.05$).

3.2. EA of LI18 Upregulates Expression of CB1 Protein. The protein expression levels of CB1 and CB2 in the spinal dorsal part at 4, 24, and 48 h after neck incision and after EA were observed, respectively. In comparison with the normal control group, the expression of CB1 protein only at 48 h after neck incision was significantly increased in the model group ($P < 0.05$, Figure 2(g)). Compared with the model group, the expression levels of CB1 protein at 4 and 24 h after neck incision were significantly increased in the EA LI18 group ($P < 0.05$, Figures 2(c) and 2(e)). No significant changes were found in the expression levels of CB1 at 4, 24, and 48 h in both the LI4-PC6 and EA GB36-GB34 groups in contrast to the model group ($P > 0.05$, Figures 2(c), 2(e), 2(g)), and such is the case in the expression levels of CB2 at the 3 time points after neck incision in both model and 3 EA groups ($P > 0.05$, Figures 2(d), 2(f), 2(h)).

Regarding the outcomes of QRT-PCR, the mRNA levels of CB1 in the cervical spinal dorsal portion were significantly downregulated at 24h after neck incision ($P < 0.05$, Figure 3(b)) but considerably upregulated at 48h in the model group in contrast to the control group ($P < 0.05$, Figure 3(c)). Compared with the model group, the CB1 mRNA expression levels at 4 h in the LI18 group and at 24 and 48 h in both EA LI18 and EALI4-PC6 groups were significantly increased ($P < 0.05$, Figures 3(a)–3(c)), whereas no significant changes were found at 4h in the EA LI4-PC6 group and at the three time points in the EA ST36-GB34 group ($P > 0.05$).

3.3. EA Upregulates Immunoactivity of CB1 Expressing in Astrocytes. Compared with the normal control group, the mean immunofluorescence intensity (immunoactivity) of

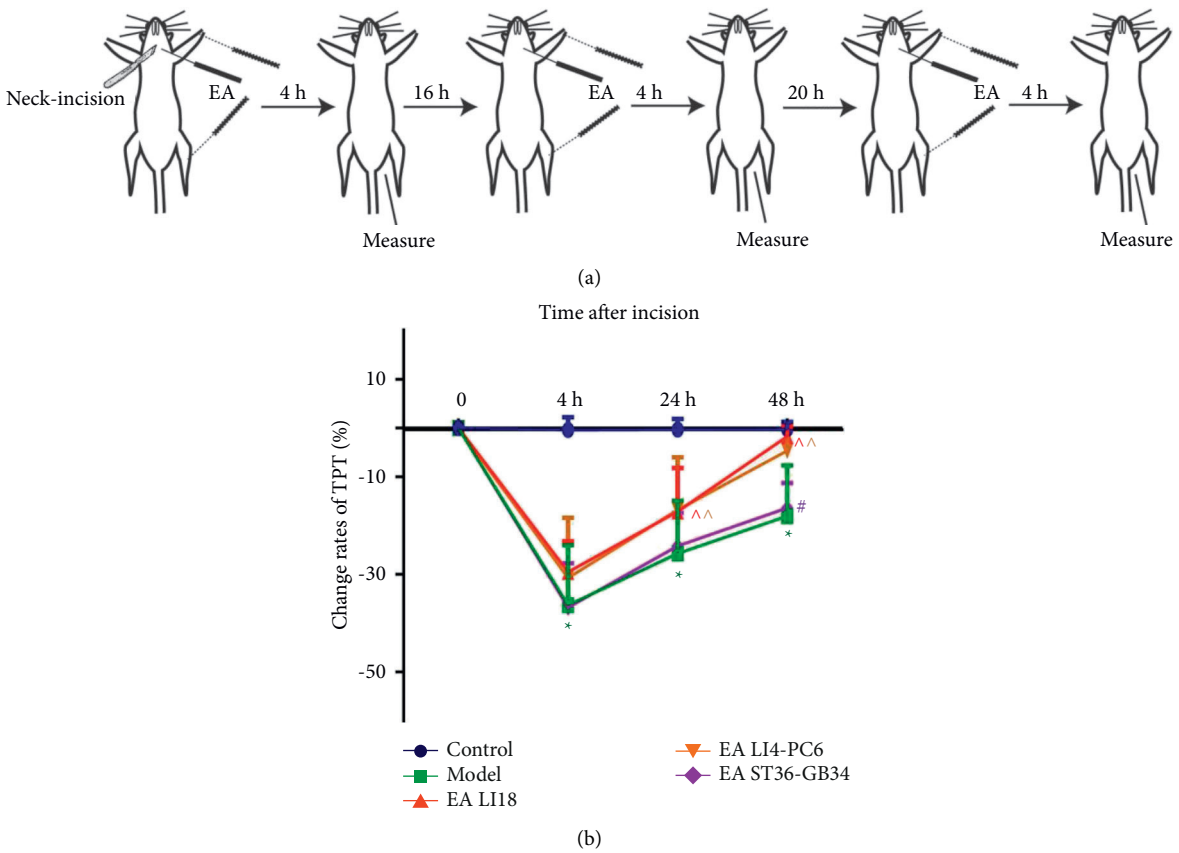

Figure 1: EA increases thermal pain threshold (TPT) in rats with neck incisional pain. (a) Schematic diagram of experimental procedures of EA intervention and TPT measurements: TPT was measured before neck incision, and 4, 24, and 48 h after EA intervention. (b) Histograms showing the effect of EA at Futu(LI18), Hegu (LI14)-Neiguan(PC6), and Zusanli(ST36)-Yanglingquan(GB34) on the change rate of TPT at different time points after modeling (mean ± SD, $n = 13$/group). The change rate of TPT (TPT%) = (TPT after neck incision − TPT before neck incision)/TPT before neck incision × 100%. Repeated measures ANOVA revealed that TPT% was significantly decreased from 4 to 48 h after modeling (vs the control group) and considerably increased at 24 and 48 h after EA of LI 18 and LI4-PC6, rather than EA of ST36-GB34 (vs the model group). $^{*}P < 0.05$, vs the control group, $^{\wedge}P < 0.05$, vs the model group, $^{\#}P < 0.05$, vs the EA LI18 group, all by the LSD test.

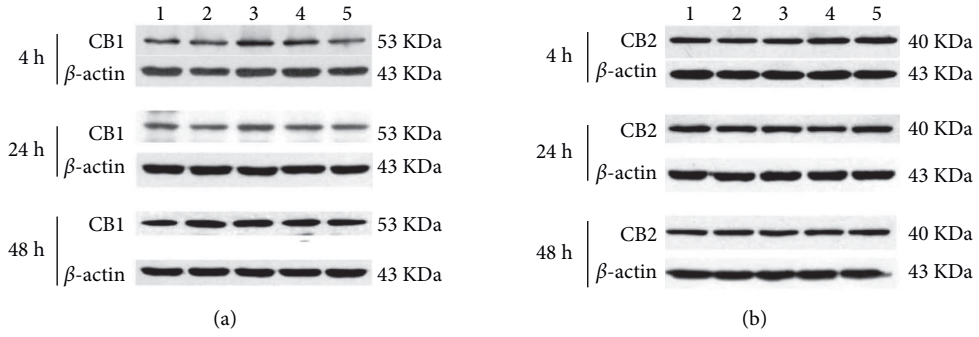

Figure 2: Continued.

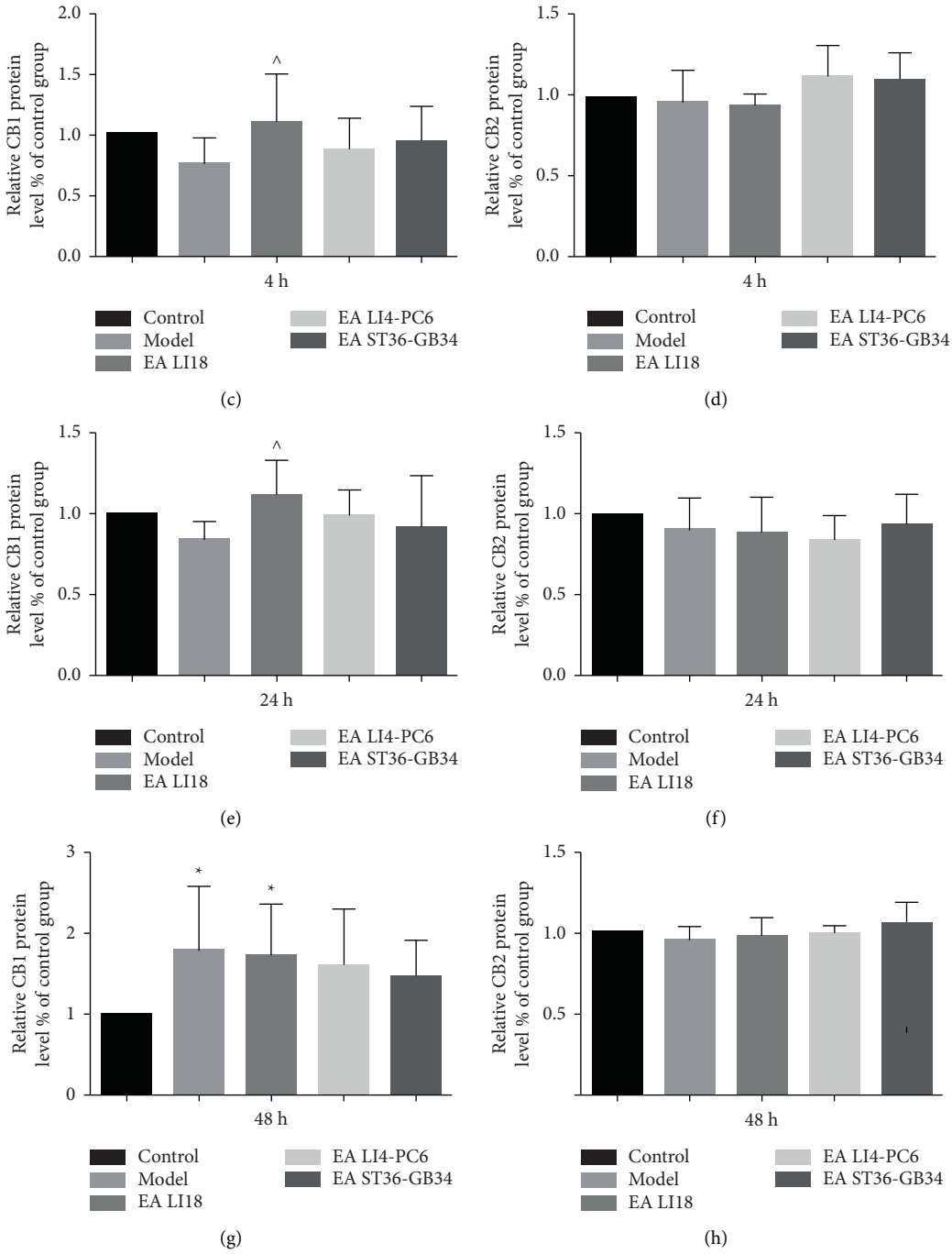

Figure 2: Effect of EA on the expression levels of endocannabinoid receptor CB1 (a, c, e, g) and CB2 (b, d, f, h) proteins at the 3 time points after modeling in the dorsal portion of the cervical spinal cord (C2-C5) in neck incisional pain rats. Representative gel images showing the protein level of CB1 (a) and CB2 (b) receptors in the cervical spinal cord tissues in different groups: (1) control group, (2) model group, (3) EA LI18 group, (4) EA LI4-PC6 group, and (5) EA ST36-GB34 group. The expression of CB1 protein was significantly increased at 48 h in the model group (vs the control group), and at 4, 24, and 48 h in the EA LI18 group (VS the model group), and the expression levels of CB2 protein at 4, 24, and 48 h had no notable changes after modeling (vs the control group) and after EA of the 3 acupoint groups (vs the model group). Data are expressed as means ± SD ($n = 8$). $^{*}P < 0.05$, vs the control group, $P < 0.05$, vs the model group.

cannabinoid receptor CB1 at 24 h after neck incision in the superficial layer of cervical spinal DHs was significantly decreased in the model group ($P < 0.05$, Figure 4(d)). Compared with the model group, the levels of immunoactivity of CB1 in the spinal DHs were significantly increased in both EA LI18 and EA LI 4-PC6 ($P < 0.05$, Figure 4(d)), but rather than in the EA ST36-GB34 group ($P > 0.05$).

In order to dissect the roles of the CB1 receptor on astrocytes during EA, the colocalization and

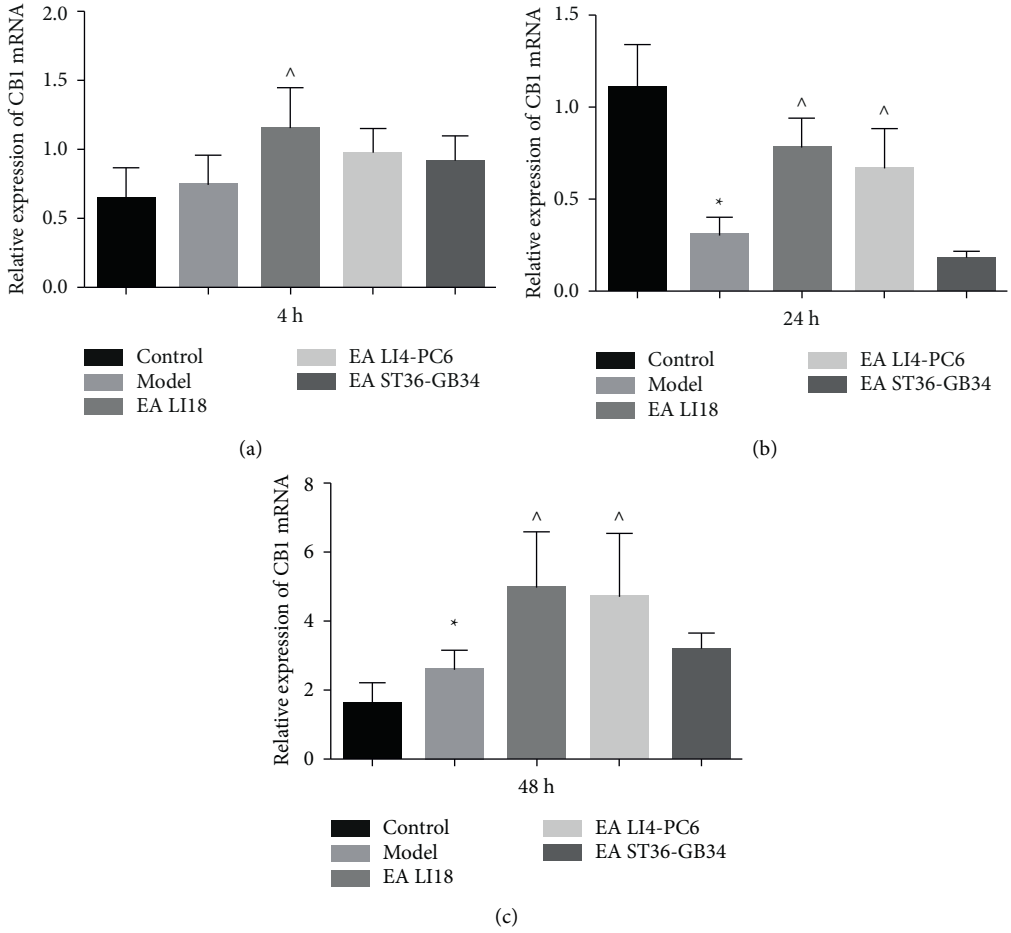

Figure 3: Effect of EA on the expression of endocannabinoid receptor CB1 mRNA at 4 (a), 24 (b), and 48 h (c) after modeling in the dorsal portion of the cervical spinal cord in neck incisional pain rats. The expression levels of CB1 mRNA were evidently decreased at 24 h and remarkably increased at 48 h after modeling (vs the control group), and considerably upregulated at 4h in the EALI18 group, at 24 and 48 h in both EALI18 and EALI4-PC6 groups, rather than in the EAST36-GB34 group (vs the model group). Data are expressed as means ± SD ($n = 8$). $^{*}P < 0.05$, vs the control group, $^{\wedge}P < 0.05$, vs the model group.

immunoreactivity of CB1 and GFAP in each group were investigated. In the control group, CB1 was expressed on astrocytes in the spinal DHs, and after neck incision surgery, nearly no co-expression of CB1 and GFAP was visualized in each slice of the observed spinal cord. In the EA LI18 group, the co-expression of CB1 and GFAP was found in laminae I and II of the cervical spinal DHs, whereas no co-expression was visualized in both EA LI4-PC6 and EA ST36-GB34 groups. These results suggest that EA at LI18 upregulated the expression of CB1 in astrocytes, which may contribute to the process of EA analgesia.

3.4. CB1 Receptor Antagonist Weakens the Analgesic Effect of EA. In order to confirm the involvement of CB1 receptors in the analgesic effect of EA, both the intraperitoneal injection and intrathecal injection of CB1 antagonist (AM251) were conducted in the present study. The results showed that either intraperitoneal or intrathecal injection of DMSO did not influence the increase in the change rates of TPT after EA at LI18. It indicated that administration of DMSO had no impact on the analgesic effect of EA. Compared with the DMSO + EA group, the change rate of TPT had no marked alteration at 4 and 24 h after neck incision ($P > 0.05$) and significantly decreased at 48 h and 72 h after intraperitoneal or intrathecal injection of AM251 ($P < 0.05$, Figures 5(a) and 5(b)). These results indicated that the CB1 receptor antagonist AM251 but not DMSO significantly reversed the analgesic effect of EA in incisional neck pain rats.

4. Discussion

Postoperative pain caused by surgical injury is a major challenge for healthcare providers. Opioid analgesics are commonly used to treat postoperative pain. However, these drugs have some adverse side effects. Therefore, more and more attention has been paid to the management and treatment of postoperative pain. It was reported that percutaneous peripheral nerve stimulation can be used to treat acute postoperative pain of various types of surgeries [32]. Acupuncture intervention and transcutaneous electrical nerve stimulation (TENS), being similar to percutaneous

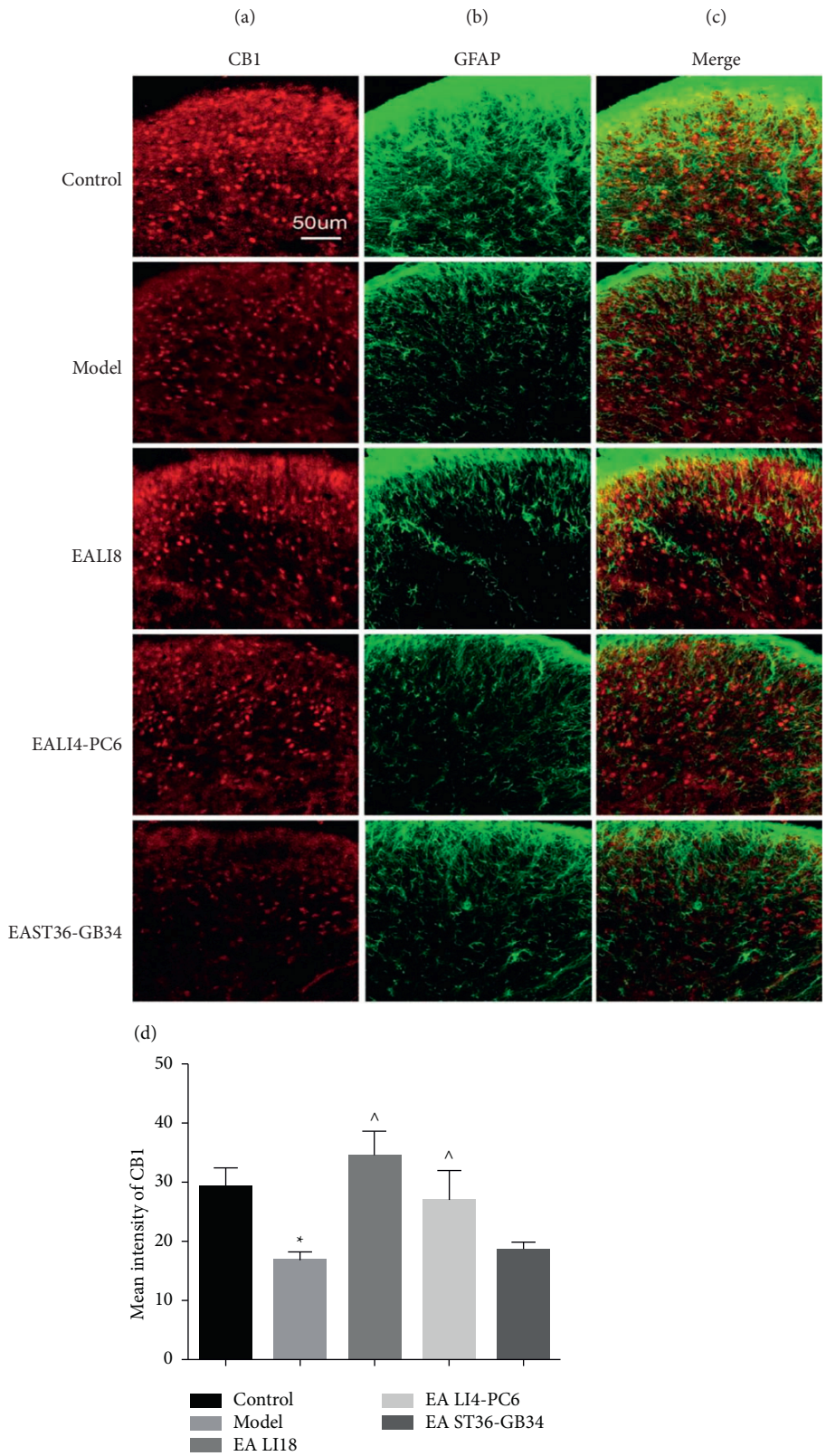

Figure 4: Effect of EA on the immunoactivity of endocannabinoid receptor CB1 at 24 h after modeling in neck incisional pain rats. Double immunolabeling of CB1 receptor and glial fibrillary acidic protein (GFAP, marker of astrocytes) in the superficial layer (laminae I and II) of cervical spinal dorsal horns (DHs): (a). CB1 positive labeling (red), scale bar, 50 μm; (b) GFAP-positive labeling (green), (c) double-labeled cells(yellow). (d): Histograms showing the intensity of the immunoactivity of CB1 receptors in the 5 groups. One-way ANOVA revealed that the expression level of CB1 was notably downregulated in the model group (vs the control group) and significantly upregulated in both EALI18 and EALI4-PC6 groups but not in the EAST36-GB34 group (vs the model group). Data are expressed as means ± SD ($n = 5$). $^{*}P < 0.05$, vs the control group, $\hat{P} < 0.05$, vs the model group.

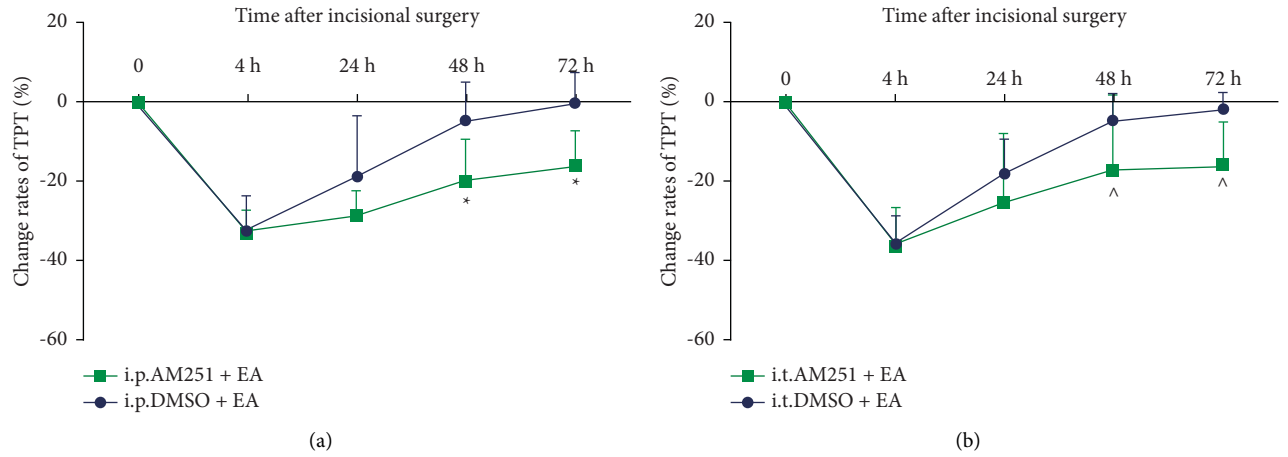

Figure 5: Intraperitoneal (a) and intrathecal (b) injection of CB1 antagonist (AM251) decreased the analgesic effect of EA LI18. Repeated measures ANOVA revealed that the PTP was significantly decreased after modeling (vs preneck incision) and obviously increased thereafter (vs 4 h), with the change rate being notably higher at 48 and 72 h in the DMSO + EA group than in the AM251+EA group. Data are expressed as means ± SD ($n = 12$). $^{*}P < 0.05$, vs the i.p.DMSO + EA group, $^{\wedge}P < 0.05$, vs the i.t. DMSO + EA group.

peripheral nerve stimulation, have been proved to be effective for relieving postoperative pain of neck surgery [9, 10, 33], tonsillectomy [7, 11, 34], back surgery [5], dental procedures [7, 35], and knee replacement [7]. Our previous clinical study [33] also showed that EA of LI18 and LI4-PC6 had a good effect in inducing analgesia and controlling the mean arterial pressure (MAP) and heart rate (HR), and reduced the anesthetic dose required by patients undergoing thyroid surgery. In this study, the PTP of rats was significantly reduced after neck incision plus repeated topical mechanical separation stimulation and considerably increased at 24 and 48 h after EA of LI18 and LI4-PC6 rather than EA of ST36-GB34, which is consistent with the results of our previous studies [6, 25, 26, 36].

The spinal cord, the primary center of nociceptive signal processing, plays a critical role in the integration and modulation of nociceptive inputs before sending to the higher central neuronal networks. Endocannabinoid is well known to be an important neuroregulatory mediator for relieving different types of pain, including chemical, mechanical, thermal, neuropathic, inflammatory, and cancer pain [37–39]. Our results of the present study showed that after neck incision, the expression levels of CB1 mRNA and protein in the cervical spinal cord had no significant changes at 4h, but were significantly increased at 48h, and those of CB1 mRNA and immunoactivity were obviously decreased at 24 h. These inconsistent results about changes of the expression levels of CB1 mRNA and protein have no suitable reasons to explain, whereas after EA interventions, the expression levels of CB1 mRNA at 4 h in the EALI18 group, 24 and 48 h in both EALI18 and EALI4-PC6 groups, and those of CB1 protein at 4, 24, and 48 h in the EALI18 group were significantly upregulated, which may contribute to the analgesic effect of EA of LI18 and LI4-PC6 in neck incision pain rats. Verification experiments demonstrated that intrathecal and intraperitoneal injection of CB1 antagonist AM251 significantly inhibited the analgesic effect of EA LI 18 in incisional neck pain rats. These findings reveal the involvement of the CA1 endocannabinoid receptor of cervical spinal DHs in the analgesic effect of EA in neck incision pain rats for the first time and are similar to those of other research studies. For example, Zheng and his colleagues [24] reported that in intrathecal (IT) morphine-induced hyperalgesia (MIH) rats, EA of ST36-GB34 reversed the reduction of mechanical and thermal TPT, Deleted and IT injection of CB1 agonist (WIN55, 212-2) combined with EA induced a significant increase of mechanical and thermal TPT and a significant increase of CB1 protein level, whereas IT CB1 antagonist (SR141716) induced the opposite results. In knee osteoarthritis (KOA) mice, EA reversed modeling-induced reduction of CB1 receptor expression and endocannabinoid 2-AG level in the midbrain, and microinjection of CB1 receptor antagonist into vlPAG (projecting to rostral ventromedial medulla-spinal neurons) could reverse the effect of EA on pain hypersensitivity and diffuse noxious inhibitory control (DNIC) function [22]. Additionally, in experimental temporomandibular joint arthritis rats, EA upregulated the expression of CB1 and CB2 mRNAs; the administration of CB1 antagonist AM251 in the temporomandibular joint significantly reversed the antinociceptive effect of EA, and the injection of CB2 antagonist AM630 reversed the anti-inflammatory effect of EA [23]. In facial thermal pain rats, EA stimulation produced an antinociceptive reaction, which was antagonized by the preadministration of AM 251, but not by AM 630, and pretreatment with an endocannabinoid metabolizing enzyme inhibitor (MAFP) and an anandamide reuptake inhibitor (VDM11) prolonged and enhanced the antinociceptive effect of EA [40]. Therefore, CB1 receptor plays an important role in mediating the antinociceptive effect of EA both in the central nervous system (in particular) and the peripheral tissues. Nevertheless, accumulated evidence [41, 42] supports that in the peripheral tissues, CB2 not CB1 induces an antinociceptive effect, possibly due to its widespread distribution in keratinocytes, macrophages, and T-lymphocytes in the epidermis and dermis of the inflamed skin tissue.

In the present study, the results of western blotting displayed that the expression of CB2 receptor protein had no significant changes at 4, 24, and 48 h after neck incision and after EA of the three acupoint groups, suggesting that CB2 in DHs of the cervical spinal cord may do not contribute to the EA's analgesic effect. Moreover, outcomes of immunofluorescence dual labeling showed that the co-expression of CB1 and GFAP was seen in the superficial layer (laminae I and II) of the spinal DHs in the control group and increased in the EA LI18 group, but nearly not visualized in the model, EALI4-PC6 and EAST36-GB34 groups, suggesting that the upregulated expression of CB1 in astrocytes in the cervical spinal DHs is involved in the process of EA analgesia in incisional neck pain rats. The distribution of CB1 receptor in the spinal DHs is consistent to Svízenská's and colleagues' results that CB1 receptors are densely expressed on the superficial layers of spinal dorsal horns, the dorsal root ganglia, and the peripheral terminals of primary afferent neurons, and within the pain descending pathway [43] and Alkaitis's and colleagues' findings that Dual blockade of CB1 and CB(2) receptor signaling prevented the resolution of postoperative allodynia and resulted in persistent overexpression of spinal GFAP and phospho-p38 in astrocytes [19].

Regarding the specific effects of EA of the three acupoint groups, the results indicated that EA LI18 and EA LI4-PC6 are basically similar in upregulating the thermal TPT and the expression of CB1 mRNA at 24 and 48 h, and increasing the immunoactivity at 24h. EA of LI18 (not LI4-PC6) also significantly upregulated the expression levels of CB1 protein at 4, 24, and 48 h, and CB1 mRNA at 4 h. Hence, EALI18 is the best in the therapeutic effect, whereas EA of ST36-GB34 had no apparent impact on the pain behavior reaction and expression of CB1 and CB2 proteins and CB1 mRNA at the three time points. These results are identical to those of our past studies in the same neck incision pain rat model [6, 25, 26, 36]. The reason is that LI18, being close to the injured neck region, is located at the same nerve segment to the incisional pain source, whereas LI4 and PC6 are at the adjacent nerve segment to the incisional pain source. Therefore, the nearer the distance, the better is the therapeutic effect.

There are some limitations in this study. First, we only explored the distribution of the CB1 receptor in astrocytes and did not observe its distribution in neurons and microglia cells in the DHs of the cervical spinal cord, and their involvement in EA analgesia in the neck incision pain model is unclear. Second, the mechanisms of EA underlying relief of postoperative pain are very complicated and need research further in depth by using more and new indexes.

5. Conclusion

The present study for the first time demonstrated that the EA stimulation of LI18-induced upregulation of CB1 receptor expression in the dorsal portion of the cervical spinal cord may contribute to its antinociceptive effect in incisional neck pain rats. EA of LI4-PC6 can also effectively relieve postoperative pain in the same animal model. The therapeutic effect of EA at LI18 is relatively better in ameliorating pain behavior and upregulating expression of CB1 protein and gene, followed by EALI4-PC6 and EAST36-GB34 being the poorest. The CB1 receptor is found in astrocytes of the superficial layers of DHs of the cervical spinal cord. These findings may provide experimental evidence for relieving the postoperative pain of thyroidectomy surgery by acupuncture treatment.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

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Review Article

Acupuncture for Primary Dysmenorrhea: A Potential Mechanism from an Anti-Inflammatory Perspective

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The low adverse effects of acupuncture for primary dysmenorrhea (PD), known as one of the most commonly reported gynecological debilitating conditions affecting women's overall health, have been thus far confirmed. Moreover, it has been increasingly recognized that inflammation is involved in such menstrual cramps, and recent studies have further shown that the anti-inflammatory effects of acupuncture are helpful in its control. This review portrays the role of inflammation in PD pathophysiology, provides evidence from clinical and animal studies on acupuncture for inflammation-induced visceral pain, and reflects on acupuncture-related therapies for dysmenorrhea with regard to their anti-inflammatory characteristics. Further research accordingly needs to be carried out to clarify the effects of acupuncture on proinflammatory factors in PD, particularly chemokines and leukocytes. Future studies on this condition from an anti-inflammatory perspective should be also performed in line with the notion of emphasizing stimulation modes to optimize the clinical modalities of acupuncture. Additionally, the effects and mechanism of more convenient self-healing approaches such as TENS/TEAS for PD should be investigated.

1. Introduction

Primary dysmenorrhea (PD), also called functional painful periods or menstrual cramps, is manifested as lower abdominal pains during menstruation without pelvic pathologies, accompanied by symptoms such as lumbago and leg pain, diarrhea, nervousness, fatigue, loss of appetite, and nausea and vomiting. PD usually occurs in adolescence after the menarche, and the pain starts a few hours before or immediately after menstruation and typically lasts for 8–72 hours [1, 2]. According to the latest epidemiological survey, about 45–90% of women in the world suffer from this condition during their menstrual period, among which 10–25% are of severe types [3]. Therefore, PD seems to be one of the most common gynecological disorders regardless of nationality and age [4]. About 1/3–1/2 of women with this debilitating disorder are also absent from work or school at least once per cycle, which significantly affects the quality of their work, study, and daily living activities, and even brings huge economic losses to any society [5, 6]. In addition, the occurrence of PD augments the risk of chronic pelvic inflammatory disease [4]. Although nonsteroidal anti-inflammatory drugs (NSAIDs) are currently the first-line medication for PD with well-grounded efficacy, the ineffective rate can reach about 20–30% [7]. Therefore, there is an urgent clinical demand to find a low-risk and effective nonpharmacological treatment option to relieve menstrual cramps.

Acupuncture has been popularly applied for a variety of diseases in China and many other countries as well, particularly for pain management [8, 9]. In fact, under the term "acupuncture", there is a family of treatment modalities, including manual acupuncture, electroacupuncture (EA), moxibustion, acupoint catgut embedding (ACE) treatment, transcutaneous electrical acupoint stimulation (TEAS), transcutaneous electrical nerve stimulation (TENS) [10]. A

large number of clinical trials and reviews have further provided evidence in support of acupuncture and its related therapies for PD [2, 11–15].

This alternative medicine has also demonstrated promising anti-inflammatory effects; therefore, it has been applied to treat inflammatory diseases [16–18], especially inflammatory pain [19–22]. Numerous studies have so far elucidated the underlying mechanism of acupuncture for various types of pain, including visceral pain [22, 23], and it has been emphasized that anti-inflammatory effects can contribute to the analgesic mechanism of this alternative therapy.

As a commonly seen among gynecological disorders, PD refers to a type of visceral pain, which develops closely related to inflammatory and immune factors [24–26]. An increasing number of studies have further examined the mechanism of acupuncture for PD from an anti-inflammatory perspective. This article aims to review the role of inflammation in PD pathophysiology and explore the potential anti-inflammatory analgesic mechanism of acupuncture as a form of alternative medicine to promote further research and optimize its clinical modalities.

2. Role of Inflammation in PD Pathophysiology

The menstrual cycle is manifested as a cyclical pattern of hormonal changes modulated by a feedback mechanism on the hypothalamus-pituitary-ovary (HPO) axis [27]. In the late secretory phase of the menstrual cycle, atrophic luteum accompanied by a rapid decline in hormone levels is the main regulatory factor shaping the destruction of the menstrual cascade [28]. Progesterone withdrawal also leads to the release of acid phosphatase and lysozyme from lysosomes into the cytoplasm. Arachidonic acid (AA) is further metabolized to prostaglandins (PGs) and leukotrienes (LTs) via the cyclooxygenase (COX) and lipoxygenase (LOX) pathways, respectively, both contributing to excessive myometrial contractions, giving rise to ischemia and hypoxia in the uterine muscle tissues [29]. The etiology of PD is multifaceted. Although numerous studies have been so far done in this respect, the pathophysiology of this alternative therapy has not been still fully clarified. The most generally acknowledged explanation is the concentration of PGs during menstruation [30]. In addition, a range of events including age, smoking habits, age at menarche, body mass index (BMI), alcohol abuse, family history, exercise, and the like can be among the risk factors for PD [4].

Notably, it has been suggested that menstruation is an inflammatory process [24, 25], before it, the endometrium exhibits inflammatory features of red with blood and edematous tissues, as a phenomenon associated with an influx of proinflammatory cytokines (viz. Interleukin-1 [IL-1], IL-6, and tumor necrosis factor-alpha [TNFα]) and leukocytes [28]. The inflammatory mediators are also an important part of the menstrual process [26], driven by a decrease in the levels of nonsteroid hormones in the late secretory phase of a nonconceptual cycle [28]. Based on previous studies, the relationship between inflammation and PD has recently attracted increasing attention even though people have a deeper understanding of the occurrence and development of PD. In the following, the relationship between PD pathophysiology and inflammation is being discussed.

2.1. Progesterone Withdrawal-Initiated Inflammation in Menstrual Cycle in PD. The current inflammatory perspective of the menstrual cycle in PD involves a complex set of events, driven by the fall in progesterone levels, which activate the release of nuclear factor-κB (NF-κB) from its inhibition by the inhibitor of IκB, leading to the downstream transduction and translation of inflammatory genes and a release of proinflammatory mediators (that is, inflammatory cytokines, chemokines, and PGs) together with an influx of inflammatory cells [31–33]. Beyond that, a cascade of inflammatory events reflected in the activation of matrix metalloproteinases (MMPs) and other degradation enzymes can participate in maintaining inflammation and eventually cause tissue destruction.

Of note, progesterone plays an anti-inflammatory role in the menstrual cycle [34, 35]. The continued presence of progesterone can significantly inhibit excessive inflammatory events in the endometrium [36–38]. Distinct uterine inflammation manifested by substantial leukocyte content has been also shown in mice lacking progesterone receptors [36]. Progesterone additionally inhibits the production and activation of MMPs [39] and reduces the inflammatory responses induced by T-cell activation [35]. The reduction of ovarian hormone levels in the endometrium is typically associated with PD [40]. One other study have further revealed that imbalances in estradiol and progesterone could also affect the synthesis of PGF2α in the endometrium and lead to menstrual pain [41]. Therefore, progesterone may enhance PD prognosis by regulating the levels of cytokines, inhibiting the activation and migration of immune cells, and reducing the oxidant activity. The inflammatory response caused by progesterone withdrawal in the first phase of the menstrual cycle is thus involved in PD occurrence.

2.2. PGs-Mediated Inflammatory Response in PD. PGs are associated with inflammatory effects and they are considered as the fundamental mechanism for PD formation [30]. PGF2α can further cause vasoconstriction and result in a decrease in the blood flow, which in turn stimulate abnormal spastic contraction of uterine smooth muscles, and ultimately induce tissue ischemia and hypoxia, and pain [42]. The function of PGE_2 depends on the type of receptors [43]. PGE_2 mediated by the PGE_2 receptor 2 also plays the role of relaxing vessels and inhibiting the contraction of uterine smooth muscles and may even work to increase edema and recruit leukocytes [44]. Studies in this line have reported that patients with PD experience significantly higher levels of PGF2α and PGE2 in the endometrial and menstrual blood than those without this condition [30]. There is even evidence that PGF2α can augment the sensitivity of nerve endings to pain and lower the pain perception threshold [45].

In addition, some studies have demonstrated that PGs can enhance the migration of inflammatory factors to the endometrium [44, 46]. $PGF_{2\alpha}$ can further promote neutrophils (NEUT) migration by increasing the release of CXCL1 [47], while PGE_2 enhances leukocyte migration by inducing the expression of CXCL8 via NF-κB signaling pathway [48, 49]. Additionally, the overexpression of inflammatory factors (such as TNFα, IL-1, and IL-6) can elevate the synthesis or release of $PGF_{2\alpha}$ in the uterus to trigger PD [50].

2.3. LTs and Inflammation in PD. LTs, as important inflammatory mediators, play an essential role in the PD process [51, 52]. Studies have accordingly shown that 10–30% of patients with PD have no obvious changes in PG levels in the uterus, while the content of LTs in the uterus and menstrual blood had significantly increased, and the LTE4 content in the urine of some young PD patients on the first day of menstruation was equivalent to three times that of women without this condition [53, 54]. LTs can also participate in the chemotaxis and activation of leukocytes, causing leukocytes to accumulate in the inflammatory area and release inflammatory mediators, which can lead to smooth muscle contractions and increased vascular permeability. Studies have further revealed that the use of LT antagonists can effectively relieve pain in patients with PD, whose traditional treatment with PG synthase inhibitors might not be much effective [55].

2.4. Changes of Cytokines and Chemokines in PD. The overexpression of cytokines and chemokines during the menstrual cycle drives the inflammatory microenvironment of PD in the uterus and plays a leading role in leukocyte recruitment.

2.4.1. TNFα. TNFα is a potent proinflammatory cytokine that mediates complex biological responses, including the upregulation of inflammatory ones [50]. The role of TNFα in PD pathogenesis has been emphasized as stimulating the synthesis or release of PGs [50, 56], resulting in hypercontraction of the myometrium, which leads to ischemic pain. Studies have further reported that women with PD have higher plasma IL-6 and TNFα levels than healthy cases [57, 58]. Targeting TNFα and other factors to regulate arachidonic acid and inflammatory signaling pathways is thus assumed as an effective approach for PD treatment [59]. Moreover, the genotype of TNFα-308 GG may be a useful tool for predicting PD susceptibility [60].

2.4.2. IL. Plasma cytokine levels, including IL-1β, IL-6, and IL-10, have been significantly altered in women with a normal menstrual cycle [61]. IL-1β and IL-6 levels have been also negatively relevant to estradiol and progesterone levels, indicating the involvement of immune inflammation in the menstrual cycle [62]. Studies have additionally shown that the IL-6 level in the luteal phase was significantly higher than that in follicular one [63], and plasma IL-6 concentration significantly increased in patients with PD on the first day of menstruation, resulting in enhanced uterine muscle contractions and reduced uterine blood flow [64, 65]. Research has further found that a reduction in IL-6 levels and an increase in anti-inflammatory factors can be induced by aerobic exercises, therefore relieving PD [50].

2.4.3. Eotaxin. Eotaxin, also known as C–C motif chemokine ligand 11 (CCL11), is a member of the CC subfamily of chemokines and acts after binding to C–C motif chemokine receptor 3 (CCR3). As a specific chemokine of eosinophil (EOS), eotaxin has the strongest chemotactic activity [66] and it has been confirmed that the local injection of CCL11 can significantly augment EOS in local tissues [67]. A recent study has shown that patients with PD have significantly higher eotaxin levels in their blood than healthy individuals, suggesting that eotaxin may be involved in PD development [68].

During the menstrual cycle, cytokines and chemokines are abundant in the endometrium, which recruits leukocytes and affects their division and activation. By regulating the composition and function of local uterine leukocytes, cytokines and chemokines can further enhance and maintain local uterine inflammation, leading to tissue damage and indirect involvement in PD.

2.5. Changes of Leukocytes in PD. Menstruation represents a highly regulated inflammatory process, manifested as substantial leukocytes before the occurrence of menstruation [44]. The major leukocyte subsets, like uterine NEUT, natural killer (NK) cells, mast cells (MCs), EOS, and macrophages, constitute up to 40% of the total cells in the premenstrual endometrium [69]. There is also growing evidence that leukocytes are closely associated with PD.

2.5.1. NEUT. In the peripheral blood of patients with PD, inflammatory metabolites of NEUT are increased [58, 70]. Serum neutrophil-to-lymphocyte ratio is significantly higher in adolescents with PD and premenstrual syndrome (PMS) [71]. In this sense, NSAIDs can bring pain-relief effects via modulating oxidative stress and ionized calcium (Ca^{2+}) levels of NEUT in patients with PD through voltage-gated calcium channels (VGCCs) and transient receptor potential (TRP) cation ones [72].

2.5.2. EOS. In addition to the classic inflammatory response, allergic inflammation dominated by EOS is also of utmost importance and usually not recognized in PD pathophysiology. Recently, the role of EOS in the reproductive system has attracted much attention. Studies have further found that EOS is present in the endometrium before and during menstruation [73, 74]. Besides, EOS can regulate local immune and inflammatory responses and play a key role in PD induction and development. The elevated levels of eotaxin can also cause EOS migration to the uterus, promoting inflammatory edema and congestion in this organ [75]. Eosinophil cationic protein (ECP), major basic protein

(MBP), neurotoxin, and eosinophil peroxidase released by activated EOS can also cause the release of reactive oxygen species (ROS) and cytotoxic molecules, promoting inflammation and inducing endothelial damage to activated platelets, which lead to vasoconstriction and blood clotting and ultimately reduced blood flow and aggravated PD. ECP can further promote MC release histamine and exacerbate pain [76]. Further explorations into the relationship between EOS and menstruation can thus help better understand the pathogenesis of PD.

2.5.3. MCs. Human MCs are derived from $CD34^+$ and $CD117^+$ pluripotent hematopoietic stem cells in the bone marrow. MC progenitor cells can be also transformed from protective immune cells to effective proinflammatory ones, thus participating in the inflammatory process of different tissues. They are additionally involved in the induction of acute inflammation and tissue repair during chronic inflammation. In the female reproductive system, MCs are mainly distributed in the myometrium and endometrium layers [77], which are closely related to uterine smooth muscles, fibroblasts, and collagens. The mediators released by MCs can effectively stimulate uterine smooth muscle contractions. Studies have accordingly shown that MC activation plays a critical role in the control of full-term and premature delivery [78, 79] and is essential in the progression of inflammatory bowel disease (IBD) [80], rheumatoid arthritis (RA) [81], and cutaneous vasculitis [82].

Therefore, inflammatory factors are directly linked with PD occurrence and development. In addition to classic pain-causing substances (such as PGs and LTs), the inflammatory response contributes to PD, mainly caused by cytokines (i.e., IL-6 and TNFα), chemokines (viz. Monocyte chemoattractant protein-1 [MCP-1] and eotaxin), and leukocytes (including, NEUT, EOS, and MC), which need further examinations.

3. Acupuncture for Inflammatory Visceral Pain

Acupuncture is widely used in treating visceral pain, in which enhancing anti-inflammatory effect is assumed as one of the important mechanisms. Considering a large number of acupuncture forms for visceral pain, the use of this alternative therapy for inflammatory visceral pain is delineated here.

3.1. Clinical Evidence. According to a randomized controlled trial (RCT), comparing EA and medical treatment in 54 patients with chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS) of category IIIB, EA (4 mA, 99 Hz) had significantly increased the scores of pain reduction, quality of life, and total Chronic Prostatitis Symptom Index (CPSI) compared with medical treatment [83]. These results were consistent with the reports that 47 patients with CPPS had been treated with EA (continuous wave, 3 Hz). After treatment, the levels of IL-8, IL-10, and TNFα in prostatic fluid had decreased, and the CPSI score had dropped. Acupuncture could thus have a significant effect on the treatment of CPPS, which could achieve anti-inflammatory and analgesic effects by reducing the levels of inflammatory factors [84]. Similarly, another RCT on 144 patients had demonstrated that EA (alternating wave, 2/15 Hz) had alleviated pain symptoms and improved quality of life concerning chronic pelvic pain in patients with the sequelae of pelvic inflammatory disease [85]. Acupuncture can further modulate the immune function in cases with irritable bowel syndrome (IBS), which is majorly manifested by downregulating the level of serum inflammatory factor IL-18, IL-23, and TNFα and reducing the number of MCs in the colon, to improve pain, intestinal gas, bloating, and stool consistency composite score [86]. In a pilot study, TEAS at Zusanli (ST36) and Neiguan (PC6) acupoints had similarly reduced rectal sensitivity in patients with IBS as manifested by increasing the threshold of rectal sensation of gas, desire to defecate, and pain [87]. One other pilot study had further suggested that acupuncture could effectively decrease subjective pain in pediatric patients with acute appendicitis and downregulate the white blood cell count. Therefore, this alternative medicine could be exploited as an effective nonpharmacological intervention for the treatment of acute appendicitis pain in children [88].

3.2. Animal Studies. Acupuncture has been shown to have several beneficial effects in animals with intestinal disorders. A recent study had accordingly revealed that EA (10 Hz, 1 mA, plus width 0.4 ms) at ST36 could reduce the production of inflammatory cytokines by activating α7nAChR-mediated JAK2/STAT3 signaling pathway in macrophages, thereby suppressing gastrointestinal inflammation and promoting its motility [17], which demonstrated the anti-inflammatory and analgesic effects of EA through vagus nerve from the point of view of the complete nerve circuit. Another recent study showed that EA at ST36 with low intensity (0.5 mA) can activate sensory neurons expressing PROKR2+, thus driving the vagal-adrenal axis to play a systemic anti-inflammatory effect, and pointed out that the anti-inflammatory effect of acupuncture was related to the intensity of stimulation and the depth of acupuncture [89]. EA at ST36 also has an ameliorating effect within inflammatory environments by decreasing inducible nitric oxide synthase (iNOS) expression, increasing serum IL-10 level by square wave pulses with 100 Hz, 1 mA, [90], and downregulating serum TNFα and IL1-β and colonic TNFα messenger ribonucleic acid (mRNA) expression by the intermittent pulse with 2 Hz frequency and 4 mA intensity [91]. EA at Tianshu (ST25), Zhongwan (CV12), and Shangjuxu (ST37) (alternating wave, 6/30 Hz) can further augment serum IL-4 content and moderate colonic NF-κB p65 protein expression [92], and EA (15/25 Hz, 0.1–0.2 mA, 2–4 V) at ST36, Guanyuan (CV4) could modulate the balance between the splenic regulatory T cells and T-helper 17 lymphocytes in ulcerative colitis [93]. Manual acupuncture at neurogenic spots with slight modification could alleviate the body weight changes and diarrhea scores and normalize the increased level of myeloperoxidase activity, TNFα, and IL-1β in the colitis rats [94].

Briefly, both clinical evidence and animal studies have proved the efficacy and reliability of acupuncture in the treatment of inflammatory visceral pain, such as CCPS, IBS, ulcerative colitis, and acute appendicitis. The effects of this form of alternative medicine on the regulation of various inflammatory factors are thus involved in its pain-relief mechanism.

4. Anti-Inflammatory Mechanisms of Acupuncture-Related Therapies for PD

As discussed earlier, acupuncture can bring a good effect on inflammatory visceral pain. PD, as a common visceral pain condition, is also closely related to inflammatory factors. Previous studies have mostly focused on the effects of acupuncture on PGs and analgesics. In contrast, there has been little research on the treatment of PD from an anti-inflammatory perspective, to the best of the authors' knowledge. Therefore, it is necessary to explore how acupuncture affects inflammatory factors in the treatment of PD and reflect on the influence of inflammatory factors on this condition. The study findings accordingly revealed that acupuncture-related therapies have shown satisfactory effectiveness in treating dysmenorrhea by enhancing the anti-inflammatory effects. The possible inflammatory mechanisms of menstrual pain and acupuncture-related therapies can thus alleviate menstrual cramps by mediating relevant inflammatory pathways, as illustrated in Figure 1.

4.1. Acupuncture. As mentioned, cytokines, chemokines, and inflammatory cells play a critical role in dysmenorrhea pathophysiology. Acupuncture has been further shown to exert a satisfactory effect on menstrual cramps; however, the mechanism of this form of alternative medicine for PD has not been fully explained. Some experimental studies have further reported that acupuncture can reduce the levels of inflammatory factors and immune cells in the rat model of dysmenorrhea.

In this respect, Zhao et al. have found that EA (dense wave, 50 Hz) at CV4 and Sanyinjiao (SP6) acupoints had significantly reduced the writhing response and the contents of IL-2, 5-HT, and substance P in the serum of rats with dysmenorrhea [95]. These results were consistent with the reports by Luo et al., in which EA (dense wave, 50 Hz) at CV4 and SP6 had mitigated the levels of TNFα and IL-1 in the serum of rats with PD, relieved uterine contractions, and alleviated pain [96], denoting that EA could alleviate menstrual pain and the mechanism was related to the reduction of peripheral inflammatory factors.

In rats with dysmenorrhea, the CD3 and CD4 levels and the ratio had further decreased, and the immune organs, namely, the thymus gland and the spleen, had shown obvious pathological changes. Li et al. have also reported that acupuncture at Zhibian (BL54) acupoint could significantly minimize the writhing response and consequently increase the levels of T lymphocyte subsets of CD3, CD4, CD4, and CD8 in the peripheral blood [97]. Similarly, Ju et al. have established that EA (dense wave, 50 Hz) could not only improve the CD3 and CD4 levels but also develop pathological changes in the thymus gland and the spleen [98]. These studies have indicated that EA could relieve pain by enhancing the immune function in rats with PD.

4.2. Moxibustion. Herbal cake-partitioned moxibustion (HM) is characterized by the combination of moxibustion with traditional Chinese medicine (TCM) and is being applied more widely than conventional moxibustion in clinical applications, especially in China [99].

HM can significantly reduce the writhing times, upregulate plasma beta-endorphin (β-EP) as well as uterus PGE_2 content and splenic NK cell activity, and even downregulate uterus $PGF_{2\alpha}$ levels in rats with dysmenorrhea [100]. MCs also have a wide range of relationships and functions in the neuroendocrine-immune network. HM can thus upregulate the number and degranulation rate of MCs in the Shenque (CV8) acupoint [101] and downregulate the expression of MCs in the uterus [102]. Another research had further shown that HM could achieve therapeutic effects by downregulating the high expression of upstream transient receptor potential vanilloid (TRPV) in the uterine MCs of rats with dysmenorrhea. Moreover, after pretreatment with MC membrane stabilizer (that is, sodium cromoglycate), the analgesic effect of HM could be weakened by the inhibitory effect of sodium cromoglycate on the function of MCs in the CV8 acupoint, suggesting the specific role of MCs in the analgesic effect of HM [103]. In addition, moxibustion could downregulate NF-κB expression and inhibit the release of TNFα and IL-2 in rats with dysmenorrhea [104].

4.3. ACE Treatment. ACE is a form of TCM external therapy with some absorbable catgut suture implants into the acupoints. In addition, it is characterized by the advantages of easier operation and durable stimulation, compared with acupuncture [105]. ACE can also have a significant effect on dysmenorrhea, which may be related to its impact on inflammatory factors. Recently, studies have shown that ACE can significantly improve symptoms and pathological damage in rats with PD, downregulate NLR family pyrin domain containing 3 (NLRP3), Caspase-1, IL-1β, and IL-18 protein expression in uterine tissues [106]; upregulate plasma β-EP, uterus PGE_2 content, and splenic NK cell activity; and even decrease $PGF_{2\alpha}$ content [100, 107] and downregulate COX-2 and NF-κB p65 protein expression in uterine tissues [108].

As a whole, acupuncture and its related therapies have partly shown that anti-inflammatory effects contribute to analgesic mechanisms on PD. It is worthwhile to study further to enrich the multitarget and multilink mechanism of clinical treatment of this debilitating condition.

5. Recommendations for Future Researches

With the understanding of the relationship between inflammation and dysmenorrhea deepens, the treatment of this common gynecological disorder from an anti-inflammatory perspective becomes promising. Given this, it is

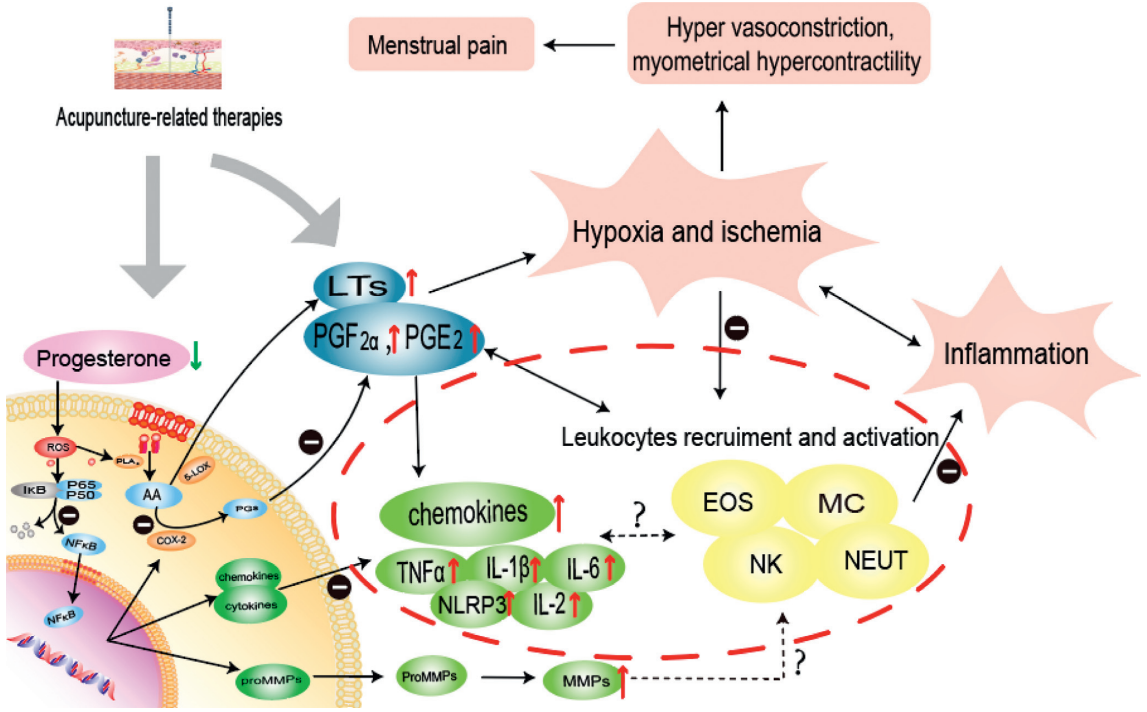

Figure 1: Possible anti-inflammatory effects of acupuncture-related therapies contributing to analgesia in menstrual pain. Progesterone withdrawal leads to the increased production of prostaglandins (PGs), leukotrienes (LTs), cytokines, chemokines, and matrix metalloproteinases (MMPs). These inflammatory factors promote the recruitment and activation of leukocytes, further increasing the inflammation and uterine hypoxia and ischemia, ultimately aggravating menstrual pain, which are considered as a vicious cycle. Acupuncture could reduce the release of PGs and downstream inflammatory cytokines by regulating nuclear factor-κB (NF-κB) signaling pathway, further relieving the inflammatory environment of uterus and uterine ischemia and hypoxia to alleviate menstrual pain. We suggest that further complexity is added to acupuncture-induced analgesia via interactions between chemokines and leukocytes. Symbols "↑" represent rising trend during menstrual pain. Symbols "—" represent inhibition of acupuncture. Abbreviations are listed at the end of the article.

speculated that enhancing anti-inflammatory effects is one of the important mechanisms of acupuncture as an alternative medicine for PD.

Based on the recent acupuncture experimental studies summarized above, we make some recommendations for future researches as follows:

1. Targeting anti-inflammatory effect of acupuncture in the treatment of PD from the perspective of chemokine-mediated inflammatory response will be a new direction to understand the mechanism of acupuncture for PD. At present, the anti-inflammatory effect of acupuncture on PD is mostly discussed from the aspects of inflammatory cells and cytokines (e.g., TNFα, IL-1, NF-κB, NK cell activity, and MC). For further studies, it is interesting to explore the effects of acupuncture on chemokines since the latest evidence has shown that the serum levels of eotaxin have an increasing trend in patients with PD [68].

(2) Optimizing acupuncture intervention methods according to classic acupuncture theory is crucial in future mechanism studies of PD. Clinical and experimental studies have indicated that alterations in needling techniques may influence the therapeutic outcomes of acupuncture [109–111]. Dysmenorrhea with the congealing-cold syndrome is the most common pattern in TCM [112]. Previous clinical and animal studies have accordingly provided evidence that transverse needling on SP6 has outperformed in relieving menstrual pain with the congealing-cold syndrome via different pathways [113–115], supporting a classic acupuncture theory of "transverse needling benefits for reinforcing yang organs of the human body". Therefore, it is recommended to conduct future acupuncture studies in line with the notion of emphasizing stimulation modes rather than acupoint selection alone. Meanwhile, it is also suggested to probe the anti-inflammatory mechanism of acupuncture with different needling methods for PD. Such studies are of great significance to increase the awareness of the benefits of acupuncture to optimize its clinical modalities for this disorder.

(3) The effects and mechanism of more convenient self-healing approaches for PD should be investigated. Although there are numerous acupuncture-like methods to reduce menstrual cramps, a more convenient procedure for patients is urgently needed because PD interferes with the activities of daily living and work of young women. TEAS/TENS are also among the noninvasive treatment options to

stimulate acupoints or sites on the surface of the body, which have the advantages of being safe, easy to use, and digitally operated. Studies have further shown that TENS/TEAS can treat many types of pain [116], including inflammatory pain [117] with satisfactory results [118]. A clinical study had also revealed that TEAS could decrease the levels of CXCL8, IL-1, IL-6, TNFα, and CCL2 to relieve inflammatory responses and decrease the injuries caused by lower limb ischemia-reperfusion [119]. Another RCT had further established that TEAS could facilitate postoperative rehabilitation and even reduce stress response, and surgical inflammation in elderly patients undergoing knee surgery [120]. An animal study had correspondingly focused on the effect of TENS on the process of wound healing from the perspective of proinflammatory cytokine expression and had found that TENS had significantly mitigated the immunoreaction of TNFα, IL-1β, and IL-6 in the dermis compared with other treatments, indicating that TENS had shortened the healing process by inhibiting the inflammatory responses [121]. Meanwhile, studies had verified the effectiveness of TENS in reducing menstrual pain and improving the quality of life in patients with dysmenorrhea [122–124]. Compared with treatment methods such as acupuncture, TEAS has also eased restrictions on in-hospital treatment. Patients can even receive treatment at home and in the workplace. Certainly, the anti-inflammatory mechanism of TEAS/TENS with proper stimulation parameters in treating PD is worthy of further study.

6. Conclusion

Acupuncture and its related therapies achieve satisfied analgesic effects on PD via the downregulation of a wide variety of inflammatory cells and cytokines (e.g., TNFα, IL-1, IL-2, IL-18, COX-2, NF-κB, NK cell activity, and MCs). The anti-inflammatory effects of acupuncture may further contribute to its analgesia in the treatment of PD, so there is a need to carry out more researches to confirm it. For further studies, it is interesting to explore the effects of acupuncture on chemokine-mediated inflammation with optimized acupuncture intervention methods according to classic acupuncture theory. Additionally, the effects and mechanism of more convenient self-healing approaches such as TENS/TEAS for PD should be investigated.

Abbreviations

PD: Primary dysmenorrhea
NSAIDs: Nonsteroidal anti-inflammatory drugs
EA: Electroacupuncture
ACE: Acupoint catgut embedding
TEAS: Electrical acupoint stimulation
TENS: Transcutaneous electrical nerve stimulation
PLA2: Phospholipase A2
AA: Arachidonic acid
PGs: Prostaglandins
LTs: Leukotrienes
COX: Cyclooxygenase
LOX: Lipoxygenase
IL: Interleukin
TNFα: Tumor necrosis factors α
NF-κB: Nuclear factor-κB
MMPs: Matrix metalloproteinases
EOS: Eosinophil
NK: Natural killer cells
MC: Mast cells
NEUT: Neutrophils
PMS: Premenstrual syndrome
ECP: Eosinophil cationic protein
MBP: Major basic protein
ROS: Reactive oxygen species
RCT: Randomized controlled trial
CP/CPPS: Chronic prostatitis/chronic pelvic pain syndrome
CPSI: Chronic Prostatitis Symptom Index
IBS: Irritable bowel syndrome
HM: Herbal cake-partitioned moxibustion
TCM: Traditional Chinese medicine
iNOS: Inducible nitric oxide synthase
mRNA: Messenger ribonucleic acid
β-EP: Beta-endorphin
TRPV: Transient receptor potential vanilloid
NLRP3: NLR family pyrin domain containing 3.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this article.

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Research Article

Comparison of the Effectiveness of Three Different Acupuncture Methods for TMD-Related Pain: A Randomized Clinical Study

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Purpose. This study aimed to compare the effectiveness of three acupuncture methods for temporomandibular disorders-(TMDs-) related pain. *Materials and Methods*. Different locations of pain, according to DC/TMD clinical assessment, were considered: temporomandibular joint (TMJ), masticatory muscles, head, and neck. Sixty patients were assigned randomly to one of three treatment groups (20 patients in each): group BA received body acupuncture, group EA received electroacupuncture, and group CA received acupuncture + cupping. The groups were compared in terms of pain (verbal numeric scale), pain-related disability (Brief Inventory Pain, BPI), and impression of the treatment's effectiveness (Patients' Global Impression of Improvement Scale, PGI-I). These were recorded before sessions of acupuncture treatment (T0), after 8 sessions of acupuncture treatment (T1), and after 4 weeks of follow-up after treatment (T2). The between-group and within-group differences in the data were analyzed statistically. The baseline characteristics were similar in all groups ($p > 0.05$). *Results*. Significant improvements were noted in all types of pain compared to baseline values in all groups (all $p < 0.05$). No significant differences were noted in the improvement of TMDs-related pain according to the different acupuncture techniques (all $p > 0.05$). All acupuncture methods used resulted to be significantly effective in improving the pain-related interference in the patient's common activities and quality of life. EA resulted to be significantly more effective than BA and CA in improving the interference of pain with patients' mood ($p = 0.015$) and quality of sleep ($p = 0.014$). *Conclusion*. BA, EA, and CA are all effective acupuncture methods in reducing pain and pain interference with common activities and quality of life in patients affected by TMD.

1. Introduction

Temporomandibular disorders (TMDs) are considered as one of the major causes of orofacial pain [1]. Pain related to TMDs is typically reported in the chewing muscles, preauricular area, or the temporomandibular joint (TMJ) [1, 2]. Often, many patients do not show localized pain but a more complex symptomatology, including headache, cervical pain, atypical facial pain, and head and neck muscle hypersensitivity [3, 4]. The presence of these symptoms may worsen the quality of life of patients and interfere with their emotional and social lives [5]. Due to the wide variety of clinical manifestations of TMDs-related pain, its treatment involves different therapeutic methods, such as splint therapy, medication, surgical therapy, physical therapy, low-level laser therapy (LLLT), transcutaneous electrical nerve stimulation (TENS), ultrasound, vibrational therapy, psychological support, and, increasingly in recent times, acupuncture [6–8].

Although many of acupuncture's physiological and neurological mechanisms are still unknown, the efficacy of acupuncture for pain therapy has been well established [9, 10]. In many clinical studies, acupuncture has been proven to be an effective form of pain management, particularly pain of musculoskeletal origin, including TMDs [9, 11–14]. Acupuncture comprises a wide range of treatment techniques, methods of point stimulation, and devices [15]. *Body acupuncture* is the most common type of

acupuncture treatment; it involves the insertion of needles into the selected acupoints, which are manually stimulated by the operator until the achievement of the proper feel of the needling with acupuncture called "Deqi." This method is actually the most investigated in the orofacial pain field, followed by laser acupuncture, as well as different microsystem acupuncture methods, such as ear, scalp, mouth, and fingers [16–20]. *Electroacupuncture* is a form of acupuncture extensively studied for its analgesic effects [21–23]. This technique involves electrical stimulation of needles, and its growing use in pain management is supported by scientific research demonstrating the differential modulation of endogenous opioids by electrical stimulation of varying frequencies [23]. Few studies investigated the effects of electroacupuncture on TMDs-related pain; although it has been proven to provide significant analgesia, its role in the management of TMDs has not been fully established [22, 23]. *Cupping therapy* belongs to traditional Chinese medicine (TCM), dating back at least 2,000 years. It consists in using one of several kinds of cups (bamboo cups, glasses, or earthen cups) placing them on the desired acupoints or sore spots on patients' skin, producing hyperemia or hemostasis, which results in a therapeutic effect [24]. This method resulted to be effective for treating several pain conditions, especially when combined with other treatments, but its use in the orofacial pain field is poorly documented [24, 25]. A single study by Choi et al. [24] analyzed the effect of acupuncture combined with medicated cupping therapy, reporting this method as effective in treating pain related to TMDs.

Although many studies have assessed the significant effects of different types of acupuncture treatment on TMD-related pain, the conclusions are inconsistent. In particular, due to the lack of direct comparison of different methods of treatment, it is not conducive to the choice of clinical application and the implementation of the best treatment. Therefore, the aim of this clinical study was to evaluate and compare the effectiveness of three different methods of acupuncture treatment (body acupuncture, electroacupuncture, and acupuncture + cupping) in alleviating pain and their interference in common activities and quality of life of patients affected by temporomandibular disorders.

2. Materials and Methods

This clinical study was conducted at the Clinical Gnathology Unit of the Department of Oral and Maxillofacial Sciences at the "Sapienza" University of Rome. The study was approved by the Institutional Ethics Committee (N.47/19/0001155); all patients signed an informed consent document before participating in the study.

During the period of February 2019–March 2021, 466 subjects under observation in our department were assessed for eligibility. All patients were screened for temporomandibular disorder (TMD) by specialists in the field and calibrated in using the DC/TMD diagnostic criteria [1]. Criteria for inclusion in the study were as follows: (1) diagnosis of at least one of the following kinds of pain at the craniocervicomandibular level, according to the DC/TMD clinical assessment procedure for the differentiation of pain location in the craniofacial area [1]: TMJ pain, masticatory muscle pain, headache, and neck pain; (2) diagnosis of at least one of the following TMDs (according to Axis I of DC/TMD classification): Myalgia (ICD-9 729.1), Arthralgia (ICD-9 524.62), Headache Attributed to TMD (ICD-9 339.89, ICD-9 784.0), and Disc Displacement with Reduction (ICD-9 524.63); (3) familiar pain intensity greater than or equal to 30 on the verbal numeric scale (VNS); (4) frequency of familiar pain greater than or equal to 1 time/week; and (5) availability to participate in the study. Patients meeting the following exclusion criteria were excluded from the study: (1) diagnosed with widespread pain; (2) chronic use of analgesic medications; (3) diagnosis of Disc Displacement without Reduction Joint disorders (ICD-9 524.63) and/or Degenerative Joint Diseases (ICD-9 715.18); and (4) receiving ongoing gnathological treatment.

2.1. Randomisation and Allocation Concealment. Since there were no data available from other clinical studies about the comparison of different acupuncture methods for the treatment of TMD-related pain, patients were recruited using convenience sampling. The patients who met the inclusion criteria were assigned randomly to three different acupuncture treatment groups. An independent investigator used a random number generator (Research Randomizer©) to allocate participants into each group by block randomisation in a 1 : 1 : 1 ratio. The results of random allocation were sealed, such that they cannot be seen from outside, stored, and managed at the Clinical Gnathology Unit. Before treatment, a random envelope was opened in front of the enrolled participant by the treatment provider. The random allocation numbers assigned to each group were recorded in an electronic chart, and no changes were allowed after allocation.

2.2. Blinding. Given the characteristics of the treatments, this study does not allow the participant or treatment provider to be blinded to their group; thus we used a single-blinded design, where only the assessors were blinded. Participants were assessed away from the acupuncture treatment area by investigators who did not participate in treatment procedures and were blinded to the treatment group. The statistician was not involved in randomisation and analyzed the data without having access to information about allocation.

2.3. Acupuncture Treatment Groups. The treatment protocol was developed according to the treatment methods used in traditional Chinese medicine (TCM) [14, 15], with the selection of application points based on TCM principles and previous studies [16, 18, 22, 25].

2.3.1. Body Acupuncture (BA Group). Patients assigned to the first group were treated with body acupuncture (BA). The acupuncture points used were ST6 (Jiache), ST7 (Xiaguan), GB20 (Fengchi), BL10 (Tianzhu), LI4 (Hegu), ST36

(Zusanli), SP6 (Sanyinjiao), and LR3 (Taichong). After asepsis of the skin with 70% alcohol at the needle penetration site, the needles were inserted bilaterally. The needles were disposable and sterilized, individually packed, with size of 0.25×25 mm (TEWA, asia-med GmbH, Pullach, Bavaria, Germany). The depth of needle penetration varied considering the anatomical differences of the application sites in each patient. The needle was manipulated clockwise and counterclockwise to achieve the proper feel of needling with acupuncture called "Deqi."

2.3.2. Electroacupuncture (EA Group). Patients assigned to the second group were treated with electroacupuncture (EA). The acupuncture points used in this group were the same acupoints selected in the BA Group, as well as the needle penetration procedure and timing, and the needle's size and characteristics. In the EA group, an electrical apparatus (Hwato SDZ-II, Suzhou Medical Appliance Factory, Suzhou, Jiangsu, China) producing a dense-dispersed wave with a frequency of 1/100 Hz was connected to the needles with alligator clips to stimulate pairs of needles inserted at ST36-SP6 and LI4-GB20. The fixed current intensity was uniformly 0.2 mA.

2.3.3. Acupuncture + Cupping (CA Group). Patients assigned to the third group were treated with acupuncture combined with cupping therapy (CA). The acupuncture points used in this group were the same acupoints selected in BA and EA groups, as well as the needle penetration procedure and timing, and the needle's size and characteristics. In the CA group, at the end of the body acupuncture session, the cupping was carried out with sterile glass cups (Mayfair Medical Supplies Ltd., Kowloon, Hong Kong, China), with size of 3 cm, at the affected side, in correspondence to the acupoints ST6 and ST7. According to the classical method of "retained cupping," the practitioner used the flaming heating power to achieve suction; the glass cups were retained for about 1-2 minutes and then were detached. The cupping procedure was carried out repeatedly for 10 min.

In all groups (BA, EA, and CA), the needles remained in place for 30 minutes and were then removed. The therapies consist of 8 sessions, administered over 4 weeks, twice a week. The needles were inserted by the same licensed acupuncturist and specialist in orofacial pain with 7-year experience (E. S.) in all treatment groups.

2.4. Outcome Measures and Data Analysis. The symptoms evaluated for all patients were the following: temporomandibular joint (TMJ) pain, masticatory muscle pain, headache, and neck pain. Each type of pain was measured at the following times:

(i) T0: Baseline, before acupuncture treatment

(ii) T1: End of treatment, 4 weeks after T0 (after the last acupuncture session)

(iii) T2: Short-term follow-up, 4 weeks after T1

The 0–100 verbal numeric scale (VNS) was used to measure pain self-assessment, with 0 indicating "no pain" and 100 "the worst imaginable pain."

At the same times (T0, T1, and T2), all patients completed the following questionnaires, in order to evaluate the general pain-related disability and the impression of the treatment's effectiveness: Brief Inventory Pain (BPI) and Patients' Global Impression of Improvement (PGI-I) Scale [1].

The primary outcome of interest was the pain level and its variation over T0, T1, and T2 in all groups. The secondary outcomes were general pain-related disability (BPI) and the patient's impression of the treatment's effectiveness (PGI-I).

Data analysis was performed with SPSS (version 23) statistical processing software. Descriptive analyses and the Chi-square test were used to compare the patient characteristics. One-way ANOVA on ranks (Kruskal-Wallis test) was used to test differences at the same time interval between groups. Bonferroni-corrected post hoc tests were used for multiple comparisons. Friedman test was used to test changes over three time intervals in the same group. Comparisons of two time intervals were performed with the Wilcoxon signed-rank test. The level of significance was set at $p < 0.05$.

3. Results

376 patients were excluded according to the inclusion/exclusion criteria. The resulting study sample consisted of 90 patients, 28 males (31.1%) and 62 females (68.9%), with an average age of 46.93 years. The patient enrollment and intervention process followed the STRICTA (Standards for Reporting Interventions in Clinical Trials of Acupuncture) criteria and is shown in Figure 1.

From the expected sample of 90 suitable patients, 30 were excluded from the analysis for discontinued intervention, mainly due to the impossibility in providing acupuncture treatments caused by COVID-19 pandemic in the period of March–November 2020 (Figure 1).

The resulting study sample therefore consisted of 60 patients (20 patients in each group). The baseline characteristics of the study groups, including age and gender, are shown in Table 1. There were no differences between the groups in terms of baseline characteristics ($p > 0.05$) (Table 1).

No significant adverse effects were seen with respect to the procedure itself. A total of two adverse events were recorded: 12 in the EA group (4 needling pain after treatment and 8 hematomas), 10 in the BA group (3 needling pain after treatment and 7 hematomas), and 11 hematomas in the CA group. These adverse events could remit spontaneously within 1 week. No other side effects or complications were evident and all patients tolerated the treatment well.

3.1. Evaluation of Pain Scores. The baseline pain VNS scores in the three groups were comparable for all different pains considered (all $p > 0.05$) (Table 1).

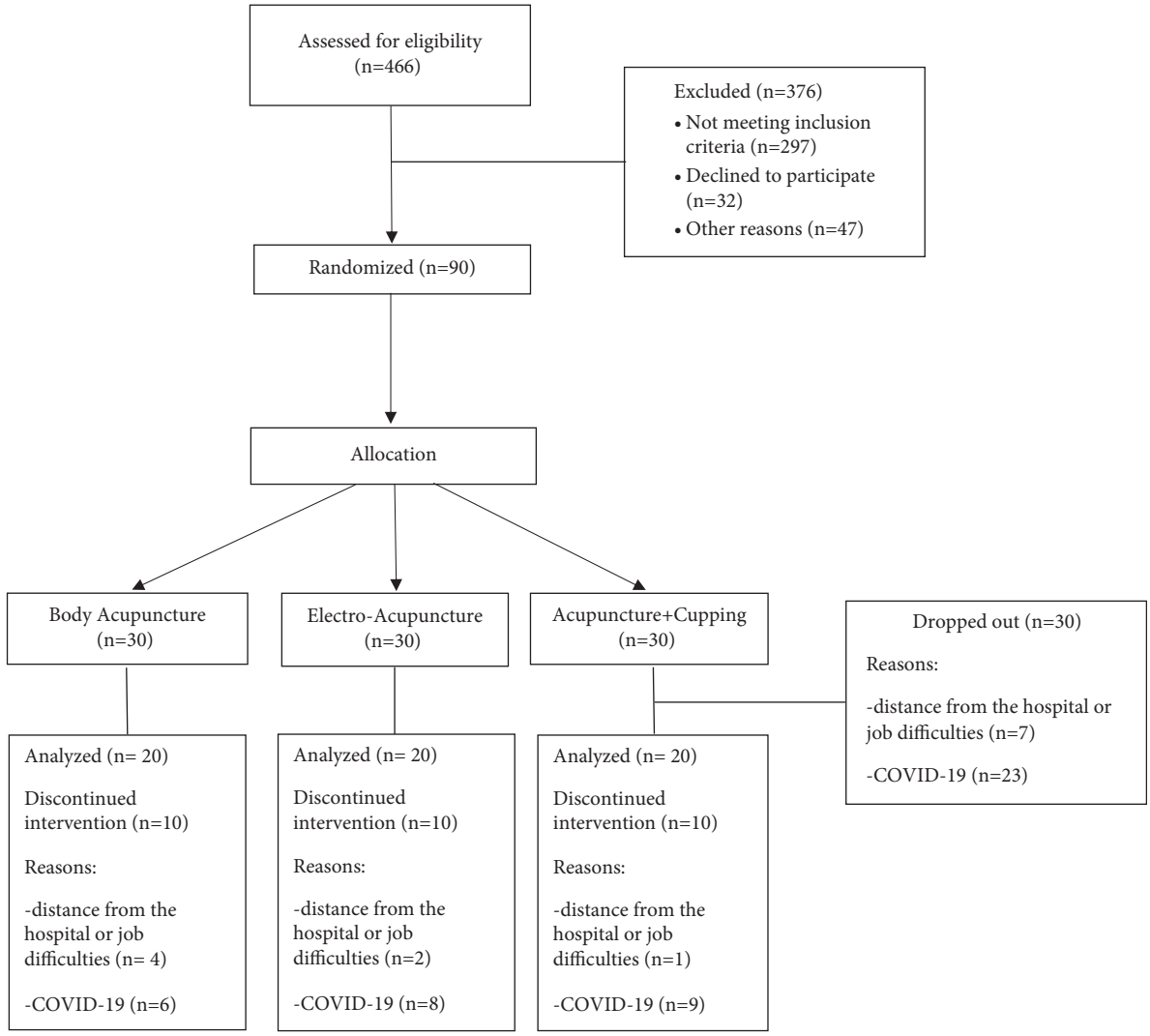

Figure 1: Flow diagram of patient enrollment and interventions.

After the treatments (T1) and during the subsequent follow-up visit (T2), no significant differences were observed between the groups (all $p > 0.05$).

Figure 2 shows the quantitative results related to pain (VNS scale), subdivided by the four types of pain analyzed (TMJ pain, masticatory muscle pain, headache, and neck pain). These results show that, in each acupuncture treatment group analyzed (BA, EA, and CA), pain shows average lower values after the treatment (T1) and at the follow-up visits (T2), compared to T0 (Figure 2).

Within-group analyses showed significant improvements in pain VNS values at T1 and T2 compared to baseline values (T0) in all study groups (all $p < 0.05$), except for TMJ pain in EA group and muscle pain in BA group (Table 2).

Wilcoxon signed-rank test for the comparison of baseline (T0) and after treatment (T1) scores and follow-up (T2) scores resulted to be significant in all study groups for all types of pain considered (all $p < 0.05$) and particularly significant for headache (all $p < 0.001$) and neck pain ($p < 0.001$ in EA and CA groups). The results of this analysis and the exact p values are shown in Table 2.

3.2. Evaluation of Pain-Related Disability (BPI). Concerning the evaluation of the pain-related interference in the patient's common activities and quality of life after the treatment (T1) and during the subsequent follow-up visit (T2), no significant differences were observed between the groups (all $p > 0.05$), except for the variable mood ($p = 0.015$). Interference values were significantly higher in BA and CA groups than in EA group for the variables "mood" and "relations with other people" after the treatment (T1) and at follow-up visit (T2) (Table 3).

Within-group analyses showed significant improvements in interference values at T1 and T2 compared to baseline values (T0) in all study groups (all $p < 0.05$), except for the variable "walking ability" (Table 3).

Wilcoxon signed-rank test for the comparison of baseline (T0) and after treatment (T1) scores and follow-up (T2) scores resulted to be significant in all study groups for all variables considered (all $p < 0.05$), except for the variable "walking ability." It resulted to be particularly significant for the variables "general activity," "mood," and "sleep"

Table 1: Baseline characteristics of the participants.

Characteristics		Groups			*p value*
		BA group ($n = 20$)	EA group ($n = 20$)	CA group ($n = 20$)	
Age, years, mean (SD)		48.25 (15.7)	38.50 (13.67)	48.05 (14.06)	0.0609*
Initial pain (VNS), mean (SD)	TMJ	38.75 (38.45)	31.00 (42.66)	24.5 (33.95)	0.5076*
	Masticatory muscle	34.00 (37.79)	33.50 (38.45)	39.5 (38.72)	0.8960*
	Head	63.25 (31.13)	69.00 (25.73)	56.00 (32.83)	0.3966*
	Neck	64.75 (33.54)	64.5 (36.77)	59.25 (33.65)	0.8522*
Gender, number (%)	Female	17 (85)	16 (80)	17 (85)	0.8869**
	Male	3 (15)	4 (20)	3 (15)	
TMD diagnosis, number (%)	Arthralgia	6 (30)	3 (15)	7 (35)	0.4181**
	Myalgia	13 (65)	13 (65)	8 (40)	
	DDWR[a]	3 (15)	6 (30)	5 (25)	
Side, number (%)	Right	3 (15)	2 (10)	1 (5)	0.5362**
	Left	3 (15)	1 (5)	1(5)	
	Both	14 (70)	17 (85)	18 (90)	

BA group, body acupuncture treatment; EA group, electroacupuncture treatment; CA group, acupuncture + cupping treatment. [a]Disc Displacement with Reduction. *p value for the comparison of the age and pain distributions among groups (one-way ANOVA). **p value for the comparison of the gender, diagnosis, and side distributions among groups (Chi-square test).

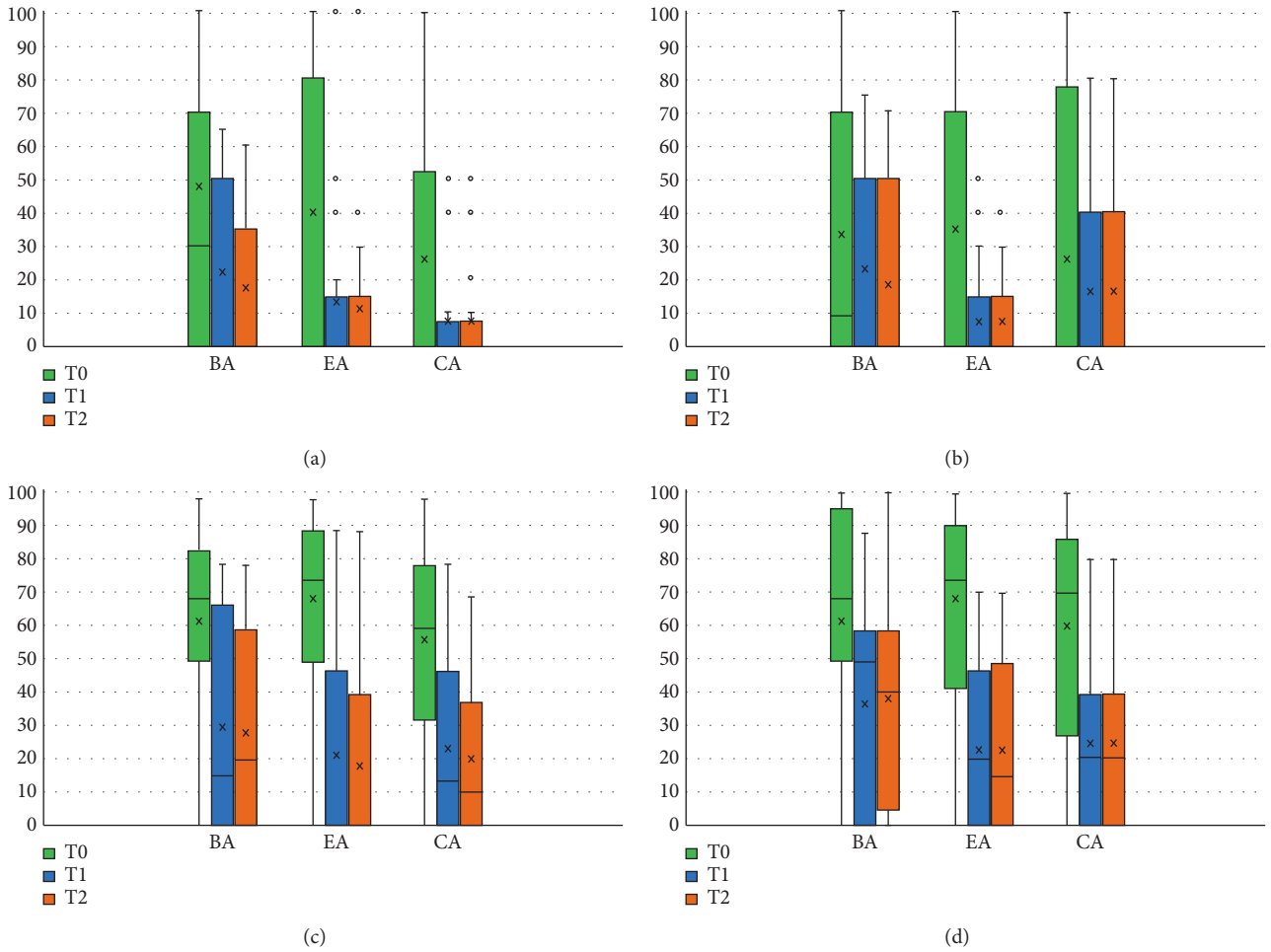

Figure 2: Pain distribution between BA, EA, and CA at T0, T1, AND T2: (a) TMJ pain; (b) muscle pain; (c) headache; (d) neck pain.

Table 2: Comparisons of pain scores (VNS) within groups after acupuncture treatments (T1) and follow-up visit (T2) ($n = 20$ in each group); mean ± SD values.

Group	T0	T1	T2	P_T	$P_{0\text{-}1}$	$P_{0\text{-}2}$
TMJ pain						
BA group	38.75 ± 38.45	18.00 ± 24.46	16.00 ± 22.57	**0.004**	**0.002**	**0.002**
EA group	31.00 ± 42.66	13.00 ± 26.77	11.00 ± 24.47	0.247	**0.035**	**0.035**
CA group	24.50 ± 33.96	8.00 ± 17.04	6.50 ± 14.24	**0.047**	**0.008**	**0.009**
Masticatory muscle pain						
BA group	34.00 ± 37.79	22.50 ± 28.40	21.00 ± 27.51	0.118	**0.022**	**0.014**
EA group	35.50 ± 38.45	9.00 ± 16.83	8.00 ± 14.72	**0.047**	**0.009**	**0.009**
CA group	39.50 ± 38.73	16.50 ± 25.19	16.00 ± 24.58	**0.004**	**0.002**	**0.002**
Headache						
BA group	63.25 ± 31.13	30.50 ± 32.84	28.50 ± 31.00	**≤0.001**	**≤0.001**	**≤0.001**
EA group	69.00 ± 25.73	22.50 ± 28.45	17.50 ± 26.73	**≤0.001**	**≤0.001**	**≤0.001**
CA group	56.00 ± 32.83	22.50 ± 25.31	19.00 ± 22.92	**≤0.001**	**≤0.001**	**≤0.001**
Neck pain						
BA group	64.75 ± 33.54	37.50 ± 31.44	38.50 ± 32.00	**0.003**	**0.002**	**0.004**
EA group	64.50 ± 36.77	24.00 ± 25.42	23.50 ± 25.19	**≤0.001**	**≤0.001**	**≤0.001**
CA group	59.25 ± 33.65	24.50 ± 25.02	25.00 ± 24.39	**≤0.001**	**≤0.001**	**≤0.001**

VNS, verbal numeric scale; SD, standard deviation. BA group, body acupuncture; EA group, electroacupuncture; CA group, acupuncture + cupping. P_T, p value for the within-group comparison (Friedman test); $P_{0\text{-}1}$, p value for the comparison of baseline (T0) and after 4 weeks (T1) scores (Wilcoxon signed-rank test); $P_{0\text{-}2}$, p value for the comparison of baseline (T0) and after 8 weeks (T2) scores (Wilcoxon signed-rank test).

($p < 0.001$) in EA and CA groups. The results of this analysis and the exact p values are shown in Table 3.

3.3. Treatment Effectiveness (PGI-I). The results of patients' self-evaluation of treatment effectiveness using the PGI-I Scale are shown in Table 4.

4. Discussion

The present study compared the effectiveness of body acupuncture, electroacupuncture, and a combination of body acupuncture with cupping therapy in the treatment of the main types of TMD-related pain: TMJ pain, masticatory muscle pain, headache, and neck pain. Based on the results, all three treatment methods yielded significantly improved outcomes regarding all types of pain considered compared to baseline, reinforcing the evidence that acupuncture is an effective treatment in patients suffering from pain with TMD origin. No significant differences emerged from the between-group analysis, suggesting that there is no specific guidance in selecting one of these three methods based on the TMD-related pain to be treated and thus the referral diagnosis. These results may be explained by the fact that, in the three study groups, different methods of needle stimulation were applied to the same acupuncture points (acupoints) in order to evaluate any differences based only on the type of needle stimulation, not on the treatment principle or scheme. In light of the TCM principle of "treating with the least effort," using the classical somatic acupuncture stimulation alone can be effective in improving the pain symptoms of the dysfunctional patient. Moreover, body acupuncture does not require any accessory tool/instrument unlike the other two methods used, resulting to be the most easily accessible and the most favorable in terms of cost/benefit. However, despite the lack of evidence of statistical significance, several differences can be highlighted among the results obtained in the three study groups, depending on the type of pain treated.

Body acupuncture represents the classic and most common stimulation method of acupuncture and the most frequently mentioned for the treatment of orofacial pain. It involves the insertion of needles into the selected acupoints, which are manually stimulated by the operator. *Zotelli* et al. [17] verified the effectiveness of body acupuncture in the treatment of pain of muscular and mixed origin in patients with TMD, showing an improvement of pain in the treated patients. A review by *Fernandez et al.* [16] evaluated the effectiveness of various body and laser acupuncture treatments for temporomandibular disorder myofascial pain, showing that both of these techniques can be effective in relieving patients' signs and symptoms. These findings are consistent with those of the present study, since the patients belonging to the BA group reported a remarkable decrease of all types of pain analyzed. Although the therapeutic efficacy of body acupuncture is mostly reported on muscle pain, in this study, it was the only type of pain that did not exhibit a statistically significant decrease when comparing the patient's average values both after treatment (T1) and at the subsequent follow-up visit (T2) ($P_T = 0.118$). Furthermore, patients of the BA group reported statistically significantly lower average values of TMJ pain ($P_T = 0.004$) overall, compared with patients of the CA group ($P_T = 0.047$) and especially those of the EA group ($P_T = 0.247$). Only one previous study by *Corcos and Brandwein (1976)* focused on the effects of body acupuncture treatment for pain related to the temporomandibular joint in 46 patients affected by rheumatoid arthritis and osteoarthritis. This study also pointed out the value of the body acupuncture method in improving TMJ pain [26]. The other two study groups (CA group and EA group) were characterized by the use of the same therapeutic scheme and procedure as the BA group but

Table 3: Comparisons of the results of the BPI questionnaire between and within groups *after acupuncture treatments (T1) and at follow-up visit (T2) (n = 20 in each group)*; mean ± SD values.

Group	T0	T1	T2	P_T	P_{0-1}	P_{0-2}
A. General activity						
BA group	4.45 ± 3.53	2.30 ± 2.66	2.40 ± 2.72	**0.002**	**0.002**	**0.002**
EA group	4.25 ± 2.99	2.00 ± 2.69	1.75 ± 2.40	**≤0.001**	**≤0.001**	**≤0.001**
CA group	4.45 ± 2.11	2.35 ± 2.18	2.45 ± 2.37	**≤0.001**	**≤0.001**	**≤0.001**
p^*	0.954	0.678	0.529			
P_{1-2}	0.785	0.587	0.469			
P_{1-3}	0.870	0.782	0.803			
P_{2-3}	0.838	0.374	0.249			
B. Mood						
BA group	6.25 ± 3.07	3.40 ± 2.94	3.25 ± 2.65	**≤0.001**	**≤0.001**	**≤0.001**
EA group	5.30 ± 2.96	2.05 ± 2.33	1.75 ± 1.94	**≤0.001**	**≤0.001**	**≤0.001**
CA group	6.25 ± 2.29	3.30 ± 2.25	3.35 ± 2.32	**≤0.001**	**≤0.001**	**≤0.001**
p^*	0.561	**0.015**	0.059			
P_{1-2}	0.279	**0.023**	0.074			
P_{1-3}	0.623	0.830	0.816			
P_{2-3}	0.576	**0.007**	**0.022**			
C. Walking ability						
BA group	3.05 ± 3.68	1.40 ± 2.66	1.45 ± 2.78	0.089	0.175	0.196
EA group	1.45 ± 2.50	0.60 ± 1.14	0.60 ± 1.14	0.549	0.491	0.491
CA group	2.30 ± 2.87	1.20 ± 1.85	1.20 ± 1.85	0.091	0.252	0.252
p^*	0.337	0.675	0.675			
P_{1-2}	0.156	0.582	0.582			
P_{1-3}	0.671	0.922	0.922			
P_{2-3}	0.276	0.340	0.340			
D. Normal work						
BA group	4.10 ± 3.51	2.30 ± 3.06	2.35 ± 3.12	**0.011**	**0.003**	**0.004**
EA group	3.60 ± 3.22	1.75 ± 2.47	1.65 ± 2.28	**0.011**	**0.004**	**0.002**
CA group	4.00 ± 2.83	2.00 ± 2.27	2.30 ± 2.41	**≤0.001**	**≤0.001**	**0.003**
p^*	0.154	0.875	0.582			
P_{1-2}	0.097	0.677	0.589			
P_{1-3}	0.097	0.988	0.673			
P_{2-3}	1	0.641	0.284			
E. Relations with other people						
BA group	3.95 ± 3.17	2.15 ± 2.60	2.30 ± 2.77	**0.023**	**0.005**	**0.005**
EA group	2.60 ± 2.76	1.05 ± 1.67	1.10 ± 1.55	**0.048**	**0.007**	**0.008**
CA group	3.70 ± 2.30	2.60 ± 2.54	2.70 ± 2.72	**0.010**	**0.003**	**0.006**
p^*	0.266	0.092	0.186			
P_{1-2}	0.193	0.239	0.390			
P_{1-3}	0.753	0.364	0.443			
P_{2-3}	0.132	**0.026**	0.053			
F. Sleep						
BA group	4.80 ± 3.30	2.95 ± 2.84	3.10 ± 2.83	**≤0.001**	**≤0.001**	**≤0.001**
EA group	4.50 ± 3.47	0.95 ± 1.05	1.75 ± 1.77	**≤0.001**	**≤0.001**	**≤0.001**
CA group	5.05 ± 3.14	3.65 ± 3.12	3.95 ± 3.05	**0.023**	**0.004**	**0.017**
p^*	0.874	**0.014**	0.062			
P_{1-2}	0.775	**0.024**	0.155			
P_{1-3}	0.796	0.484	0.317			
P_{2-3}	0.614	**0.007**	**0.022**			
G. Enjoyment of life						
BA group	5.05 ± 3.89	3.35 ± 3.98	3.35 ± 3.98	**0.047**	**0.015**	**0.015**
EA group	3.05 ± 3.27	1.35 ± 2.50	1.40 ± 2.54	**0.023**	**0.005**	**0.005**
CA group	3.65 ± 3.12	2.30 ± 2.49	2.40 ± 2.68	**≤0.001**	**≤0.001**	**≤0.001**
p^*	0.222	0.210	0.224			
P_{1-2}	0.101	0.142	0.147			
P_{1-3}	0.281	0.757	0.790			
P_{2-3}	0.419	0.107	0.118			

VNS, verbal numeric scale; SD, standard deviation. BA group, body acupuncture; EA group, electroacupuncture; CA group, acupuncture + cupping. p^*, p value for the comparison among groups (one-way ANOVA on ranks); P_{1-2}, p value for multiple comparisons of BA group and EA group (Bonferroni-corrected post hoc test); P_{1-3}, p value for multiple comparisons of BA group and CA group (Bonferroni-corrected post hoc test); P_{2-3}, p value for multiple comparisons of EA group and CA group (Bonferroni-corrected post hoc test). P_T, p value for the within-group comparison (Friedman test); P_{0-1}, p value for the comparison of baseline (T0) and after 4 weeks (T1) scores (Wilcoxon signed-rank test); P_{0-2}, p value for the comparison of baseline (T0) and after 8 weeks (T2) scores (Wilcoxon signed-rank test).

Table 4: Patients' impression of the effectiveness of treatment of the entire study population, according to the PGI-I scale, *after acupuncture treatments (T1) and at follow-up visit (T2) (n = 60)*; Pt *n* (%).

Patients' impression of the treatment	T1	T2
Very much worse	0 (0%)	0 (0%)
Much worse	0 (0%)	0 (0%)
A little worse	0 (0%)	0 (0%)
No change	9 (15%)	11 (18.3%)
A little better	16 (26.7%)	16 (26.7%)
Much better	17 (28.3%)	16 (26.7%)
Very much better	18 (30%)	17 (28.3%)

adding a "supplemental" stimulation to the selected acupoints. From the results obtained, this addition seems to determine a greater effectiveness in the treatment of pain and especially for headache and neck pain (all $p < 0.001$). Furthermore, both patients belonging to the EA and CA groups reported more significant results compared to patients treated with body acupuncture alone for all types of pain considered, except for TMJ pain.

The addition of *cupping therapy* to body acupuncture was found to be the most effective treatment method in the management of muscle pain. This evidence is consistent with the majority of systematic reviews and RCTs to date which suggest a favorable effect of cupping for pain, especially tension headache and musculoskeletal pain [24]. The negative pressure applied to the skin during the cupping procedure has been proven to induce muscle relaxation and changes in local tissue structures and in blood circulation, significantly reducing peripheral and local P substance and inflammation and thus resulting in pain reduction [24]. *Han* et al. [25] compared the therapeutic effect of medicated cupping and acupuncture combined with medicated cupping in 120 TMD patients, reporting a significant improvement of TMDs' signs and symptoms in both groups after a treatment course of 10 days ($p < 0.01$). In line with the present study, the authors suggested that the combination of the two treatments leads to superior clinical outcomes, compared to the use of single medicated cupping therapy. However, it is difficult to make a comprehensive comparison with the results obtained in this research. The authors did not specify the type of pain treated or the TMD diagnosis of these patients; moreover, the cupping therapy procedure involved the addition of medicinal herbal substances.

To our knowledge, this is the first study to analyze the effects of the classic technique of dry retention cupping combined with acupuncture on the most common types of pain associated with TMD. Given the positive results obtained, the need for further studies becomes evident to deepen and better define the therapeutic potential of this ancient medical practice in relieving pain related to TMD.

Electroacupuncture is considered a particularly effective method of acupuncture for the treatment of persistent tissue and nerve injuries, chronic pain, and visceral pain, as addressed by several research studies conducted within the last decade [21, 22, 27]. Zhang et al. [27] suggested that the electroacupuncture mechanism of action in relieving pain is the result of activation or inhibition of various bioactive chemicals in peripheral, spinal, and supraspinal pathways. Despite its popularity in pain management, few studies investigated the clinical effects of electroacupuncture on TMD-related pain. A literature search by *Kuo* et al. [22] yielded to nine publications from Chinese practitioners concerning the use of electroacupuncture for treating TMD symptoms, and all of them reported analgesic efficacy in the treatment of pain, especially of muscular origin. However, the authors highlighted the inconsistency in most of these studies, accentuating the need for more well-designed and long-term studies in this research area. The results obtained in the present study agree with these few lines of evidence, pointing out that this method was particularly effective in reducing pain of muscle origin, as well as headache and neck pain.

While evaluating the effectiveness of treatment methods for TMD, the psychological and emotional status of the patients, as well as their functioning in daily activities, should also be considered. Numerous studies highlighted the association between pain and some social, emotional, and psychological features influencing the quality of life of people affected by TMD [28–30]. Moreover, a particular interest is shown to deepen the correlations between TMD-related pain and quality of sleep and insomnia. When compared to pain-free controls, TMD patients exhibited poorer sleep quality and were mainly categorized as poor sleepers [31, 32]. In addition, poorer sleep quality has been associated with coexisting headaches, body pain, clenching habit, and reduced mouth opening [33]. Connections between mood disorders and TMD were also investigated. Several studies reported high prevalence of symptoms such as anxiety, hostility, anger, paranoid ideation, and especially depression in patients affected by TMD, in particular of muscular origin [34–36]. The presence of depressive symptoms in TMD patients was reported to be related to the presence of a painful condition [36]; the onset or the exacerbation of sufferance and pain in these patients could be generated and perpetuated by the presence of such psychological aspects [34]. In the present study, the impact of pain on all considered activities and quality of life aspects was found to be significantly decreased after the acupuncture treatment and in the short-term follow-up in all three study groups, except for the aspect of "walking ability." In particular, electroacupuncture was found to be effective in improving the influence of pain on the patient's quality of sleep and mood, with a statistically significant difference compared to body acupuncture and acupuncture combined with cupping. These results are consistent with the evidence defining EA as an effective therapeutic intervention for patients with anxiety, depression, and primary insomnia, capable of improving patients' life and sleep quality without serious adverse effects [37–41]. A multidisciplinary therapeutic approach is needed to address all factors, including sleep and mood alterations, which modulate pain experience. The results of this study indicate that acupuncture, especially electroacupuncture, rehabilitated the patients' ability to perform daily work activities, sleep quality, and emotional and social aspects, rapidly and effectively, up to 1 month after treatment.

Strengths of the present study include the randomized allocation of participants to the treatment groups, the follow-up recording, the differentiation of four types of TMD-related pain recorded, the evaluation of the participants' disability in common activities and quality of life by means of a validated questionnaire, and the evaluation of different methods of stimulation of acupoints using the same therapeutic scheme. Possible weaknesses of the study were the sample size and the duration of the acupuncture treatment. Acupuncture comprises several acupoint stimulation methods, treatment patterns, and timing, allowing the application of individualized therapies. Due to its versatility and special effectiveness in multifactorial diseases, acupuncture is particularly suitable for the treatment of TMD-related pain, which in turn is characterized by a very complex and varied symptomatology. For these reasons, despite the positive results obtained, we think that the patient sample size examined is too limited to present reliable results regarding the comparison of the effectiveness of three different acupuncture methods in reducing TMD-related symptoms. Furthermore, our results correspond to a single course of 4 weeks of therapy. Due to the tendency of TMD to become chronic, a prolonged course evaluation with additional long-term follow-up is necessary.

5. Conclusions

(i) Body acupuncture, electroacupuncture, and acupuncture combined with cupping therapy are all effective methods in reducing pain and pain interference with common activities and quality of life in patients affected by TMD.

(ii) For the first time, the classical method of retained cupping is reported to be effective in the management of TMD-related pain, when combined with body acupuncture.

(iii) Electroacupuncture reduces the interference of pain in patients' mood and sleep quality more effectively than body acupuncture alone or combination with cupping therapy.

(iv) Further studies are needed to confirm the results obtained and to better define the eligibility of one of these methods in improving the complex symptomatology connected with TMD.

Data Availability

All the data are contained and described within the manuscript. The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors report no conflicts of interest in this work.

Supplementary Materials

Table 4: STRICTA 2010 checklist of information to include when reporting interventions in a clinical trial of acupuncture (Expansion of Item 5 from CONSORT 2010 checklist). Table 5: CONSORT 2010 checklist with the Nonpharmacological Trials Extension to CONSORT (with STRICTA 2010 extending CONSORT Item 5 for acupuncture trials). (*Supplementary Materials*)

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Research Article

Effect of Acupoint Embedding on Serum Leptin and Hypothalamus Leptin Receptor Expression in Rats with Simple Obesity

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Background. Acupoint embedding treatment on obesity has been applied in clinical practice for many years and has achieved obvious efficacy. However, animal experimental studies on acupoint embedding are relatively few, and its mechanism remains unclear. *Methods*. We established a simple obese rat model using a high-fat diet for 8 weeks. Acupoint embedding therapy was performed once a week for 4 weeks. After the treatment, serum leptin, triglyceride (TG), cholesterol (TC), low-density lipoprotein (LDL), and high-density lipoprotein (HDL) levels were detected by radioimmunoassay, HE staining was used for fat morphology analysis, and immunohistochemical method was used to detect the expression of leptin receptor in hypothalamus. *Results*. Compared with model group, acupoint embedding treatment can reduce body weight and Lee's index, reduce serum leptin, TG, TC, and LDL level, increase HDL level, change the morphology and number of adipocytes, and increase the expression of leptin receptor in hypothalamus. *Conclusion*. Acupoint embedding therapy can reduce the level of leptin in blood, increase the number of leptin receptors in hypothalamus, enhance the biological effect of leptin, alleviate the leptin resistance in obese body, change the shape of fat, and regulate the level of blood lipid, so as to achieve the goal of weight loss.

1. Introduction

Obesity is a chronic disease characterized by excessive amount of fat in the body, increased volume of fat cells, and abnormal distribution. It is a recognized risk factor or pathogenic factor of a variety of chronic diseases in modern society. The latest authoritative figures put the proportion of the population as high as 12 percent as obese [1], and obesity has become one of the most serious health problems in the 21st century [2]. Obesity not only affects physical appearance, but also increases the incidence of cardiovascular and cerebrovascular diseases, diabetes, hyperlipidemia, infertility, and tumors, which seriously affects human physical and mental health and weakens body resistance [3].

The most fundamental cause of obesity is that energy intake is greater than consumption, and excess energy is stored in the body in the form of fat, resulting in excessive accumulation of body fat [4]; that is, the imbalance between energy storage and consumption is the basis of obesity [5] Leptin is one of the most important energy regulation signals, which is not only present in the blood, but also widely distributed in the brain tissue and gastrointestinal tract. It can suppress appetite, reduce energy intake, and increase energy consumption, and it plays an important role in inhibiting fat synthesis [6]. When leptin binds to hypothalamic receptor (OB-R), it alters the expression of specific neuropeptides produced by other genes in hypothalamic neurons, thereby regulating the body's energy balance [7]. However, clinical studies have found that the leptin level of

obese patients is generally higher than that of people with normal weight, which is positively correlated with BMI [8]. Hyperleptinemia in obese patients does not exert the function of leptin as an appetite suppressant, and this phenomenon is considered to be leptin resistance. Some scholars believe that the mechanism of leptin resistance is the result of inhibition on multiple levels of leptin signal transduction pathway, including changes in leptin receptor expression, blocking of leptin receptor postsignaling pathway, or inhibition of downstream signaling factors [9]. So leptin and its receptors play a central role in weight regulation, and any disruption of this signaling system can lead to obesity.

Acupoint embedding treatment on obesity has been applied in clinical practice in recent years and has achieved obvious curative effects. Acupoint embedding therapy is a continuation of acupuncture, which embeds biological protein lines that can be absorbed by the body into the acupoints and stimulates the acupoints continuously through the protein lines for a long time to prevent and cure diseases. Compared with ordinary acupuncture, acupoint embedding has the advantages of longer duration of effect, fewer visits, and good long-term efficacy [10], which improves the quality of life of patients and saves medical costs. However, animal experiments on acupoint embedding are relatively few, and its mechanism is still unclear.

In this study, we established a simple obesity rat model by using a high-fat diet for 8 weeks and used this model to explore the mechanism of acupoint embedding on simple obesity.

2. Materials and Methods

2.1. Animals. Sprague Dawley (SD) female rats (150–180 g) were purchased from Hunan Slike Jingda Experimental Animal Co., Ltd., license number: SCXK (Hunan) 2016-0002. The study was conducted in accordance with the local code of ethics for animal care and use. According to Meng et al. [11], rats in the high-fat group were fed with high-fat diet (Table 1) for 8 weeks of continuous feeding. The criteria for successful modeling of obese rats were as follows: the body weight of obese rats was 20% higher than that of normal rats. Rats were randomly divided into 3 groups, 10 in each group: normal control group: normal feeding without intervention measures; model control group: no intervention measures, high-fat feed feeding; acupoint embedding group: high-fat feed feeding, with acupoint embedding.

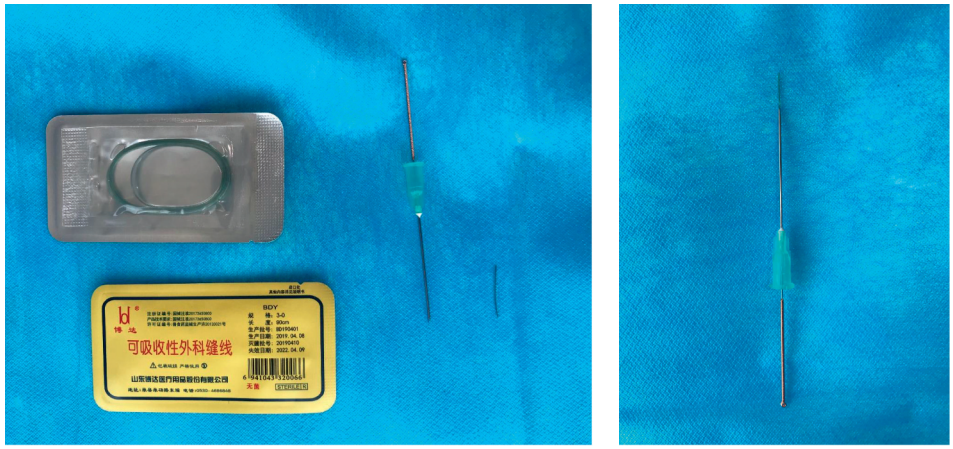
2.2. Acupoint Embedding Operation. According to the orientation of animal acupuncture points commonly used in ≪Experiment Guidance and Skill Training of Experimental Acupuncture and Moxibustion≫ [12], before embedding the thread, put the medical absorbable thread (JinHuan medical gut, 3-0) on the disinfectant bending plate, cut it into 3 mm equal length segments with the disinfectant thread shears, and put the thread body into the tip of the embedding needle (No.8 disposable injection needle, 0.8 × 38 TWLB, OuJian disposable sterile injection needle). A flat-head needle (Shunhe flat-head acupuncture needle, size 0.35 × 35 mm) was inserted into the pinhole on the other side to form a thread embedding needle (Figure 1). After disinfecting the skin at the acupoints of rats, we selected "Zhongwan," "Tianshu," and "Hou Sanli" acupoints for oblique pruning. According to the study of Tekus E [13], the average thickness of subcutaneous fat layer in rats can be more than 5 mm, so 4 mm was selected as the predetermined depth of embedding wire to ensure that the embedding wire reaches the fat layer. We slowly pulled out the needle while injecting the thread into the acupoints. The absorbable line was buried in the acupoints of rats after observing whether the gut was exposed outside the body. Acupoint embedding therapy was performed once a week for 4 consecutive weeks. All rats were sacrificed immediately after treatment.

Table 1: Rat chow composition (%).

Ingredients	Standard diet (%)	High-fat diet
Barley powder	20	10%
Bran	16	8%
Corn flour	16	8%
Flour	10	10%
Fish meal	10	5%
Bone meal	5	2.5%
Salt	2	3%
Yeast	1	0.5%
Alfalfa meal	20	10%
Lard	—	12%
Milk powder	—	10%
Egg yolk powder	—	5%
Casein	—	5%
Peanut	—	5%
Sucrose	—	5%
Sesame oil	—	1%
Vitamin A + D	—	10 drops

2.3. Body Weight and Lee's Index. Body weight and body length of all rats were measured every 7 days during modeling and intervention, using an electronic balance with an accuracy of 0.5 g. Lee's index = $3\sqrt{}$ (weight × 1000)/body length. Note: Lay the rats on the table with the back facing up to straighten the body. Place cardboard barriers around the rats to restrict their activities and keep them relatively still. The length from the tip of the nose to the anus is the length of the body, and it is measured with a ruler.

2.4. Biochemical and Hematological Analysis. After treatment, rats in each group were fasted overnight, blood samples were taken from abdominal aorta the next day and centrifuged at 2000 r/min for 20 min, serum separation was followed by immunoassay for leptin, as previously reported [13], and the kit of Cloud-Clone Corp. (CCC, USA) was used. Triglyceride (TG), cholesterol (TC), low-density lipoprotein (LDL), and high-density lipoprotein (HDL) were detected by ChemRay 240 and ChemRay 800 automatic biochemical analyzer (Shenzhen Redu Life Science and Technology).



(a) (b)

Figure 1: Structure of embedding needle. Cut the medical absorbable thread into 3 mm equal length line segments (a), and insert the thread body through one side of the embedding needle, and the other side was inserted by the flat-head needle (b).

2.5. Measurement of Fat Coefficient (White Fat and Brown Fat). After the treatment, the rats were sacrificed. The white fat around the abdomen, genitalia, and the kidney and the brown fat around the shoulder were separated from each group. The wet weight of the fat was measured by an electronic balance (accuracy was 0.1 mg) after filtration with a filter paper and recorded.

2.6. Morphological Evaluation by HE Staining. Adipose tissue with a thickness of 0.2–0.3 cm and a size of 1.5 cm ∗ 1.5 cm ∗ 0.3 cm in the same part of the rats' groin in each group was selected and immersed in 4% paraformaldehyde solution for 48 h. After that, the adipose tissue was dehydrated with conventional gradient alcohol, xylene was transparent, and it was paraffin embedded. Routine sectioning was done on a microslicer. The thickness of the slices was controlled at about 5 μm, the slices were spread, baked (baked at 60°C for 3 hours), dewaxed, and hydrated by xylene step by step and then stained with hematoxylin-eosin (HE), and the slices were sealed with gum, as reported by Li et al. [14]. Ten fields were randomly selected under a 400x light microscope, and the number of adipocytes in the whole field was recorded. The size of adipocytes in each group was measured and compared, and the average value of each case was taken.

2.7. Detection of Expression of Leptin Receptor by Immunohistochemistry. After blood collection, the rats were fixed and perfused to extract the hypothalamus. The samples were quickly placed into a fixed solution containing 0.2 kg/mol PBS of 4% paraformaldehyde containing 1/1000 DEPC, dehydrated with gradient alcohol at room temperature, transparent with xylene, embedded in paraffin after paraffin immersion for 30 min, and sected (the thickness was 5 μm). Next, the following procedures were performed to complete the operation: (1) The tissue sections were hydrated and dewaxed. ② 3% H_2O_2 deionization was used to incubate it for five to ten minutes to block the peroxidase in it, and distilled water was used to wash it for three times. (3) The heat repair antigen was carried out by microwave, repeated twice, and then cooled and washed. (4) Rat awntibody drops were added to the prepared working solution overnight. (5) IgG (biotin sheep anti-rabbit) was added, and 0.1 kg/mol PBS was used for repeated washing for three times. ⑥ DAB was used for color rendering. ⑦ Hematoxylin was used for redyeing, sealing, transparency, and dehydration. (8) After observing and taking pictures, CMIAS image analysis system was used to conduct directional analysis of cell count. Immune positive cells were yellow brown cells, as reported by Yang et al. [15]. In each group, five slices of rat hypothalamus with a field of vision of more than 40 times were selected.

2.8. Statistical Analysis. In this experiment, SPSS25.0 statistical software was used for data sorting, screening, and statistical analysis. Measurement data were recorded in $\overline{x} \pm s$ form when obeying normal distribution. Paired t-test was used for intragroup comparison before and after treatment, and analysis of variance was used for intergroup comparison. The test level of 0.05 was used, and $P < 0.05$ indicated that the difference was statistically significant.

3. Results

3.1. Effects of Acupoint Embedding on Body Weight and Lee's Index. Changes in body weight and Lee's index of the rats during the 12-week experiment are shown in Figures 2 and 3. The general trend of body weight and Lee's index among each group were shown in Figures 2(a) and 3(a). Two-way repeated ANOVA and post hoc test were conducted for experimental rigor. Time and group interaction effects of body weight and Lee's index between control and model group, control and AE group, and model and AE group were significant ($P < 0.05$). After simple effect analysis, the simple effect analysis line chart of body weight and Lee's index between each group were shown in Figures 2(b)–2(d), 3(b)–3(d), respectively. In the first 7 weeks, the body weight and Lee's index in each group increased steadily. From the 8th

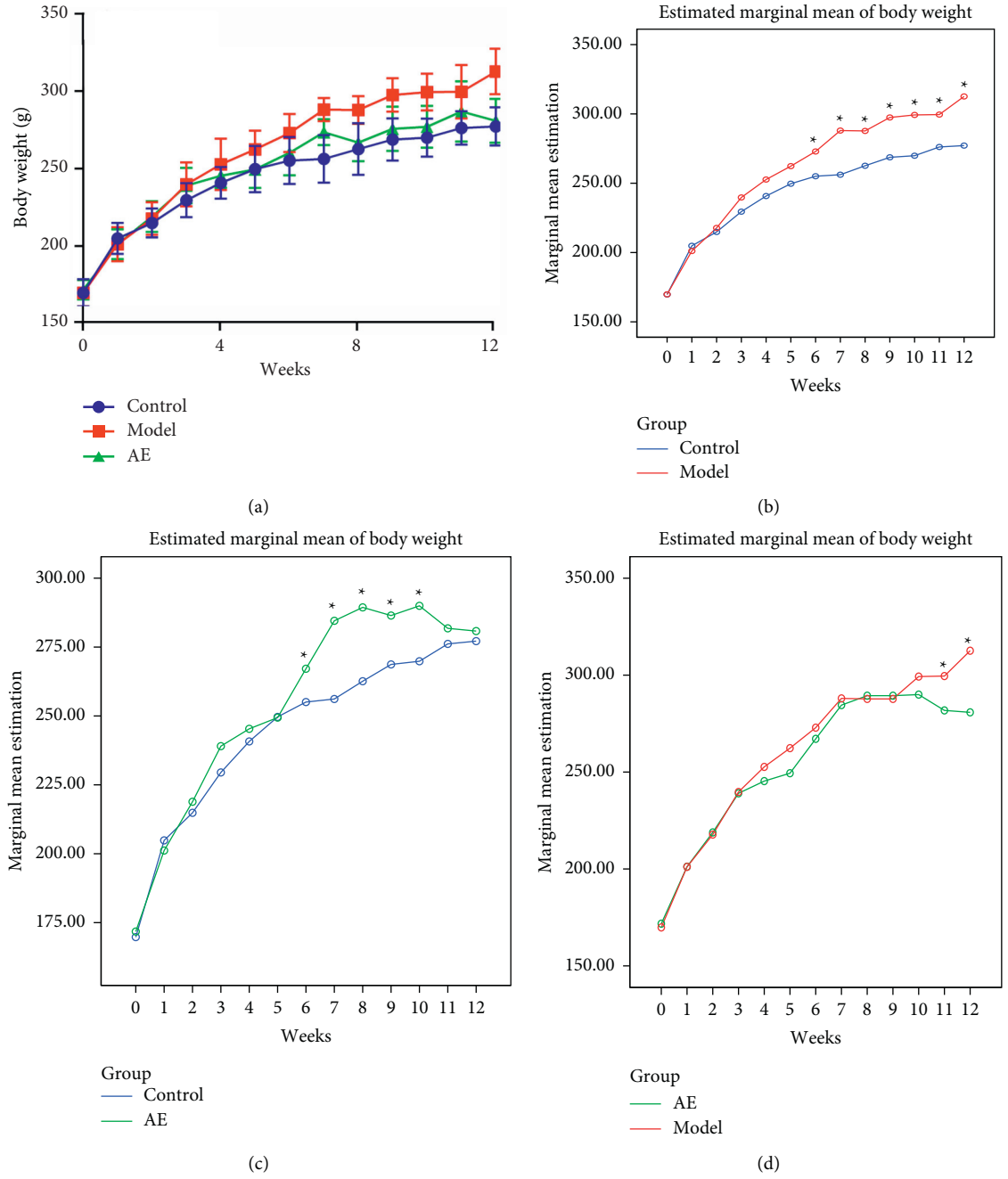


Figure 2: Effect of acupoint embedding on body weight of 12W rats. Control: normal control group. Model: model control group. AE: acupoint embedding group. $^{*}P < 0.05$. The general trend of weight change in each group (a). Line chart of simple effect analysis on body weight of control group and model group (b). Line chart of simple effect analysis on body weight of control group and AE group (c). Line chart of simple effect analysis on body weight of model group and AE group (d).

week, the body weight and Lee's index in the two high-fat diet groups were significantly higher than those fed normal diet. And the body weight in the model group was 20% higher than that in the control group, with statistically significant differences ($P < 0.01$), indicating that the modeling was successful. From the 9th week (start of the acupoint embedding intervention) to the 12th week (end of the experiment), the average body weight and Lee's index of the model group were significantly increased compared with the control group, and the difference was significant ($P < 0.05$); see Figures 2(b) and 3(b). From the 11th week, the average body weight and Lee's index of acupoint embedding group increased slowly, which had significant difference compared with the model group ($P < 0.05$); see Figures 2(d) and 3(d). But they had no significant difference compared with the control group ($P > 0.05$); see Figures 2(c) and 3(c). Compared with the model group, acupoint embedding can reduce body weight and Lee's index.

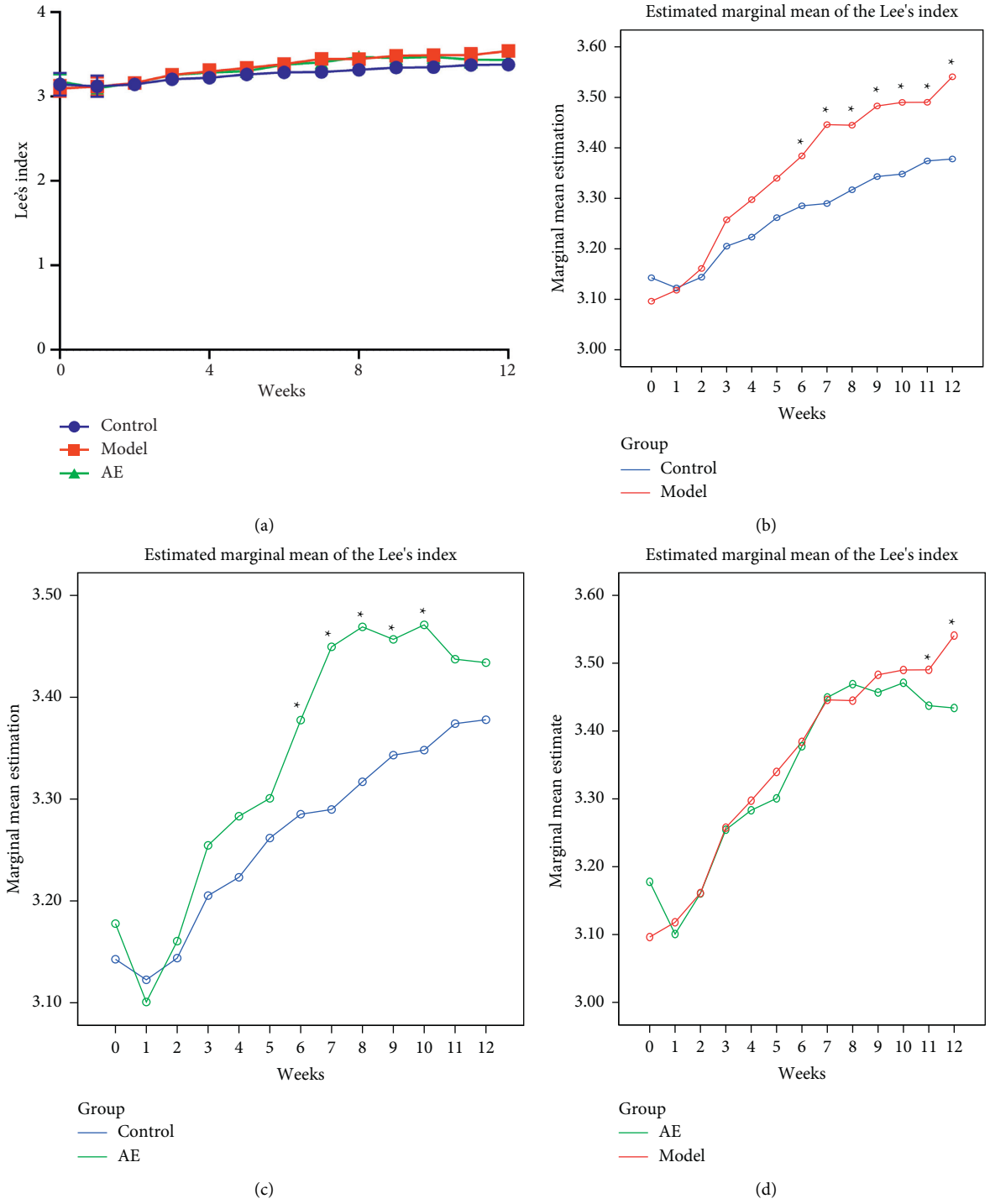


Figure 3: Effect of acupoint embedding on Lee's index of 12W rats. Control: normal control group. Model: model control group. AE: acupoint embedding group. $^{*}P < 0.05$. The general trend of Lee's index change in each group (a). Line chart of simple effect analysis on Lee's index of control group and model group (b). Line chart of simple effect analysis on Lee's index of control group and AE group (c). Line chart of simple effect analysis on Lee's index of model group and AE group (d).

3.2. Effects of Acupoint Embedding on Serum Leptin. As shown in Table 2, compared with the control group, the serum leptin level of model group rats was significantly increased ($P < 0.05$), whereas the serum leptin level in acupoint embedding group was significantly decreased after treatment compared with model group ($P < 0.05$). These results suggested that obesity may be related to the increase of leptin level and the failure of leptin to exert its normal biological effect, which is consistent with previous studies [16]. Acupoint embedding therapy can reduce serum leptin level.

Table 2: Comparison of fat coefficient of each group after treatment ($\overline{x} \pm s$).

	Normal control group	Model control group	Acupoint embedding group
WAT	0.0213 ± 0.0078	$0.0301 \pm 0.0045^{*}$	$0.0207 \pm 0.0062^{\#}$
BAT	0.0016 ± 0.0005	$0.0031 \pm 0.0006^{*}$	$0.0020 \pm 0.004^{\#}$

Note. $^{*}P < 0.05$ compared with normal control group; $^{\#}P < 0.05$ compared with model control group.

3.3. Effects of Acupoint Embedding on Blood Lipids. As shown in Table 2, the levels of serum TG, TC, and LDL were significantly increased in the model group relative to the controls, whereas the effects were blocked by acupoint embedding treatment ($P < 0.05$). The levels of serum HDL were significantly decreased in the model group relative to the controls, and the effects were blocked by acupoint embedding treatment ($P < 0.05$). The above results suggested that simple obesity rats may have abnormal lipid metabolism and lipids. Acupoint embedding therapy can regulate the level of blood lipid and alleviate dyslipidemia.

3.4. Effects of Acupoint Embedding on Fat Coefficient. As shown in Table 3, the white fat coefficient and brown fat coefficient were both increased in the model group relative to the controls ($P < 0.05$). And the white fat coefficient and brown fat coefficient of acupoint embedding group decreased after treatment compared with the model group ($P < 0.05$), but there was no difference compared with the control group ($P > 0.05$). The above results suggested that the white fat and brown fat in obese rats were increased compared with control rats, and the white fat and brown fat in obese rats were decreased after acupoint embedding treatment.

3.5. Effects of Acupoint Embedding on Adipose Morphology. As shown in Figure 4(a), the morphology of groin adipocytes of rats in each group stained with HE was observed under the field of 400x light microscope. In the adipocytes of the control group, the volume of adipocytes was generally large, and the cell margins were irregular and scattered. In the adipose tissue of model group, adipose cells were large, round, and compact. In the adipose tissue of acupoint embedding group, the adipose cells were small, uniform, and full.

As shown in Figure 4(b), compared with the control group, the adipocyte count of model group rats was significantly different ($P < 0.05$). Compared with the obesity control group, the adipocyte count in acupoint embedding group was significantly different after treatment ($P < 0.05$). Compared with the control group, the adipocyte count in acupoint embedding group had no statistical difference ($P > 0.05$).

As shown in Figure 4(c), compared with the control group, the size of adipocytes in the model group was significantly different ($P < 0.05$). Compared with the model group, the size of adipocytes in acupoint embedding group was significantly different after treatment ($P < 0.05$). Compared with the control group, there was no significant difference in the size of adipocytes in acupoint embedding group ($P > 0.05$).

The comparison of adipocyte morphology between the model group and the acupoint embedding group showed that, after the treatment, the adipocytes in the acupoint embedding group showed an increasing trend, while the cell morphology showed a decreasing trend, suggesting that the treatment could change the morphology and number of adipocytes.

3.6. Effects of Acupoint Embedding on Leptin Receptor Expression in Hypothalamus. The immunohistochemical results were shown in Figure 5(a). As shown in Figure 5(b), compared with the control group, the expression of leptin receptor in hypothalamus of obese control group rats was significantly decreased ($P < 0.05$). Combined with the above significant increase of serum leptin level in obese control rats, it suggests that there may be leptin resistance in obese rats. But after 4 weeks of treatment, the expression of leptin receptor in hypothalamus of acupoint embedding group was significantly increased compared with the model group ($P < 0.05$), and there was no difference compared with the control group ($P > 0.05$). These results suggest that the leptin resistance can be reversed by acupoint catgut embedding therapy, which can improve the expression of leptin receptor in hypothalamus and reduce the level of leptin in blood.

4. Discussion

The latest epidemiological data suggests that, by the end of 2015, about 800 million people which means 17 percent of the population were obese [17]. There are about 4 million deaths each year as a direct result of high BMI, accounting for about 7.1 percent of all deaths [18]. At present, the medicaments that treat obesity use methamphetamine, amino acid mostly which are with strong side effects. Other treatments focus on lifestyle changes such as diet and physical activity [19]. It is urgent to find effective and less side-effect treatment methods due to the great harm and insufficient treatment methods of simple obesity. In recent years, TCM therapy has achieved remarkable curative effect in treating simple obesity. Among them, the acupoint embedding method is the synthesis and development of the acupuncture and embedding needle. It produces immediate and lasting effects on human body, makes up for the short stimulation time of acupuncture, and reduces the number of times of patients visiting a doctor. It is especially suitable for modern busy obese people [20]. The latest meta-analysis shows that acupoint embedding has a significant effect on simple obesity with less incidence of adverse reactions [21]. Qi and Cheng [22] showed that, after acupoint catgut embedding treatment, 32 of the 60 female patients were significantly effective, 22 were effective, and the effective rate was as high as 90%. Tao et al. [23] used acupoint embedding therapy combined with traditional Chinese medicine to treat obese PCOS patients. After treatment, the levels of BMI and adipocytokine TNF-α in patients in the acupuncture group were significantly reduced.

Table 3: Blood lipid indexes after treatment.

Items	Normal control group	Model control group	Acupoint embedding group
Leptin	0.42 ± 0.25	$5.82 \pm 1.65^{*}$	$0.82 \pm 0.37^{\#}$
TG	0.171 ± 0.180	$0.679 \pm 0.098^{*}$	$0.188 \pm 0.006^{\#}$
TC	1.114 ± 0.093	$2.859 \pm 0.072^{*}$	$1.594 \pm 0.074^{\#}$
HDL	0.930 ± 0.077	$0.543 \pm 0.089^{*}$	$0.883 \pm 0.037^{\#}$
LDL	0.180 ± 0.033	$0.267 \pm 0.031^{*}$	$0.203 \pm 0.006^{\#}$

Note. $^{*}P < 0.05$ compared with normal control group; $^{\#}P < 0.05$ compared with model control group.

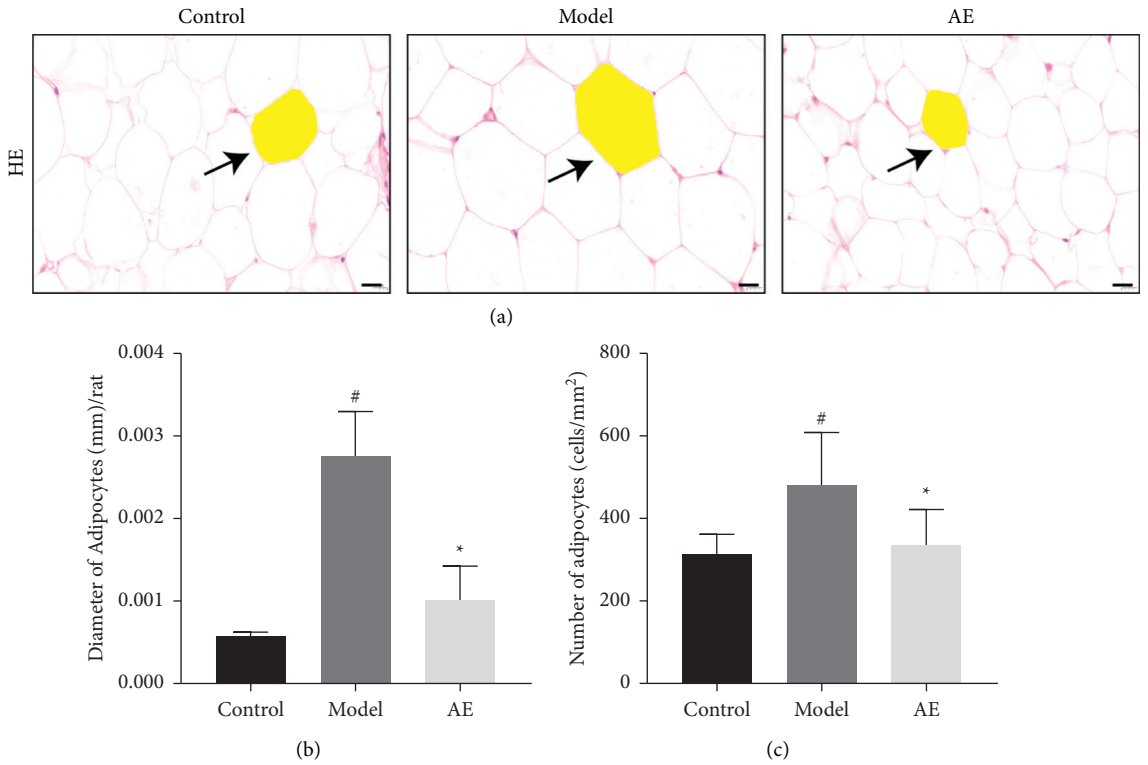


Figure 4: Effects of acupoint embedding on adipose morphology. At least 3 400-fold visual fields were randomly selected from each section of each group for photographing (a). When photographing, try to fill the entire field of view with tissue to ensure the same background light for each photo. Using Image-Pro Plus 6.0 software and 400x scale as the standard, an appropriate field of vision was selected. Diameters of 5 adipocytes (mm) were measured for each section, and the average value was calculated (c). The visual field area of each section was measured and analyzed (mm^2), the number of adipocytes in the visual field was counted (number), and the density of adipocytes per unit area (number/mm^2) = the number of adipocytes/visual field area (mm^2) which was calculated (b). $^{\#}$Compared with normal control group, $P < 0.05$; $^{*}P < 0.05$ compared with model control group.

As a product secreted by adipocytes, leptin is a peptide hormone that acts on many tissues and organs through its receptors. Zhang et al. [24] successfully cloned the obesity-related gene in mice and identified the corresponding obesity-related gene and its protein product, leptin. It is a circulating hormone secreted by adipocytes, and it is currently believed that leptin can regulate fat deposition in the body through three ways [25]: (1) regulation of energy balance through several brain mechanisms, such as decreased appetite, decreased energy intake, and increased energy expenditure; (2) promoting the expression of uncoupled protein mRNA in brown adipose tissue, which can improve the heat production, energy consumption, and metabolic rate; (3) inhibit fat synthesis. It acts on the weight regulation center of the hypothalamus, thereby causing appetite reduction, increasing energy consumption, and finally reducing weight. Leptin resistance (LR) refers to the insensitivity or unresponsiveness of tissue to the regulation of leptin. Most obese people have hyperleptinemia [26], and the possible mechanism of obesity LR is as follows: (1) LP deficiency, hyperleptinemia in 5% of obese patients; (2) LP receptor mutation. LP receptor defect cannot accept LP information [27]; (3) LP transport disorder. LEP enters central nervous system through the blood-brain barrier. (4) LP signal inhibition, LP receptor postsignal transduction disorder [28]. In this study, we found that leptin levels were significantly increased, but hypothalamus leptin receptor expression decreased in high-fat induced obese rats,

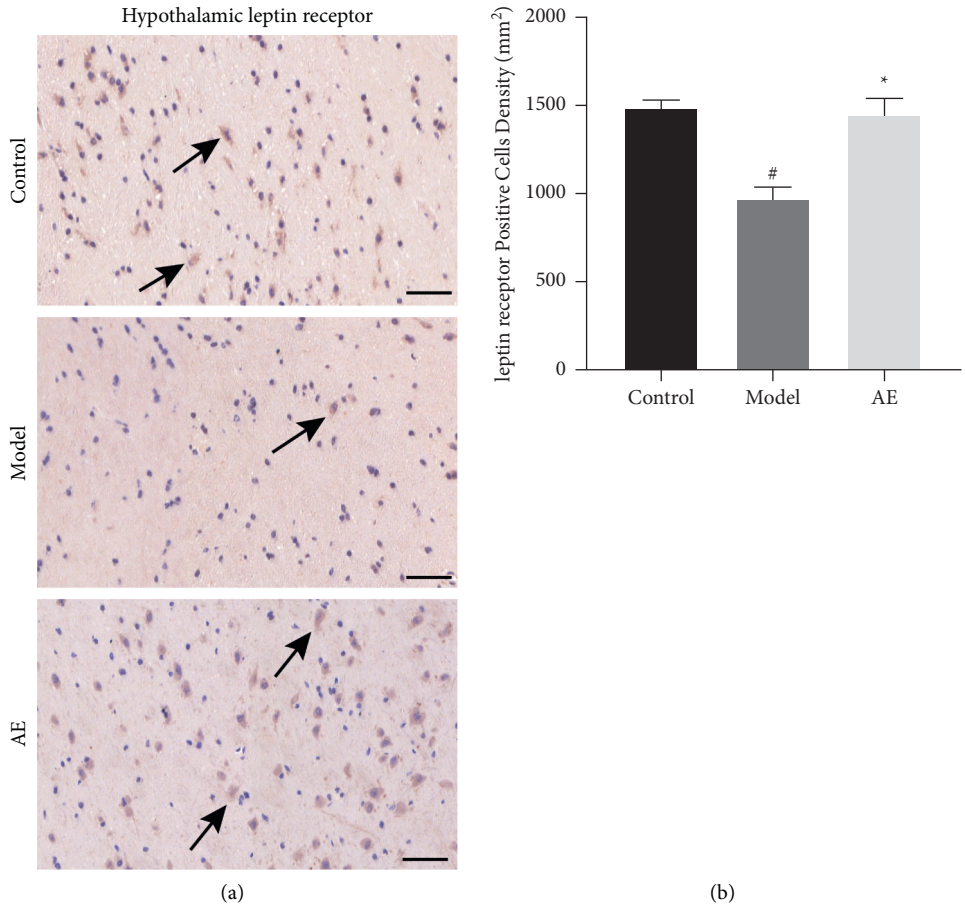


Figure 5: Effects of acupoint embedding on leptin receptor expression in hypothalamus. At least three 400-fold fields were randomly selected from the lateral hypothalamic region of each rat in each group for photography (a). CMIAS image analysis system was used to conduct directional analysis on the number of cells, and the number of weak and strong positive cells in the measured area was analyzed and calculated. The immune positive cells were yellow brown cells. Leptin receptor-positive cell density in hypothalamus: number of positive cells/tissue area to be measured (b). $^{\#}$Compared with normal control group, $P < 0.05$; $^{*}P < 0.05$ compared with model control group.

indicating there exists leptin resistance in the obese model group. Endogenous leptin is not effective in increasing energy expenditure, reducing food intake, and regulating energy balance which is consistent with previous research. After treatment, the serum leptin in acupoint embedding group decreased, and the difference was statistically significant compared with the model group ($P < 0.05$), indicating that acupuncture can effectively reduce the excessive leptin in the blood of obese rats and alleviate the leptin resistance.

Leptin receptor (OB-R) is widely distributed in the hypothalamus [29]. The combination of leptin and OB-R in the hypothalamus changes the expression of specific neuropeptides produced by other genes in the hypothalamus neurons, thus regulating the balance of energy in the body. Leptin is secreted by surrounding adipose tissue, and its levels reflect fat storage. It reaches brain tissue through blood circulation, binds to leptin receptors that feed on the central nervous system, and sends signals to the central nervous system, producing a series of effects: Inhibitory neuropeptide Y (NPY) promotes the combination of melanocorticoid and melanocorticoid receptor 4 and produces the completely opposite effect to S protein, so as to inhibit feeding and the occurrence of obesity, increase the expression of uncoupling protein in adipose tissue, and consume excess energy in the form of heat energy [7]. Leptin and leptin receptors therefore play a central role in weight regulation, and any disruption of this signaling system can lead to obesity. In this study, immunohistochemical analysis of the hypothalamic leptin receptor in obese rats induced by high-fat diet was conducted, and it was found that the expression of the hypothalamic leptin receptor was significantly decreased in the obesity model group, indicating that the leptin resistance in obese rats may be caused by the lack of receptors in the central part and the decreased expression of the hypothalamic receptor. After treatment, the expression of leptin receptor in hypothalamus was significantly increased in acupoint embedding group, indicating that acupoint embedding therapy can significantly increase the number of leptin receptor in hypothalamus, enhance the biological effect of leptin, and alleviate the leptin resistance in obese bodies, so as to achieve the goal of weight loss.

Previous studies have shown that increased adipocyte volume is an important histological feature of obesity [30, 31]. The results of this study showed that the diameter, area, and volume of adipocytes in the model group were increased compared with those in the normal group ($P < 0.05$), and the body weight and Lee's index were also significantly increased. This phenomenon suggested that obese rats in the model group had the characteristics of increased adipocyte volume and significantly increased lipid storage capacity, which was consistent with the histological changes of human obesity. Compared with the model group, the diameter of adipocytes in acupoint embedding group was significantly decreased ($P < 0.05$), and the body weight and Lee's index were also significantly decreased, which was consistent with the appearance of flat and round adipocytes distributed in sheet shape. This phenomenon can reflect the decrease of the storage capacity of adipose cells, indicating that the structure of adipose tissue changes after the overall adjustment of fat metabolism after acupoint embedding. It should be noted that the results of this study showed that, after acupoint embedding treatment, the number of adipocytes in the acupoint embedding group was higher than that in the normal control group or model control group in the field of vision ($P < 0.05$), which was inconsistent with previous studies [32]. This phenomenon was considered to be related to the level of embedding at the acupoints. Li et al. [14] conducted relevant studies, and the results showed that acupoint catgut embedding at the fat layer, muscle layer, and mixed layer had different influences on the count of adipocytes. This study aimed to study the mechanism of acupoint embedding at the subcutaneous fat layer, which was consistent with the clinical level of acupoint embedding, so embedding at the fat layer was selected.

There are two types of fat in the human body, brown adipose tissue and white adipose tissue, which play opposite roles in energy metabolism. The function of WAT is mainly to store the excess energy in the body in the form of fat, while BAT is mainly to emit the energy generated by lipid oxidation in the form of heat through the uncoupling of its mitochondria, thus playing its role [33]. This study suggested that the white fat coefficient and brown fat coefficient of obese rats were higher than those of normal rats, and the trend of decrease after acupoint embedding treatment indicated that acupoint embedding could reduce the white and brown fat coefficient of obese rats, so as to play the role of energy metabolism of fat. Obesity is the result of the imbalance of energy metabolism, which will inevitably lead to the disorder of lipid metabolism. This study showed that, compared with normal rats, the high-fat diet induced the increase of lipid level in obese rats ($P < 0.05$). After acupoint embedding treatment, with the weight reduction of obese rats, TC, TG, and LDL significantly decreased, and HDL-C increased, indicating that acupoint embedding can alleviate lipid while reducing body weight. It can reduce weight and adjust lipid metabolism and reduce the incidence of cardiovascular and cerebrovascular diseases. Acupoint embedding reduces the level of blood lipid and changes the fat structure which is an important part of weight loss. It is also the result of energy metabolism and fat metabolism tending to be normal.

In conclusion, our results show that, in leptin resistance, reduction of hypothalamic leptin receptor expression exists in the obese rats induced by high-fat diet; this makes the leptin and leptin receptor cannot effectively play the role of energy balance regulation and cause the change of the adipose cell morphology and secondary elevated blood lipid levels and lipid metabolism disorders. This process is similar to the deposition of fat and food in traditional Chinese medicine, which cannot be absorbed by the body, that is, the pathogenesis of "dysfunction of spleen in transportation" in connotation and correlation. Acupoint embedding therapy can reverse this process, reduce the level of leptin in blood, increase the number of leptin receptors in hypothalamus, enhance the biological effect of leptin, alleviate the leptin resistance in obese body, change the shape of fat, and regulate the level of blood lipid, so as to achieve the goal of weight loss. Further research is needed to determine the molecular mechanism of acupoint embedding.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Disclosure

Qiming Yang and Liwei Xing are co-first authors.

Conflicts of Interest

All the authors state that they have no conflicts of interest.

Authors' Contributions

Qiming Yang and Liwei Xing contributed equally to the manuscript.

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Research Article

The Efficacy and Safety of Acupuncture for Treating Osteoporotic Vertebral Compression Fracture- (OVCF-) Induced Pain: A Systematic Review and Meta-Analysis of Randomized Clinical Trials

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Background. Osteoporotic vertebral compression fractures (OVCFs) are common health issues in the elderly that cause chronic pain in over one-third of patients. This study was sought to evaluate the efficacy and safety of acupuncture for alleviating pain caused by OVCFs. *Methods*. We performed a search of 8 electronic databases for publications from the inception to 30th March 2021. Eligible studies were randomized clinical trials (RCTs) that evaluated the effect of acupuncture for the treatment of OVCFs. Two investigators evaluated literature quality and extracted data independently. RevMan V.5.4.1 was used for data analyses, with pooled risk estimates presented as mean difference (MD) or relative risk (RR) along with corresponding 95% confidence intervals (CIs), as appropriate. *Results*. Fourteen RCTs involving 1,130 patients were included in this meta-analysis. Compared with the control group, acupuncture showed a greater benefit on pain reduction caused by OVCFs (1 week: MD = −1.26, 95% CI: (−1.82,−0.70); 1 month: MD = −1.63, 95% CI: (−1.82,−1.43); 6 months: MD = −1.13, 95% CI: (−1.55, −0.70)). Acupuncture treatment was also associated with fewer adverse events, lower ODI index, and higher bone density than the control group (safety: (RR: 0.30, 95% CI: 0.12–0.75); ODI: MD = −3.19, 95% CI: (−5.20, −1.19); bone density: MD = 0.15, 95% CI: (0.05, 0.26)). The GRADE quality of these results was assessed as low or very low. *Conclusions*. Compared with the control treatment, acupuncture was more effective and safer in relieving the pain caused by OVCF and made a greater improvement on patient's ODI score and bone density. Given the low level of our study evidence, future high-quality studies are needed to verify our study findings.

1. Background

With population ages, the proportion of the elderly population (>60 years old) will continue to increase [1–3]. It is estimated that the proportion of the elderly population will reach 21.1% (approximately 2 billion) by 2050 [4–6]. Osteoporotic vertebral compression fracture (OVCF) is a common type of fracture in the elderly, with risk increasing continuously with age [7, 8]. Chronic pain is the major symptom of OVCF [9], which not only largely reduces patients' quality of life, but also imposes a heavy medical burden to aged healthcare [10, 11]. Analgesics are commonly used treatments in clinical practice for alleviating the pain symptoms of patients with OVCF, but there are still 40%–60% of patients who experienced intolerable pain within 2 years of the fracture [12]. Even for cured patients, their levels of pain are higher than the levels of their prefracture state [12]. Therefore, novel therapeutic approaches are required to further relieve pains and improve the patient's health [12].

Acupuncture is a traditional treatment approach in China that has been widely acknowledged worldwide [13]. Existing evidence showed that acupuncture is effective in treating most chronic pains, with and without being compared with other standard treatments [14–16]. Informed by it, the World Health Organization recommends use of acupuncture to treat a variety of pains including low back pain [13, 17]. In view of this, acupuncture has also been used by many clinicians for relieving pains caused by OVCF. However, there is lack of systematic evidence showing the effectiveness and safety of acupuncture on the management of pains caused by OVCF. To fill the evidence-practice gap and inform evidence-based clinical practice, we conducted this meta-analysis of randomized controlled trials assessing the clinical efficacy and safety of acupuncture versus controls in patients with OVCFs who underwent pain symptoms.

2. Methods

2.1. Data Sources and Selection Strategy. We searched published studies from the following electronic databases (4 Chinese and 4 English databases): PubMed, EMBASE, Cochrane Library, Web of Science, China BioMedical Literature (CBM), China National Knowledge Infrastructure (CNKI), Chinese Scientific Journals Database (VIP), and Wanfang database. Randomized controlled trials (RCTs) of acupuncture in the treatment of OVCF published from inception of the databases to 30 March 2021 were considered in the current study. The search was only limited to human studies, and no language restrictions were made. We used subject ("Osteoporosis" "Fractures, Compression" and "Acupuncture") and free words ("Osteoporoses" "Bone Loss" "Bone Losses" "Compression Fracture" "Fracture, Compression" "Compression Fractures" "Acupuncture and moxibustion" "needle therapy" "needle" "Electroacupuncture") jointly to search the titles and abstracts in the databases aforementioned. The search strategy was as follows, taking PubMed as an example:

(1) "Osteoporosis"[Title/Abstract] OR "Osteoporoses"[Title/Abstract] OR "bone loss"[Title/Abstract] OR "bone losses"[Title/Abstract]

(2) Fractures compression"[Title/Abstract] OR "compression fracture"[Title/Abstract] OR "fracture compression"[Title/Abstract] OR "compression fractures"[Title/Abstract]

(3) Acupuncture"[Title/Abstract] OR ("Acupuncture"[Title/Abstract] AND "moxibustion"[Title/Abstract]) OR "needle therapy"[Title/Abstract] OR "needle"[Title/Abstract] OR "Electroacupuncture"[Title/Abstract]

(4) (1) and (2) and (3)

Inclusion criteria: the following criteria were considered for the inclusion of RCTs in the present study (PICO format): (1) participants: No restrictions on country, race, language, age, and gender. Patients should be clearly diagnosed with OVCF by X-ray, CT or MRI examination, etc., with no established serious cardiovascular and cerebrovascular diseases and contraindications to acupuncture. Patients did not have cognitive impairment and pains caused by other diseases such as tumors and tuberculosis. (2) Intervention: acupuncture as the main treatment; a comparative study carried out between acupuncture and the control group. (3) Control: any type of control group including surgery, western medicine, etc. (4) Outcomes: Visual Analogue Scale (VAS, 0 point for painless and 10 points for extreme pain. Higher scores indicated more serious pains); Oswestry disability index (ODI); bone density and safety. (5) Study type: RCTs.

Exclusion criteria: studies with incomplete data and information, studies combined with other traditional Chinese methods. Also, duplicate articles were excluded.

2.2. Data Extraction. We used standard data extraction methods to extract data. The basic information, sample characteristics, intervention measures, outcome indicators, and other data, which were included in the article, were extracted by two investigators. In case of any inconsistency occurring in the result, this was further discussed by the two investigators or scrutinized by a third person.

2.3. Methodological Quality of Assessment. The quality of included studies was assessed by two investigators independently, using Cochrane risk assessment tool. Seven domains used for the quality assessment include: random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective reporting, and other bias. Each part can be graded into three categories: low risk, unclear risk, and high risk. Researchers made judgments based on the bias risk evaluation criteria of the Cochrane Handbook bias risk assessment tool.

The scoring system provided by The Grading of Recommendations Assessment, Development and Evaluation (GRADE) Working Group was used to assess the evidence quality for a specific outcome across studies. Five criteria were evaluated including study limitations, inconsistency, indirectness, imprecision, and publication bias. The evidence quality level of RCT is assigned an a priori as high but may be downgraded to intermediate, low-level, or very low-level if there is any identifiable bias.

2.4. Statistical Analysis. Data for the included literature was analyzed using RevMan 5.4.1 software downloaded from the Cochrane website. Data merging chose different methods according to the type of data. We chose the mean difference (MD) for continuous outcomes and risk ratio (RR) for categorical outcomes. Random effect model and fixed effects model were used to perform analysis, as appropriate. The I^2 statistic was calculated to describe between-study heterogeneity. When $I^2 \geq 50\%$ and $P < 0.05$, we used the random effects model to pool the data; otherwise, the fixed effects model was selected for data merging. According to the different time lengths for VAS evaluation in each study, we formulated 3 different evaluation time length: 1 week (or 7

days), 1 month (4 weeks or 30 days), and 6 months. The ODI score and bone density measurement are based on the data collected after one month of acupuncture treatment. Subgroup analysis was performed according to different treatments combined with acupuncture (acupuncture combine with drugs, acupuncture combine with surgery). Sensitivity analysis was performed when necessary. Publication bias was estimated using a funnel plot (articles ≥10).

3. Results

3.1. Literature Screening. We retrieved 584 articles initially. We then removed 251 duplicate articles manually leaving 333 articles. Out of 333 articles, 231 were excluded after reading title and abstract. Out of the remaining articles, five articles lack full text, and 83 were excluded because they failed to meet the inclusion criteria through complete reading. The present study eventually included 14 articles [18–31] (Figure 1), in which a total of 1,130 patients were randomly assigned to acupuncture-based ($n = 573$) or control therapy ($n = 557$).

3.2. Characteristics of Included Studies. Among the 14 (Table 1) RCTs that met the inclusion and exclusion criteria, 2 [25, 31] were in English, and 12 were in Chinese. Two [18, 27] were master's thesis, and the other twelve were journal articles. The study type of all included papers was single-center randomized controlled trial undertaken in China. Nine [18, 20, 23, 24, 26, 27, 29, 31] studies used surgery combined with acupuncture (including 4 percutaneous kyphoplasty (PKP) surgeries and 5 percutaneous vertebroplasty (PVP) surgeries). The intervention group received acupuncture analgesia after the operation, and the control group did not use acupuncture analgesia after the operation. Five studies used conservative treatment, the observation group of 4 studies [19, 21, 25, 28] used drugs combined with acupuncture treatment, and the control group only used drugs. Only one study [22], the observation group, was treated with acupuncture, and the control group was treated with medication. The shortest acupuncture intervention treatment time was 6 days, and the longest was 6 months. The intervention frequency of 11 studies was once a day, 1 study [26] every other day, 1 study [23] twice a week, and one study [18] based on different stages of the patient's disease course use of different frequencies (early stage (1 month after surgery): Qd in the first week; Qod in the second week; Biw in the third to fourth weeks. Mid-term (2 to 3 months after surgery): the first Week Qd; 2nd week Qod; 3rd to 6th week Biw. Late stage (3 to 6 months postoperatively): 1st to 4th week Biw). The observation time of outcome indicators (VAS) was not consistent across studies. The shortest VAS evaluation time was 48 hours, and the longest was 2 years. 2 [18, 23] out of 14 studies had an initial VAS evaluation time of less than one week, and 3 studies [18, 23, 30] had the last follow-up over 3 months,

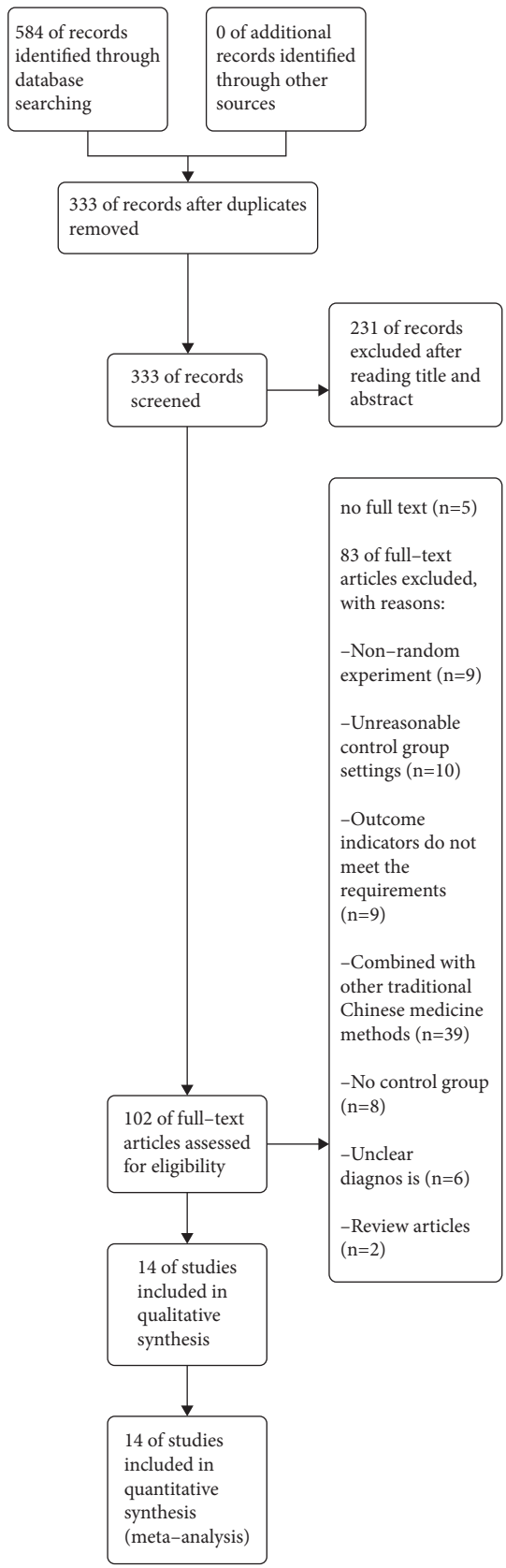

Figure 1: A flowchart showing the selection process.

Table 1: Characteristics of included studies.

Author, year	Random method	Blinding	No. (acupuncture/control)	Age (years)	Acupuncture group			Control group	VAS evaluation time
					Intervention	Frequency	Treatment cycle	Intervention	
Zhang W, 2015	RNT	UC	30/15	55–80	M + A	QD	3 weeks	M	1, 2, 3 weeks
Liu K, 2017	UC	UC	36/36	≥65	PKP + A	Compound therapy	6 months	PKP	3 days, 1, 6, 12 months
Huang M, 2016	UC	UC	20/20	65–88	PKP + A	QD	6 days	PKP	1 week, 1, 6 months, 1, 2 years
Liu G, 2019	RNT	UC	43/43	57–77	PVP + A	QD	30 days	PVP	7 days, 30 days
Feng MG, 2017	Draw lots	UC	75/75	50–79	PVP + A	QD	30 days	PVP	7 days, 30 days
Chen QF, 2020	RNT	UC	60/60	52–79	PVP + A	QD	30 days	PVP	7 days, 30 days
Liu ZG, 2020	RNT	UC	43/43	60–71	PVP + A	Every other day	12 weeks	PVP	12 weeks
Chen SS, 2019	RNT	UC	36/36	50–65	PKP + A	QD	2 weeks	PKP	2 weeks, 1 month
Chen ZS, 2018	RNT	UC	32/31	48–80	PVP + A	BIW	6 months	PVP	2 days, 6 months
Zhang LQ, 2020	RNT	UC	30/30	50–85	PKP + A	QD	2 weeks	PKP	1,2 weeks
Feng ZW, 2020	RNT	UC	41/39	49–72	M + A	QD	6 months	M	3 months
Ping D, 2020	RNT	UC	35/35	60–80	M + A	QD	2 month	M	1 month
Li Y, 2020	RNT	UC	45/45	61–78	M + A	QD	3 months	M	30 days
Li SM, 2018	RE	UC	50/50	62–74	A	QD	2 months	M	7,14 days

M: medicine; A: acupuncture; RNT: random number table; RE: randomized envelope; UC: unclear; QD: Quaque die; BIW: twice a week; compound therapy, early stage (1 month after surgery): QD in the first week; Qod in the second week; BIW in the third to fourth weeks. Midterm (2 to 3 months after surgery): the first week QD; 2nd week Qod; 3rd to 6th week BIW. Late stage: 3 to 6 months postoperatively.

and the most time for outcome evaluation of VAS was 1 month after treatment. 8 studies [18, 20, 21, 24, 28–31] conducted VAS evaluation at this time. Only 3 [22, 25, 27] of 14 studies reported adverse reactions.

3.3. Methodological Quality. The quality of the included studies is generally low (Figures 2 and 3). All studies were randomized controlled studies, 2 studies [18, 30] did not introduce specific randomization methods, 10 studies [19–21, 23–28, 31] used low-risk random number table grouping, 1 study [29] used lottery grouping, and 1 study [22] used the envelopes that were randomly grouped. No study used blinding for the allocation process. Due to the nature of acupuncture treatment, it was impossible to blind patients and practitioners in acupuncture-related RCTs. Among all the studies, the data of 13 studies were complete, and one study [23] reported missing data and was judged as high risk. Because no studies were registered in advance, it is not clear whether there is a risk of reporting bias and other risks.

We did not test the publication bias in this meta-analysis due to the insufficient number of studies for the analysis for each time period (<10).

3.4. Meta-Analysis of Effectiveness. A total of 1,130 patients were studied in the selected 14 studies, of which 573 in the acupuncture group and 557 patients in the control group. According to the different VAS evaluation times, we performed the evaluation at 5 time points.

First, we evaluated the pain relief in a week after acupuncture treatment of OVCF (Figure 4), with 7 studies [20, 22, 24, 25, 27, 29, 30] of 601 patients included in the analysis. The combined results showed that the acupuncture-based treatment had a significantly greater efficacy in treating pains caused by OVCF than the control treatment (MD = −1.26, 95% CI: [−1.82 to −0.70], $I^2 = 92\%$).

We evaluated the VAS after one month of acupuncture treatment for OVCF. A total of 8 studies [18, 20, 21, 24, 28–31] conducted VAS testing after one month of treatment. The heterogeneity among the studies was moderate ($I^2 = 67\%$). Random effects model was used to merge the data. The results suggest that the acupuncture has a greater efficacy than the control group (MD = −1.63, 95% CI: (−1.82, −1.43)) (Figure 5).

Finally, we evaluated the studies with a VAS evaluation time of 6 months (Figure 6). There were three studies [18, 23, 30] that had the VAS evaluation time in 6 months, and between-study heterogeneity was large. The pooled results showed a greater analgesic efficacy of acupuncture treatment for treating pains caused by OVCF than the control group.

3.5. Safety Analysis. Three studies [22, 25, 27] reported adverse events. Compared with the control group, the risk of any adverse event was significantly lower in the acupuncture group (RR = 0.30, 95% CI: 0.12–0.75, $I^2 = 55\%$) (Figure 7).

3.6. ODI Score Analysis. Six of the 14 studies reported ODI scores of patients in different periods. We pooled the data during the period over which the ODI scores were collected

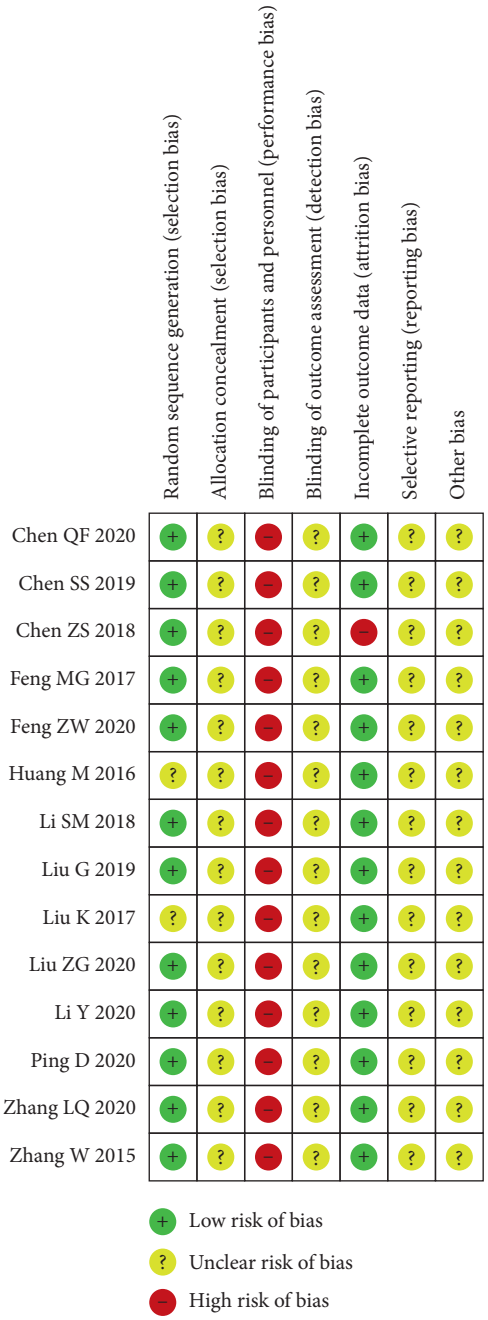

Figure 2: Risk of bias summary. +: low risk of bias; ?: unclear risk of bias; −: high risk of bias.

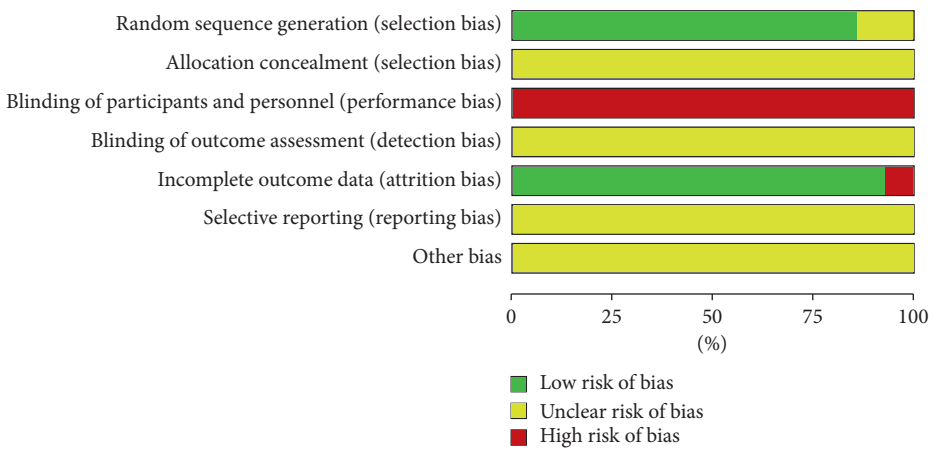

Figure 3: A graph showing the risk of bias.

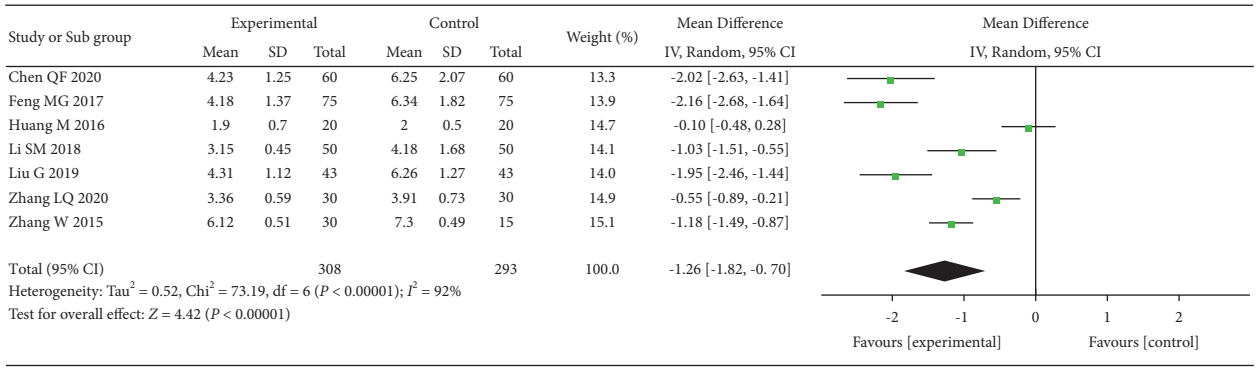

Figure 4: The forest plot shows the comparison of VAS between the acupuncture and control group in one week of treatment for pain caused by OVCF.

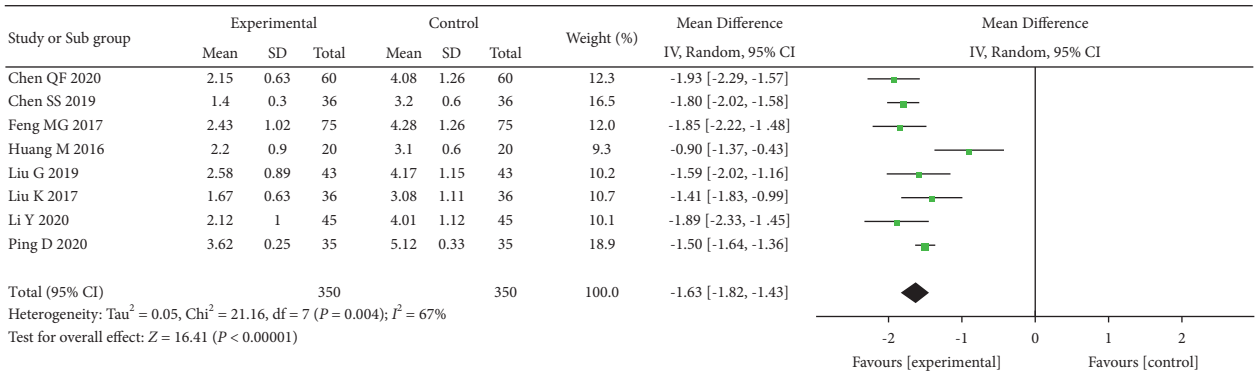

Figure 5: The forest plot shows the comparison of VAS between the acupuncture and control group in one month of treatment for pain caused by OVCF.

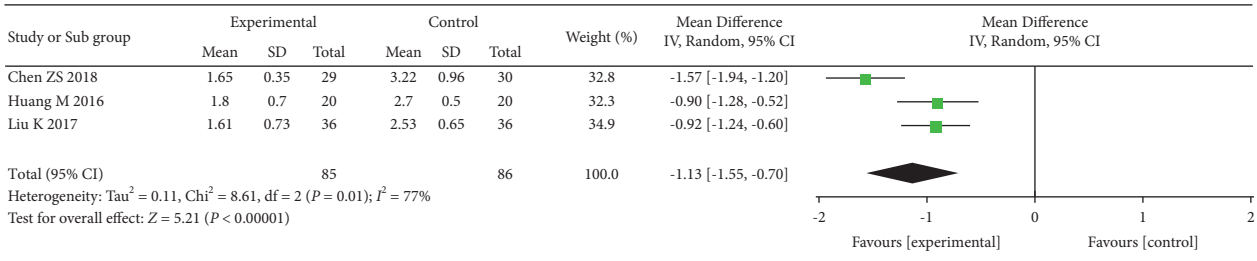

Figure 6: The forest plot shows the comparison of VAS between the acupuncture and control group in 6 months of treatment for pain caused by OVCF.

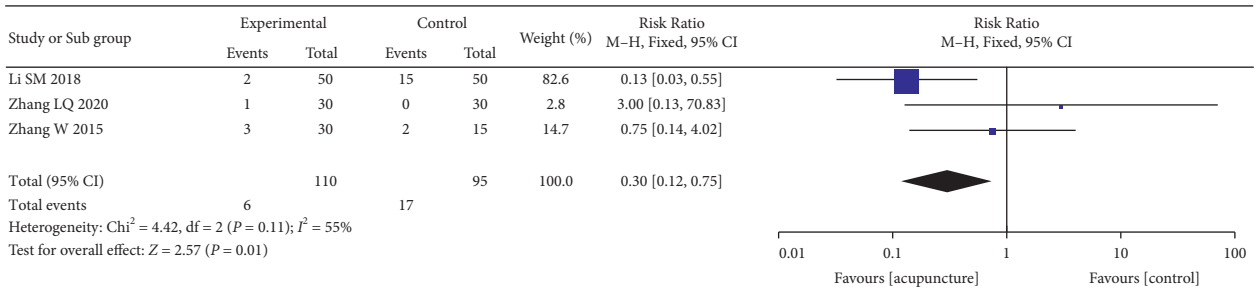

Figure 7: The forest plot shows the comparison between the acupuncture and control group in safety.

most frequently (1 month). A total of 304 patients from 4 studies [18, 20, 30, 31] were included in this analysis. The analysis results show that, compared with the control group, acupuncture significantly reduces patients' ODI scores (Figure 8).

3.7. Bone Density Analysis. Seven of the 14 studies measured the bone mineral density of patients. However, because of the inconsistency in the measurement method and the measurement time across studies, we only included 4 studies [20–22, 29] of 440 patients that used the same measurement method and the measurement time (1 month after acupuncture treatment). The results show that acupuncture significantly increased the bone density of patients compared with the control (Figure 9).

3.8. Subgroup Analysis. Subgroup analysis was conducted based on different treatment methods: acupuncture combined with surgery and acupuncture combined with drug (or only use acupuncture). Two time points were chosen to perform the subgroup analysis (1 week and 1 month). For both time points, combining the acupuncture with drug or with surgery was more effective in reducing pains caused by OVCF than the control treatment (Figures 10 and 11).

3.9. Sensitivity Analysis. Given the high heterogeneity between the studies, a sensitivity analysis was performed at the time point of 1 month. Through the analysis, we found that one study [30] may be the heterogeneity source. After removing this study, the between-study heterogeneity was no longer statistically significant (Figure 12).

3.10. GRADE Evidence Quality Evaluation. The quality of evidence applied for each outcome was summarized in the 'Summary of findings' table based on the GRADE approach (Table 2). The quality of evidence on the efficacy (one month), safety, the ODI score, and bone mineral density associated with acupuncture was rated as low, very low, very low, and very low, respectively.

4. Discussion

In this systematic review and meta-analysis, we found that acupuncture is better than the control group in treating pains caused by OVCFs over both short term and long term (from 1 week to 6 months), despite the large between-study heterogeneity for most outcomes. The pooled data from three studies reporting adverse events showed that acupuncture was safer than the control treatment in reducing pains caused by OVCFs. The study results also showed that, compared with control treatment, acupuncture significantly reduced the ODI score and increased the bone density of patients, suggesting a better clinical prognosis in those who received acupuncture. The subgroup analyses found that combining the acupuncture with either drugs or surgery was more effective than the control treatment in reducing pains caused by OVCFs. However, it is worth noting that the levels of evidence quality for outcomes ranged from low to very low.

By pooling the results from existing studies, our study found that acupuncture can effectively relieve pain caused by OVCFs, although the level of evidence was low. This study has several implications for patients with OVCFs. For patients who are treated conservatively, acupuncture can reduce the amount of use of analgesic drugs and the occurrence of side effects. For patients treated by surgery, acupuncture can promote earlier rehabilitation and improve clinical prognosis [18]. Although the mechanism of acupuncture alleviating pains is not fully explained, most studies suggested that it works by affecting the conduction of pain signals or by controlling inflammatory responses. Xu et al. [32] found that acupuncture could play a rapid analgesic role by inhibiting the upward conduction system of pain or promoting the downward inhibitory system and play an analgesic role in chronic pain by controlling the peripheral and central inflammatory responses. This finding is consistent with the research results of Lu et al. [17]. Liang et al. [33] and Wang et al. [34] studied the analgesic effect of acupuncture at the molecular level and found that the inactivation of spinal microglia and astrocytes mediated the immediate and long-term analgesic effects of electroacupuncture. Yang et al. [35] found that acupuncture can improve local blood circulation and promote the absorption of inflammation and edema, thus relieving pain at local sites. Abraham et al. [36] found that transient receptor potential vanilla-1 (TRPV1) may mediate the local analgesic effect of electroacupuncture. In addition to this, Chen et al. [37] showed that endogenous opioids, cholecystokinin octapeptide (CCK-8), 5-hydroxytryptamine (5-HT), noradrenalin, dopamine, glutamate, γ -aminobutyric acid (GABA), acetylcholine (ACTH), orexin A (OXA), and other substances mediate the analgesic effect of acupuncture.

Safety of acupuncture: our study concluded that the safety of acupuncture was higher than that of the control group. The favorable safety profile of acupuncture will promote more patients to choose it as a preferential treatment [15], especially those who are vulnerable and intolerable to the standard treatment. Although some patients experience fainting and bruising during the acupuncture, these adverse events are generally mild and faded away shortly after treatment. In this meta-analysis, only three studies reported adverse events related to study treatment assignment; thus, the reporting bias cannot be ruled out. More robust evidence is still needed to inform the safety of acupuncture in clinical practice.

Effect of acupuncture on ODI index: acupuncture can reduce the ODI index of OVCFs patients, a gold standard for the self-reported evaluation of the quality of life of patients with lumbar spine [18]. Since the pain is a major factor that affects the ODI index, the decreased ODI index with acupuncture is a likely result of its beneficial effect on pain relieving.

Effect of acupuncture on BONE mineral density: decreased bone mineral density is an important factor leading to OVCF in patients. Our study suggested that acupuncture could improve bone mineral density of OVCF patients,

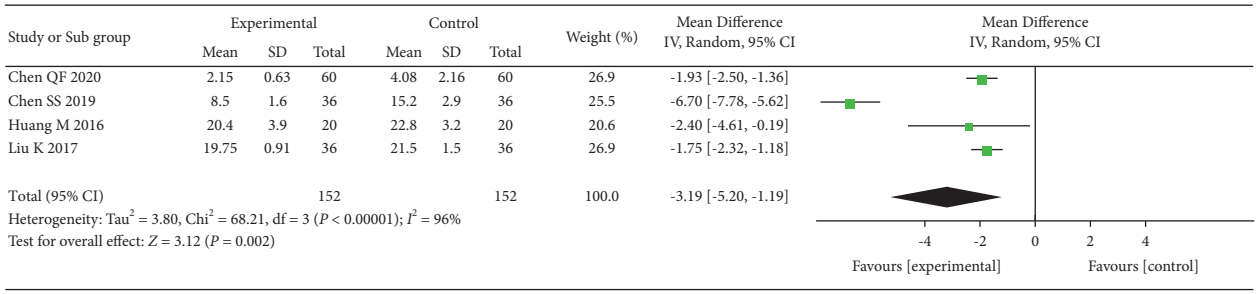

Figure 8: The forest plot shows the comparison between the acupuncture and control group in the ODI score.

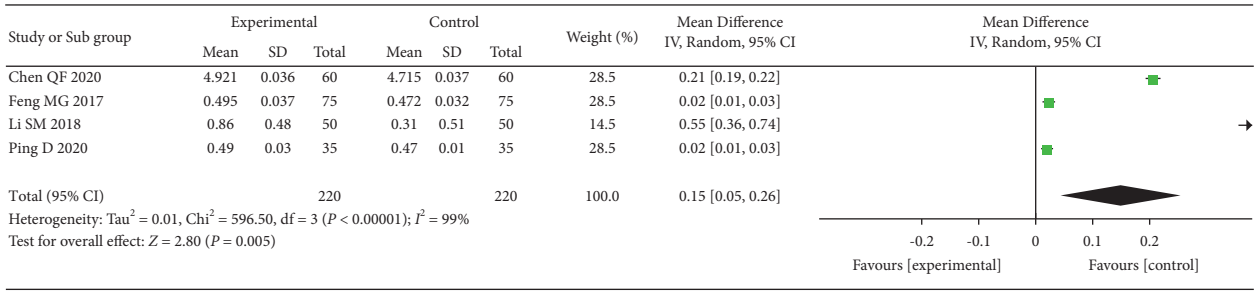

Figure 9: The forest plot shows the comparison between the acupuncture and control group in bone density.

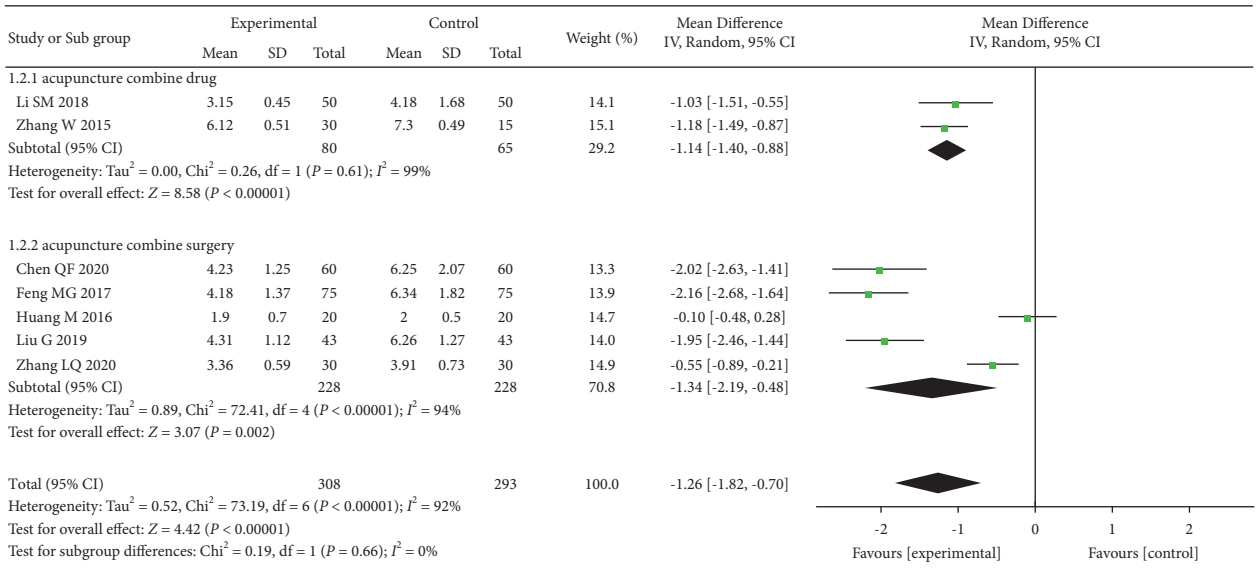

Figure 10: The forest plot shows the comparison of VAS between the acupuncture and control group in 1 week of treatment for pain caused by OVCF, subgroup analysis based on different treatment methods.

which was consistent with the research results of Wang et al. [38] and Zeng et al. [39]. Wang Gang et al. found that warm acupuncture at Back-Shu points and Jiaji (EX-B 2) points, the common points in OVCF, could reduce the BGP and IL-6 levels in serum, thus reducing bone absorption and inhibiting excessive bone turnover, and finally increasing bone mineral density and improving clinical symptoms. Through animal experiments, Wu et al. [40] found that acupuncture at Back-Shu points and Mingmen point could delay bone loss and improve bone strength and ultrastructure.

Factors influencing acupuncture: the effect of acupuncture can be affected by intervention frequency, number of acupuncture, acupuncture retention time, etc. Taking acupuncture retention time as an example, studying the time of acupuncture retention for tinnitus treatment, Wen et al. [41] found that the effective rate of acupuncture retention for 60 minutes was higher than that for 30 minutes, a timing used frequently in clinical practice. By studying the retention time of acupuncture in the treatment of perimenopausal syndrome, Yang et al. [42] found that the effective rate of electroacupuncture

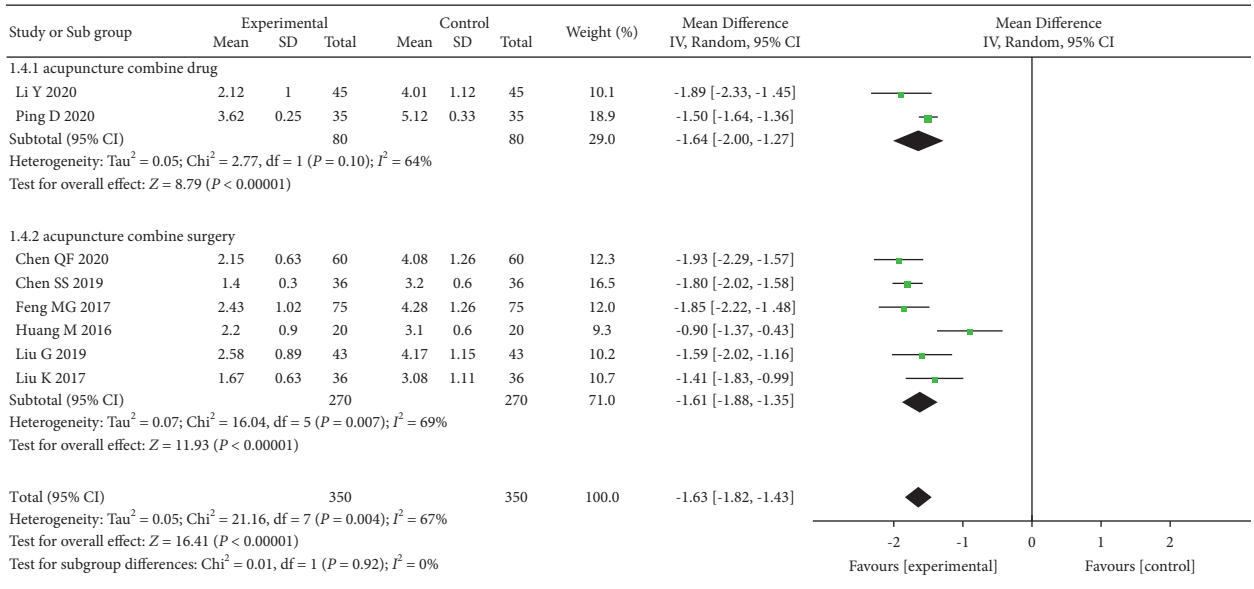

Figure 11: The forest plot shows the comparison of VAS between the acupuncture and control group in 1 month of treatment for pain caused by OVCF, subgroup analysis based on different treatment methods.

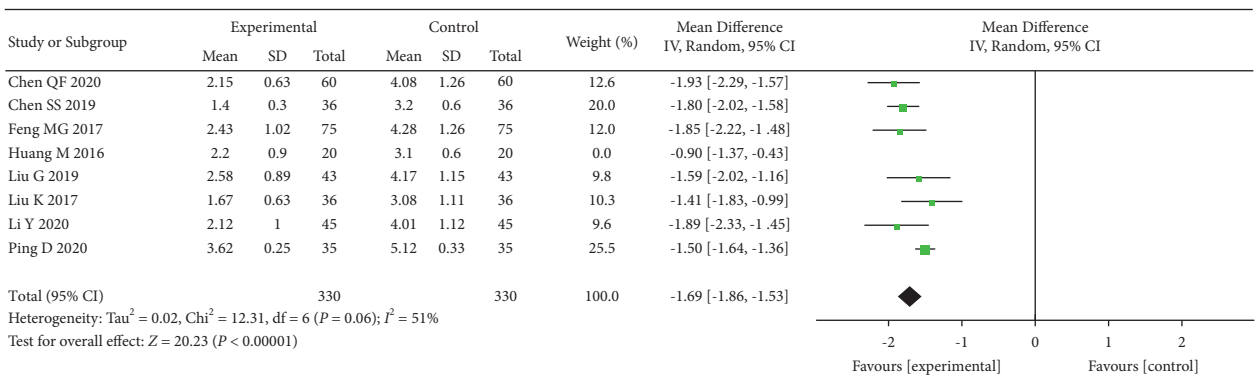

Figure 12: The forest plot shows the comparison of VAS between the acupuncture and control group in 1 month of treatment for pain caused by OVCF; one study is not calculated.

after 25 minutes was 100%, the effective rate of electroacupuncture after 40 minutes was 95.1%, and the effective rate was 92.31% when the needle was not retained (1–5 minutes). The timing of acupuncture plays a role on its effect. However, the benefit obtained does not simply increase with increasing timing. The times of acupuncture used also affect its efficacy. In our included literatures, there is a study [30] that the retention time of acupuncture was 20 minutes, and the times of acupuncture used were six; also, in this study, we found that it was the main source of heterogeneity when we conducted sensitivity analysis. Our analysis results showed that the commonly used acupoints were Back-Shu points and Jiaji (EX-B 2) points at the level of bone fracture, and the frequency was commonly once per day. Treatment duration was longer than 2 weeks in all but one study; retention time: 12 studies had retention time greater than 30 minutes (one was not recorded), and the longest retention time was 45 minutes.

4.1. Limitations. Several limitations of this study should be noted. First, all the included studies were single-center RCTs conducted in China; thus, our study results may not be generalizable to patients from other regions or countries. Second, except that the study itself is difficult to implement blinding, all studies lacked blinding of allocation methods and evaluators, and no studies were registered in advance before trial conducted. These might have largely reduced the quality of evidence generated from our analysis. Third, the heterogeneity between studies was large. Subgroup analysis and sensitivity analysis revealed that acupuncture combined with different treatment methods, retention time of acupuncture, and frequency of acupuncture were possible sources of heterogeneity. We only analyzed the heterogeneity at one point in time and did not analyze the heterogeneity in other time points because of the small number of studies. Fourth, the acupuncture points used in the studies included in this meta-analysis were different. This might have led to the differences in effectiveness of acupuncture

Table 2: Summary of findings.

Acupuncture for OVCF						
Patient or population: patients with OVCF Settings: inpatient Intervention: acupuncture						
Outcomes	Illustrative comparative risks* (95% CI) Assumed risk Control	Corresponding risk Acupuncture	Relative effect (95% CI)	No. of Participants (studies)	Quality of the evidence (GRADE)	Comments
One-week VAS VAS Follow-up: mean 1 weeks		The mean 1-week VAS in the intervention groups was 1.26 lower (1.82 to 0.7 lower)		601 (7 studies)	⊕⊝⊝⊝ very low[1,2,3]	
One-month VAS VAS Follow-up: mean 1 months		The mean 1-month VAS in the intervention groups was 1.69 lower (1.86 to 1.53 lower)		660 (7 studies)	⊕⊕⊝⊝ low[1,3,4]	
Six-month VAS VAS Follow-up: mean 6 months		The mean 6-month VAS in the intervention groups was 1.13 lower (1.55 to 0.7 lower)		171 (3 studies)	⊕⊝⊝⊝ very low[1,2,3,5]	
Safety	Study population 179 per 1000 Moderate 133 per 1000	54 per 1000 (21 to 134) 40 per 1000 (16 to 100)	RR 0.3 (0.12 to 0.75)	205 (3 studies)	⊕⊝⊝⊝ very low[1,3,5]	
ODI Oswestry dysfunction index (ODI) Follow-up: mean 1 months		The mean ODI in the intervention groups was 3.19 lower (5.2 to 1.19 lower)		304 (4 studies)	⊕⊝⊝⊝ very low[1,2,3]	
Bone density X-ray determination of bone density Follow-up: mean 1 months		The mean bone density in the intervention groups was 0.15 higher (0.05 to 0.26 higher)		440 (4 studies)	⊕⊝⊝⊝ very low[1,2,3]	

*The basis for the assumed risk (e.g., the median control group risk across studies) is provided. The corresponding risk (and its 95% confidence interval) is based on the assumed risk in the comparison group and the relative effect of the intervention (and its 95% CI). OVCF: osteoporotic vertebral compression fracture; CI: confidence interval; RR: risk ratio; VAS: visual analogue scale; ODI: Oswestry disability index; GRADE working group grades of evidence: high quality: further research is very unlikely to change our confidence in the estimate of effect. Moderate quality: further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate. Low quality: further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate. Very low quality: we are very uncertain about the estimate. [1]No description of allocation concealment and blinding. [2]The heterogeneity is large and cannot be explained. [3]Publication bias is not tested, but there is a lot of dedication. [4]The heterogeneity was explained after we performed sensitivity analysis. [5]Sample size is too small ($n < 300$).

between studies and contributed to the between-study heterogeneity. Last, the level of evidence for our findings was from low to very low. More high-quality studies are needed to validate our study results.

4.2. Implications for Future Research. This study suggests that acupuncture can be used as a useful supplementary approach adding to the drugs and surgery in alleviating the pain caused by OVCFs. Future studies with a more rigorous trial design are needed to improve trial quality, such as implementing allocation concealment to reduce selection bias. RCTs that are designed to compare the effectiveness of using different selected acupoints for acupuncture are also needed to facilitate the selection of the acupuncture points with greatest benefits for treatment OCVF-related pains. Given that all the studies included in this meta-analysis were conducted in China, relevant RCTs examining the effects of acupuncture on treating OVCFs-induced pains recruiting participants from other countries are needed. For the studies included in this meta-analysis, few evaluated the effect of acupuncture on the quality of life of patients. Future studies may target this area.

5. Conclusions

Compared with the control group, acupuncture is more effective in alleviating the pains caused by OVCFs over both short term and long term and has a favorable safety profile. At the same time, acupuncture can reduce the ODI score and increase the patient's bone density. Acupuncture combined with drugs and acupuncture combined with surgery are also more effective than simple drugs and

surgery alone. However, given the low-level evidence quality, these results need to be interpreted with caution, and more high-quality studies are needed to provide more robust evidence.

Data Availability

The table data used to support the findings of this study are included within the article. The figure data used to support the findings of this study are included within the figure files.

Ethical Approval

This study was based on previously published studies; therefore, ethical approval and patient consent are not relevant.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

J-LL and X-BZ contributed to the conception and design of the review. J-LL, SR, and ZZ carried out the search strategy. J-LL and SR developed the search criteria and carried out data extraction quality evaluation. X-BZ and J-LL analyzed the data. J-LL wrote the first draft of the manuscript. W-HL, Z-HT, ZZ, and Q-SH critically edited the manuscript. J-LL and W-HL were responsible for the overall project. All authors read and approved the manuscript.

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Research Article

Pain Changes Induced by Acupuncture in Single Body Areas in Fibromyalgia Syndrome: Results from an Open-Label Pragmatic Study

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To date, there is considerable evidence of the effectiveness of acupuncture in fibromyalgia syndrome (FM). However, it is not known in which body areas acupuncture is more effective. The objective of this study was to assess the improvements of pain induced by acupuncture in single body areas in patients with FM. In this open-label pragmatic study, FM patients in a state of high disease severity were consecutively enrolled and treated with a course of 8 weekly sessions of manual acupuncture. Patients were assessed with the Self-Administered Pain Scale (SAPS) of the Fibromyalgia Assessment Status at baseline and at the end of eight acupuncture sessions. Acupuncture sessions were all conducted with the same acupuncture formula (LV3, SP6, ST36, LI4, CV6, CV12, Ex-HN-3, and GV20) in each session and in each patient. Ninety-six FM patients completed the course of treatment. All the 16 body areas assessed by SAPS showed improvement in pain. A statistically significant improvement was achieved in 12 of the 16 body areas investigated, with the best results in abdomen and forearms ($p = 0.001$), while the worst results were registered for neck ($p = 0.058$), chest ($p = 0.059$), left buttock ($p = 0.065$), and right thigh ($p = 0.052$). The treatment has also shown significant effectiveness in improving fatigue and sleep quality ($p < 0.0001$). Acupuncture has a beneficial effect on pain in all body areas in FM patients with high disease severity, with the greatest effects in the abdominal region and in the forearms, allowing a personalization of the treatment.

1. Introduction

Acupuncture, a therapeutic technique that goes back thousands of years, is still an effective treatment strategy for chronic musculoskeletal pain [1]. In particular, in the field of fibromyalgia syndrome (FM), there are growing clinical experiences oriented towards its integration into multimodal treatment schemes [2]. Indeed, the demand for integrating alternative therapy modalities into complex treatment regimes is growing rapidly, even for inpatients [3].

At present, randomized controlled trials (RCTS) have demonstrated the effect of acupuncture in the treatment of FM [4–7]. The latest EUropean League Against Rheumatism (EULAR) guidelines for the management of FM have suggested a "weak for" recommendation regarding the use of acupuncture [8]. The main advantages of acupuncture are the excellent tolerability and the almost absence of adverse events when compared to the standard drug therapy of FM [4]. A recent meta-analysis of RCTs documented the benefits of acupuncture on pain and quality of life in FM, demonstrating the superiority of verum acupuncture over sham acupuncture, and concluding that it may be a recommended treatment in FM patients [9].

However, in evaluating the effectiveness of acupuncture in FM, the majority of studies have focused mainly on generic patient-reported outcomes (e.g., numerical rating scale [NRS] of pain) [4], or disease-specific instruments (e.g., Fibromyalgia Impact Questionnaire [FIQ]) [5, 10]. Other authors have also documented improvements in serum biomarkers [6, 11].

The clinical manifestations of FM are numerous: they are proteiform, it is often difficult to frame them, and the

complexity of the clinical picture is also responsible for a significant diagnostic delay [12]. However, the key symptom that characterizes FM is the presence of chronic widespread pain (CWP). The concept that FM is a condition primarily characterised by chronic pain is emphasised in the classification proposed by the International Classification of Diseases (ICD). In the 11th version of the ICD, FM is grouped into the conditions of chronic primary pain [13]. CWP remains the main criterion in the diagnostic definition of FM, although there are differences in its definition and assessment depending on the set of diagnostic/classification criteria used. Based on the criteria of the American College of Rheumatology (ACR) of 1990, the CWP was essentially evaluated by counting tender points [14]. The CWP was thus "measured" by the count of 18 myofascial tender points. Tender point count remained valid for many years, to be abandoned mainly because of the difficulties of objective assessment by general practitioners and the arbitrary nature of the assessment. Since 2010, with the new ACR diagnostic criteria, CWP evaluation has undergone a complete revolution with the introduction of the widespread pain index (WPI), a score between 0 and 19 in which the patient independently reports the presence or absence of pain in specific body regions [15]. The WPI has undergone some minor changes until the full definition of 2016 [16].

Recently, the ACTTION-American Pain Society-Pain Taxonomy (AAPT) initiative proposed a revision of the ACR criteria, introducing the concept of multi-site pain (MSP), which defines FM if present in at least six of the nine possible body areas [17].

Beyond the diagnostic definition and classification purposes of this complex sensory phenomenon that is CWP, there are no studies that have investigated in detail the improvements that acupuncture treatment can produce in different areas of the body. It is recognized that painful symptoms are more prevalent in some body regions in FM patients and occur more in the neck, upper back, and lower back [18].

For proper clinical application, it is important to identify whether the beneficial effects of a general acupuncture formula on pain are greater in certain body regions than others.

Starting from these considerations, the primary aim of this study is to assess the response to a general scheme of manual acupuncture, in terms of pain changes in single body areas in subjects with FM. The secondary aims of the study are to estimate the changes of fatigue and quality of sleep at the end of the treatment.

2. Materials and Methods

2.1. Setting and Study Design. This open-label, pragmatic, non-control study involved adult FM patients diagnosed according to 2010 ACR criteria [14]. Patients were consecutively recruited from January 2018 to June 2019 at a tertiary reference centre for the diagnosis and treatment of FM.

Patients underwent a course of eight weekly acupuncture sessions according to a pre-established schedule. The outcomes studied were assessed one week after the end of the acupuncture treatment course compared to baseline, i.e., at the beginning of the treatment course.

Acupuncture was not performed blindly and only one intervention group was chosen, in the absence of control groups. The reasons for this decision are explained later in Discussion.

All patients voluntarily participated in the study and signed informed consent. The study was approved by the local ethics committee (Comitato Etico Unico Regionale, number 1970/AV2) and conducted in accordance with the 1964 Helsinki Declaration and subsequent amendments.

2.2. Inclusion and Exclusion Criteria. For the purposes of this study, patients had to be in a status of severe FM, defined by the concomitant presence of a revised FIQ (FIQR) ≥39 and a Patient Health Questionnaire 15 items (PHQ15) ≥5 (Table 1 provides a synthetic description of the clinimetric instruments) [19].

In addition to these clinimetric criteria, drug therapy-related inclusion criteria were added. In particular, at the time of enrolment, patients had to show unresponsiveness or intolerance to reference drug therapy at stable dosages for at least three months. A combination of pregabalin 300 mg/day and duloxetine 60 mg/day was considered as reference drug therapy. Patients on lower dosages of the respective drugs were also admitted, provided that intolerance to titration of the drugs up to the dosages mentioned was recorded.

During the period of acupuncture treatment, patients were required to keep the reference drug therapy unchanged, while analgesics were allowed on demand (paracetamol up to 3 g/day or tramadol up to 150 mg/day).

All patients who had already undergone acupuncture even for indications outside FM, all patients with concomitant chronic painful conditions which could hamper patient evaluation or which could confound FM symptomatology (chronic inflammatory joint diseases, vasculitis, inflammatory muscle diseases or metabolic myopaties, thyroid diseases or other poorly controlled endocrinopathies, inflammatory bowel diseases, coeliac disease, Alzheimer's disease and other dementias, Parkinson's disease, and opioid-induced hyperalgesia), and all patients with relevant internistic diseases (uncontrolled heart failure, severe chronic renal failure, liver failure, and active infections) or cancer (uncontrolled active neoplasms) were excluded.

FM is frequently associated with other conditions such as lumbar or cervical spondylosis, peripheral osteoarthritis, or radiculopathies. These conditions were not considered as exclusion criteria as long as the dominant symptomatology was related to CWP.

2.3. Acupuncture Treatment Modalities. The procedures of this study were outlined according to the STandards for Reporting Interventions in Clinical Trials of Acupuncture (STRICTA) checklist [20].

The manual acupuncture sessions were all conducted by a single physician (MDC), a rheumatologist with nine years' experience in acupuncture, with the certification to practice acupuncture in accordance with Italian law obtained in 2014 at the end of a four-year training period.

Table 1: Description of the clinimetric characteristics of the instruments used as inclusion criteria (FIQR and PHQ15) and as outcome assessment (FAS).

Instrument	Number of items	Calculation	Interpretation
FIQR	21 items investigating 3 domains of health: 9 physical function, 2 overall health status, 10 symptoms	Each item is an 11-point NRS. The final score is the algebraic sum of the scores of the three domains: the sum of the 9 NRS in the physical function domain is divided by 3, the 2 NRS in the overall health status domain are considered as they are, and the sum of the 10 NRS of the symptoms domain is divided by 2.	Final score ranging from 0 to 100. Disease severity is defined as follows: remission (below 23), mild disease (between 24 and 40), moderate disease (between 41 and 63), severe disease (between 64 and 82), and very severe disease (above 83).
PHQ15	15 items, 13 investigating somatic symptoms, 2 psychological symptoms	Each item is scored from 0 (no disturbance) to 2 (severe disturbance). The final score is the algebraic sum of the items.	Final score from 0 to 30. The severity of symptoms is considered as follows: low (below 5), medium (between 5 and 10), and high (above 15).
FAS	It contains 2 parts. The first is made by 2 11-point NRS investigating fatigue and sleep quality, and the second is the SAPS. SAPS evaluates 16 body regions for the presence of pain on 4-point NRS (0 = no pain, 1 = mild pain, 2 = moderate pain, 3 = severe pain) for each area.	The final score is the mean of the algebraic sum of the 2 NRS for fatigue and sleep quality and the SAPS normalized (SAPS is scored from 0 to 48; then score is normalized to a 0–10 scale).	Final score from 0 to 10. No cut-off points for disease severity states are available.

FIQR = revised Fibromyalgia Impact Questionnaire; PHQ15 = Patient Health Questionnaire 15 items; FAS = Fibromyalgia Assessment Status; SAPS = Self-Assessment Pain Scale, NRS = numerical rating scale.

The acupuncture scheme was performed according to the rules of Traditional Chinese Medicine (TCM). All patients were treated with the acupoints LV3, SP6, and ST36 on the lower limb, LI4 on the upper limb, CV6 and CV12 at abdominal level, Ex-HN-3 (Yintang), and GV20 at head level (Figure 1). Based on TCM, the proposed treatment formula aimed to move Qi, raise Qi, tonify Qi and blood, and calm Shen [21]. Pair acupoints were treated bilaterally. This acupuncture formula was performed in all patients for each treatment session, and all of the listed points were used simultaneously. Each point was inserted with sterile single-use needles 0.25 × 25 mm (Huanqiu®) equipped with a guide tube, and the needles were manipulated only at the beginning of the session until the so-called de Qi was reached. De Qi refers to those sensations (generally described as paresthetic sensations such as aching, soreness, numbness, tingling, fullness, distention, pressure, or heaviness) reported by the patient at the time of needle insertion and manipulation [22]. Each session lasted 30 minutes, and eight sessions were carried out weekly. In total, each patient received 240 minutes of acupuncture treatment.

The acupuncturist was allowed only minimal interaction with the patients at the beginning and end of the sessions, but was not allowed to make any questions about the state of health of the patients. The acupuncturist had no clinical and clinimetric information about the patients throughout the study.

2.4. Clinimetric Assessment. For the objectives of this study, the clinimetric evaluation was focused on Fibromyalgia Assessment Status (FAS) (Table 1) [23]. FAS has proven to be a valid, reliable, and responsive tool in the assessment of FM patients. Recently, a modified version of FAS has been used with excellent results also for diagnostic purposes [24].

FAS contains three evaluations: two 11-point NRS scales related to fatigue and sleep quality and the Self-Administered Pain Scale (SAPS). This latter scale was the one used for the primary aim of this study. SAPS evaluates 16 body regions for the presence of pain on 4-point NRS for each area, where 0 represents no pain, 1 mild pain, 2 moderate pain, and 3 severe pain. The reference to painful symptoms is relative to the last week. SAPS is administered on paper with a front and back representation of a manikin, and for the 16 body areas the patient indicates the pain level in the scales from 0 to 3 for each area. The final SAPS score ranges from 0 to 48 and is transformed by a nomogram to a 0–10 scale to be computed within the FAS. In this study, the improvement of pain symptoms in the single body areas was evaluated on the SAPS.

All questionnaires were administered by a fellow in rheumatology (GB), with experience in managing patient-reported outcome measures.

2.5. Statistical Analysis. For the purposes of this study, no formal sample size assessment was carried out. The case study enrolled is of convenience, determined by reasons of feasibility.

The data of this study showed a nonparametric distribution (verified with the Kolmogorov–Smirnov test); therefore, the results are presented as median and interquartile range for FAS and subscales. The homogeneity of the case study was assessed with Levene's test for SAPS, FAS fatigue, and FAS sleep quality.

In order to estimate the effect of acupuncture in each of the 16 body areas of the SAPS, and also to provide a graphical representation of acupuncture effects through a spidergram, mean SAPS values, not transformed by the

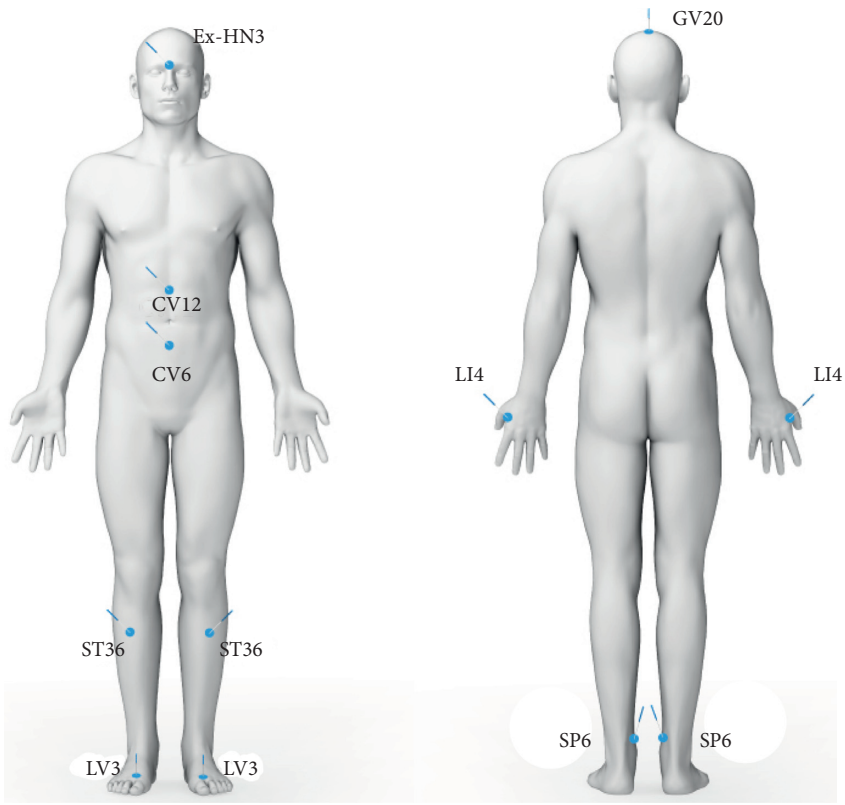

Figure 1: Front-back manikin with localization of acupoints.

nomogram, were calculated for each area at baseline and at the end of treatment.

Changes between baseline and endpoint were evaluated using the Wilcoxon test for paired data.

The statistical analysis was conducted with MedCalc, version 18.0.0, and statistical significance was considered for values of $p < 0.05$.

3. Results

The analysis was conducted on 96 of the 102 patients (94.1%) who started acupuncture treatment. In six patients, the course was suspended, at the second session in two patients due to poor tolerance to needle insertion, during the following sessions in the other four patients due to intervening logistics difficulties in carrying out the sessions on a weekly basis. Among the 96 patients treated, 85 (88.5%) were women and 11 (11.5%) men, with a mean age (±standard deviation) of 50.6 ± 12.3 years and a disease duration of 5.6 ± 6.2 years. The reference "optimal" drug therapy (combination of duloxetine and pregabalin) was taken by 32 (33.3%) patients, 22 (22.9%) patients were taking only pregabalin, 13 (13.5%) patients were taking only duloxetine, and 29 (30.2%) patients were not taking either molecule.

SAPS and FAS sleep disturbances data were homogeneous, whereas FAS fatigue data were not homogeneous.

SAPS, considered in its entirety, showed a significant improvement at the end of the acupuncture treatment, with median values that reduced from 5.8 at the beginning to 4.4 at the end of the course ($p < 0.0001$) (Table 2). Analyzing the data from the non-normalized and split SAPS in each body area, at baseline the highest mean scores were found at the axial skeleton level, with 2.24 (range 0–3) for the low back and 2.20 for the neck, respectively.

Comparing the mean values of the 16 body areas between basal and final, an improvement was recorded for each body area (Figure 2), with a mean difference of −0.43 points. A statistically significant improvement was achieved in 12 of the 16 body areas investigated, with the best results in abdomen and forearms ($p = 0.001$). The four areas where the statistical significance of the improvement was not achieved were neck ($p = 0.058$), chest ($p = 0.059$), left buttock ($p = 0.065$), and right thigh ($p = 0.052$) (Table 3).

With regard to the secondary objectives of the study, fatigue and sleep quality showed also a significant improvement at the end of treatment compared to baseline ($p < 0.0001$) (Table 2).

4. Discussion

To the best of our knowledge, this is the first study that investigated the effects of acupuncture, in terms of pain improvement, in the single areas of the body in patients with FM. This study showed that improvements induced by a general acupuncture formula are widespread, even in areas of the body not directly stimulated by needles. However, in some areas improvements are greater. It can therefore be affirmed that acupuncture has a global effect on CWP, the key symptom experienced by FM patients. Acupuncture demonstrated also to be effective to treat fatigue and sleep disturbances.

Table 2: Data at baseline and at the end of the acupuncture treatment regarding FAS and its subscales.

	Baseline		End of acupuncture treatment		Significance (p)*
	Median	Interquartile range	Median	Interquartile range	
FAS total score	7.45	5.35–8.30	5.05	3.55–7.05	<0.0001
Fatigue	8.00	6.75–9.00	7.00	4.00–8.00	<0.0001
Sleep quality	8.00	4.75–9.00	6.00	3.00–8.00	<0.0001
SAPS	5.80	4.60–7.10	4.40	2.65–5.85	<0.0001

* = Wilcoxon test (paired samples). FAS = Fibromyalgia Assessment Status; SAPS = Self-Assessment Pain Scale.

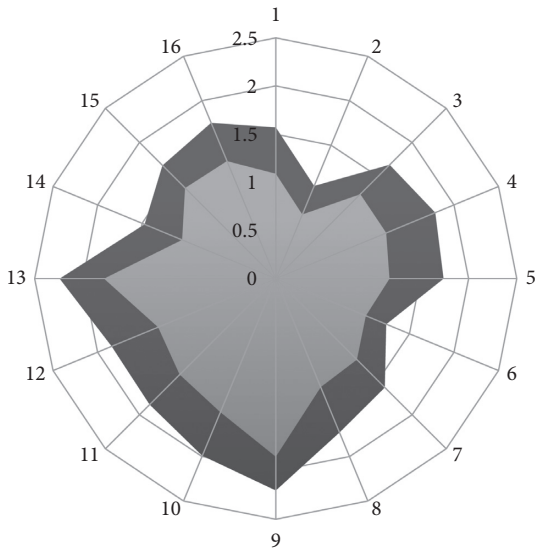

Figure 2: Spidergram showing improvement in final mean values (light grey spidergram area), after acupuncture treatment, compared to initial mean values (dark grey spidergram area) in the 16 body areas assessed by the Self-Assessment Pain Scale. 1 = head; 2 = chest; 3 = left arm; 4 = left forearm; 5 = abdomen; 6 = left buttock; 7 = left thigh; 8 = left leg; 9 = neck; 10 = upper back; 11 = right arm; 12 = right forearm; 13 = low back; 14 = right buttock; 15 = right thigh; 16 = right leg.

Chronic musculoskeletal pain is the main reason why patients rely on acupuncture, followed by systemic symptoms such as fatigue [25, 26]. The effect of acupuncture in chronic pain is clinically relevant as demonstrated by the metanalysis of randomized controlled trials, and true acupuncture is more effective than sham acupuncture and non-acupuncture [27]. The mechanisms underlying the effectiveness of acupuncture in pain are multiple. Analgesia derives from integrated mechanisms involving the nervous system, the painful stimuli, and the stimuli resulting from skin infission with needles. Several neurotransmitters are involved, including serotonin, adenosine, gamma-aminobutyric acid, opioid peptides, calcitonin gene-related peptide, and substance P [28]. The above list of substances involved in acupuncture-induced analgesia is certainly not exhaustive, but the molecules act through pathways involving segmental and supraspinal spinal mechanisms [29].

These integrated mechanisms ensure that, through a general formula in accordance with the TCM, such as the one applied in this study, generalized benefits can be achieved. For example, improvements can also be documented in areas of the body where no needles are inserted, such as the lumbar region. This finding has important clinical consequences because, while it is essential that the treatment is effective, it must be optimally tolerated and, specifically, the formula used in this study allows the patient to lie comfortably on his back during the sessions.

However, it should be noted that the biggest improvements in terms of SAPS score difference between final and baseline were at abdominal level, where two acupoints (CV6 and CV12) were used. Our acupuncture formula included the treatment of 12 acupoints per session. Within the context of CWP of FM, where painful symptoms are also connoted by the presence of allodynia and hyperalgesia (as well as a major psychological burden), it is essential to balance the use of needles since it is virtually impossible to treat every single painful body area. Probably the integration of a general therapeutic scheme such as or similar to that used in this study, with a microsystem-based technique (e.g., auricular acupuncture, focusing on areas corresponding to the cervical region, where minor improvements were recorded), can provide even better results in terms of efficacy. However, this represents a theoretical speculation that should be the topic of future research.

The use of SAPS as a clinimetric instrument has allowed a detailed analysis of the changes induced by acupuncture in each body area. There are several ways to measure CWP, such as the WPI in the ACR 2016 criteria or the AAPT criteria manikin [16, 17]. In this study, we preferred to use the SAPS. This tool provides a synthetic result but also analyzes the pain in each body area, allowing the patient to describe it in 16 NRS. The result is a detailed mapping of the CWP. The studies that have verified the effectiveness of acupuncture on pain in FM are several; however, in our view, they have done so in an overly synthetic way, most of the cases using only a single VAS or NRS scale [4–6]. The description of a sensory phenomenon as complex and multifaceted as CWP in FM cannot be reduced to a single scale. Targino and colleagues revealed that the positive effects of acupuncture last for more than six months after the end of the treatment. In addition to general measures of quality of life, the beneficial effects of acupuncture showed also to reduce pain pressure threshold at tender points [7]. Mist and Jones documented in their RCT that acupuncture is superior to other non-pharmacological modalities such as patient education, demonstrating improvements in all generic and disease-specific indices [5]. Acupuncture demonstrated improving biochemical parameters, such as increased serotonin levels and reduced substance P [6, 11].

Table 3: Mean values at baseline and at the end of acupuncture treatment for each of the 16 numerical rating scales (range 0–4, where 4 identifies the most severe painful symptoms) that describe body areas considered in the SAPS.

SAPS bodily areas	Mean values baseline	Mean values end treatment	Difference	Significance (p)*
1. Head	1.57	1.09	−0.48	0.008
2. Chest	1.04	0.72	−0.32	0.059
3. Left arm	1.67	1.24	−0.43	0.002
4. Left forearm	1.79	1.24	−0.55	0.001
5. Abdomen	1.74	1.18	−0.56	0.001
6. Left buttock	1.24	1.01	−0.23	0.065
7. Left thigh	1.60	1.19	−0.41	0.009
8. Left leg	1.73	1.22	−0.51	0.005
9. Neck	2.20	1.85	−0.35	0.058
10. Upper back	1.99	1.50	−0.49	0.001
11. Right arm	1.85	1.41	−0.44	0.011
12. Right forearm	1.84	1.32	−0.52	0.001
13. Low back	2.24	1.78	−0.46	0.009
14. Right buttock	1.47	1.05	−0.42	0.012
15. Right thigh	1.66	1.33	−0.33	0.052
16. Right leg	1.75	1.32	−0.43	0.009
			Large sample test statistic Z, 3.558550	
			Two-tailed probability, $p = 0.0004$**	

* = Wilcoxon test (paired samples); ** = p value for nonnormalized SAPS. SAPS = Self-Assessment Pain Scale.

Not least, our study confirmed the effectiveness of acupuncture on fatigue and sleep quality, the other two variables considered in FAS. It should also be emphasised that this cohort of patients belonged to a category with severe symptoms refractory to conventional pharmacological therapy.

To date, there are no universally accepted treatment modalities for FM. What is widely accepted is the need for multimodal treatment approaches. Multimodal approaches, also suggested by international recommendations, are particularly useful for personalization of treatment, which is so important in FM, especially in patients with long-lasting symptoms [8, 30].

This study confirms that acupuncture, integrated with pharmacological therapy, is beneficial for pain management in FM patients. Several studies have already demonstrated the effectiveness of acupuncture in FM. However, no previous study had demonstrated improvement in pain in individual body areas.

Citing the limitations of the study, a first criticism that can be made is that no control group was used, in particular no group in which sham acupuncture was employed. We have chosen to treat all patients, first of all for the severity of their symptoms, since true acupuncture showed to be more effective than sham acupuncture in FM from recent meta-analyses [9, 31]. Secondly, based on a certain orientation in the literature, the effectiveness of acupuncture in chronic pain should be investigated with pragmatic real-life studies. Acupuncture represents a complex intervention that cannot be compared to a non-inert placebo such as sham acupuncture, which adds potential bias instead of removing it [32, 33]. A second criticism may be the single-centre recruitment. However, most of the studies dedicated to acupuncture are monocentric and this fact may also represent a strength since a certain uniformity of treatment has been guaranteed. A third criticism could be related to the acupuncture formula used, as one of several possible formulas was used. Virtually, the possibilities of combining acupoints are unlimited. We used a scheme in accordance with TCM, effective, with acupoints well known by acupuncturists, and easily repeatable in every patient.

A fourth limitation may be the fact that most of the patients were taking pharmacological treatment. The integration of acupuncture into multimodal schemes makes it more difficult to assess its therapeutic effect. However, all patients came from a failure/intolerance of drug therapy.

5. Conclusion

This study showed how eight sessions of manual acupuncture treatment, using a general formula in accordance with TCM, has whole body beneficial effects on CWP in FM patients. Improvements can be documented in each body area, with the greatest effects in the abdominal region and in forearms. Acupuncture is confirmed as a complementary method that can be effectively integrated with other treatment strategies in FM.

Data Availability

The data are available upon reasonable request to the corresponding author.

Conflicts of Interest

The authors declare no conflicts of interest.

Authors' Contributions

MDC and FS gave a significant contribution to the study concept and design, and to the analysis and interpretation of data; GB gave a significant contribution in acquisition of

data; MDC drafted the manuscript; GB and FS revised the manuscript for important intellectual content; all the authors approved the final version to be published; all the authors agreed on the journal to which the article will be submitted; all the authors reviewed and agreed on all versions of the article before submission, during revision, the final version accepted for publication, and any significant changes introduced at the proofing stage; all the authors agree to take responsibility and be accountable for the contents of the article.

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Research Article

Investigation and Analysis of the Key Objectives of WFAS "Technical Specifications of Acupuncture and Moxibustion: General Rules for the Drafting"

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Objective. To determine the key objectives of WFAS "Technical Specifications of Acupuncture and Moxibustion: General Rules for the Drafting" (hereinafter referred to as General Rules). *Methods*. From the medical institutions, colleges, and scientific research organizations at major levels in China and overseas, leading researchers and experts in the field of acupuncture-moxibustion standardization, as well as some experienced foreign specialists in acupuncture-moxibustion, were selected as the respondents. The questionnaire was prepared by using the website of Questionnaire Star, and 60 links of the questionnaire were sent out through e-mail. Excel was used to set up the database and conduct statistical analysis. *Results*. Fifty-one valid questionnaires were collected with effective recovery rate of 85%, involving 9 countries (China, South Korea, Italy, Spain, Sweden, Norway, Netherlands, United States, and Canada) from 3 continents. Most experts agreed with us on the target people, structural elements, and text structure proposed in General Rules and held that the General Rules should emphasize the safety and international applicability and should stipulate in details the common problems (contraindications, indications, taboo crowd, target people, therapeutic effects) of various techniques and the specific contents of technical operation (manipulating techniques, selection of patient's body position and treated areas, disinfection and environmental requirements, selection of acupuncture-moxibustion instruments, cautions, needle retention time, treatment frequency, precise location of the acupoints, treatment course) in order to enhance the practicality and operability. *Conclusion*. The key objectives of General Rules mainly include target people, structural elements, text structure, safety requirements, common technical problems of acupuncture-moxibustion, and specific details of technical manipulations.

1. Introduction

Acupuncture-moxibustion technique has been applied in 183 countries and regions around the world [1]. As a very practical subject, the operational techniques of acupuncture and moxibustion are considered to be extremely important, and the safety and standardization of acupuncture-moxibustion manipulation have always been the major determinants affecting its spread worldwide [2]. Lack of guidance on technical specifications will result in a variety of confusion during operations, leading to a negative impact on the application. At present, there are great variations in the specifications of important acupuncture techniques among countries, such as treatment frequency and the depth of needling. Take acupuncture treatment of osteoarthritis of the knee as an example, some patients are treated once a day or once every other day [3], while other patients being treated once a week [4]. For the protrusion of lumbar vertebra disc, Jiaji acupoints are punctured 50 mm to 75 mm in depth [5], or sometimes shallowly and subcutaneously [6]. Nonuniform and nonstandardized operations will not only affect the effectiveness of acupuncture and moxibustion but also increase the chance of adverse events and even mislead the public understanding and evaluation of acupuncture and

moxibustion treatment. Therefore, in order to promote the effective utilization and further development of acupuncture and moxibustion technique in the world, it is very necessary to regulate the technical specifications and formulate international standards in this field.

However, the current international standards for acupuncture and moxibustion technique are quite insufficient. There are only two documents "Standardized Manipulations of Acupuncture and Moxibustion: Part 1 Moxibustion" and "Standardized Manipulations of Acupuncture and Moxibustion: Part 2 Scalp Acupuncture," published by WFAS in 2012. Few countries have issued national standards or professional standards in this field, including China, Japan, South Korea, the United States, and Australia, with their own focused issues [7]. Except for China, the operation standards in other countries mainly focus on the safety and infection control, while the core technical aspects, such as the procedures and methods of acupuncture and moxibustion related to curative effect, are rarely involved, which is unable to meet the demands of global application of acupuncture technique. Accordingly, it is imperative to formulate international standards for acupuncture and moxibustion technical operation specifications. Compiling the "Technical Specifications of Acupuncture and Moxibustion" to formulate the corresponding international standards is an important measure for the long-term, in-depth, and continuing development of acupuncture-moxibustion [8].

In accordance with international practices, the development of General Rules should be given priority before the formulation of "Technical Specifications of Acupuncture and Moxibustion." This General Rules is considered to be the basic standard and the basis of other standards within the series. It also offers broad and basic guidance for the formulation of other standards in this series. For this reason, the World Federation of Acupuncture-Moxibustion Societies (WFAS) authorized our research group to host the development of WFAS "Technical Specifications of Acupuncture and Moxibustion: General Rules for the Drafting" (hereinafter referred to as General Rules). Our group has already conducted extensive preliminary survey on the international needs of the General Rules through online questionnaires. In order to reflect the common and key objective problems in the drafting process of "Technical Specifications of Acupuncture and Moxibustion," to strengthen the guidance and normative effect of the General Rules for meeting the needs of writing specifications of different acupuncture and moxibustion techniques within the worldwide scope, we carried out this investigation. The investigation has further clarified the common goals and issues related to safety and effectiveness in the operation of acupuncture and moxibustion techniques and consulted representative domestic and foreign experts about the related issues. Their opinions will be important basis for forming a new "General Rules" and will provide guidance and reference for the development of specific "standardized manipulations of acupuncture and moxibustion" and lay a foundation for further standardizing acupuncture and moxibustion in clinical operations and promoting a wider application. The results of the survey were analyzed and reported as follows.

2. Materials and Methods

2.1. Respondents. From the medical institutions, colleges, and scientific research organizations at major levels in China and overseas, leading researchers and experts in the field of acupuncture-moxibustion standardization, as well as some experienced foreign specialists in acupuncture-moxibustion, were enrolled as respondents. It includes seven types of people: leading experts in our research group, the members of WFAS acupuncture standardization working committee, foreign experts from the International Organization for Standardization (ISO)/Chinese Medicine Technical Committee (TC249)/the third and the fourth working groups (WG3 and WG4), the main drafts men of the current criteria of acupuncture and moxibustion, the members of the National Technical Committee on Acupuncture and Moxibustion of SAC, the members of the Third Committee of Standardization Work Committee of Chinese Acupuncture and Moxibustion Society, and acupuncture-moxibustion experts who have been engaged in clinical work abroad for a long time.

2.2. Questionnaire Method. The questionnaire was bilingual in Chinese and English. All questions were set as semiopen multichoice ranking questions. The respondents were required to rank each answer according to its importance from highest to lowest on the basis of selecting appropriate answers. Meanwhile, each question was supplemented with a blank option to fill in.

2.3. Questionnaire Contents. Our investigation questionnaire concentrated on the key objectives in the developing process of "Technical Specifications of Acupuncture and Moxibustion: General Rules for the Drafting," aiming at 12 questions, including target people, structural elements, text structure, safety requirements, common problems of acupuncture-moxibustion techniques, specific details of technical manipulations, international applicability, practicality and operability, etc. Different options are listed at the end of each question, and the respondents are required to select the options and rank them according to the importance.

2.4. Evaluation of the Reliability and Validity of the Questionnaire. The research group entrusted statistics experts from the Data Center of China Academy of Chinese Medical Sciences to analyze the reliability and validity of this questionnaire.

2.4.1. Reliability Analysis. According to the overall data of the twelve issues in this questionnaire, the research group used SPSS Ver.19.0 to analyze Cronbach's alpha of the questionnaire. The reliability test shows that the alpha coefficient is 0.834 and the alpha coefficient calculated based on standardized data is 0.785, both of the data represent a

relatively high level of reliability, indicating that the design of the questionnaire is highly reliable and the results are relatively accurate.

2.4.2. Validity Analysis. The validity is combined by the content validity and face validity. Generally, the KMO value (Kaiser–Meyer–Olkin) and Bartlett's test of sphericity are used to measure the data validity.

Since most of the questions in this questionnaire are multiple choices, the statistical experts believe that the validity analysis is not very suitable for this survey.

Therefore, only some of the questions are involved into the statistical results (such as the third and the eleventh question). All sig values of Bartlett's test of sphericity are 0.000 (<0.05), indicating that the correlation matrix is not an identity matrix. The two parameters of the KMO test statistic are 0.564 and 0.554, which are bigger than 0.5, the recommended standard of the test. Combined with these results, it can be confirmed that the data are suitable for factor analysis and the questionnaire data validity is qualified.

Interpretation of the validity of other issues: all of the issues in this questionnaire are derived from the results of the first-round international demand surveys about the "Technical Specifications of Acupuncture and Moxibustion: General Rules for the Drafting."

Through the analysis and screening of the survey results, twelve targeted questions (issues) were finally determined. During the formation of this questionnaire, we invited experts who were engaged in the development of relevant standards for acupuncture and moxibustion techniques to participate in the demonstration. After being discussed and optimized by a number of experts in the field, all the items in the questionnaire could express the research content as accurately as possible. The experts assessed that the questionnaire possessed good face validity and content validity.

2.5. Investigation Method. This investigation was prepared in both Chinese and English through the platform of electronic questionnaire, the website of Questionnaire Star (https://www.wjx.cn). The questionnaire link was sent through e-mail.

2.6. Investigation Time. The preparation and demonstration of the survey questionnaire started from February 9th to 14th, 2020. The investigation period is 1 week (February 15 ~ 22, 2020) in which two rounds of reply reminders were sent through e-mail, WeChat, and short message service (SMS).From February 22nd to 26th, 2020, the original data were exported and statistically analyzed.

2.7. Data Sorting and Analysis. Microsoft Excel 14.0 was used to establish a database for statistical analysis.

3. Results

3.1. Questionnaire Collection. Aimed at the key objectives in General Rules, a total of 60 investigation questionnaires were sent to 11 countries of 4 continents. Among them, 51 valid questionnaire replies were collected from 9 countries of 3 continents, including 2 Asian countries (China 40, South Korea 1), 5 European countries (Italy 2, Spain 2, Sweden 2, Norway 1, the Netherlands 1), and 2 American countries (the United States 1, Canada 1). The collective rate was 85%.

3.2. General Information of the Respondents

(i) Age: averaged 55.69 ± 10.10 years, the youngest 29 years, the oldest 75 years

(ii) Affiliation: 17 people (33.33%) in TCM hospitals, 12 people (23.53%) in medical colleges, 10 people (19.62%) in scientific research institutions, 4 people in TCM clinics and 4 people in specialized hospitals (7.84% respectively), and 2 people in Western medicine comprehensive hospitals and 2 people in private clinics (3.92% respectively)

(iii) Occupation: the majority of participants were Chinese medicine doctors and scientific researchers, including 40 TCM physicians (78.43%), 7 scientific researchers (13.73%), 2 medical teachers (3.92%), and 1 Western medicine physician and 1 in other occupation (1.96% respectively)

(iv) Department: 38 people (74.51%) in acupuncture department, 4 people (7.84%) in TCM department, and 9 people (17.65%) in other departments

(v) Professional title: 48 people are senior (94.12%), 2 people are intermediate (3.92%), and 1 person is junior (1.96%)

(vi) Working years in the acupuncture-moxibustion field: 30.67 ± 11.18 years on average

3.3. The Selection Criteria of the Survey Object (including Identity and Country). This survey aims at the experts who focus on the research and development of "Technical Specifications of Acupuncture and Moxibustion." Through preliminary investigations, it is found that only a few foreign experts are specialized in developing the technical standard of acupuncture and moxibustion. Given this situation, the selection criteria are expanded to include domestic experts who focus on developing the technical standard of acupuncture and moxibustion and international experts who are engaged in developing the standards in acupuncture-and-moxibustion-related fields. It is confirmed that authoritative experts in relative fields from all over the world have basically participated in this survey. The participants are as follows:

(i) Chinese leading researchers who are focused on the technical standard of acupuncture and moxibustion

(ii) Experts in the field of international standards (not limited to the field of acupuncture and moxibustion technical specifications): including SC-WFAS members and foreign representatives of ISO/TC249 WG3 (the group of acupuncture needles) + WG4

(other working groups except for the group of acupuncture needles)

(iii) Foreign experts in the research group

(iv) Foreign experts of acupuncture and moxibustion

Some experts have also been recruited in different organizations, and those duplicate identities have been removed from this survey.

Explanation of the surveyed countries: Considering the different levels of the development of acupuncture and moxibustion in each country, and the uneven distribution of countries participating in the formation of international standard, it turns out that some countries are interested in participating in this survey, while some countries are unwilling to be involved. After excluding those with no response, it can be confirmed that we have already involved sufficient countries and experts that could represent the authoritative group in the field of "Technical Specifications of Acupuncture and Moxibustion" in this survey.

3.4. Investigation Results

3.4.1. Target People of General Rules. More than 75% of the experts believed that General Rules should aim at meeting the various needs of the practitioners, compilers, and researchers of the "Technical Specifications of Acupuncture and Moxibustion."

3.4.2. Structural Elements. More than 70% of the experts believed that General Rules should contain all the structural elements listed in the investigation, as shown in Table 1. The structural elements relating to safety, such as "adverse reactions," "precautions," and "contraindications," occupied the highest proportion of importance, reaching the same level as "cover," "table of contents," and "standard title" did (all 94.12%), indicating that experts attached great importance to the safety of acupuncture practice. "Theoretical basis" was in the lowest selection frequency (74.51%). Some experts come up with additional suggestions: (1) editors should be listed and (2) images and videos (electronic version) should be added.

On the basis of the current China's national standard "Technical Specifications of Acupuncture and Moxibustion: General Rules for the Drafting," it was planned to add two structural elements, "adverse reactions" and "theoretical basis of acupuncture-moxibustion techniques," and meanwhile, it was stipulated that "diagnosis methods applicable to acupuncture-moxibustion techniques" could be presented in the appendix. With further investigation on these three elements, in order of importance, experts ranked the contents of the "adverse reactions" and "diagnosis methods applicable to acupuncture-moxibustion techniques" and the perspective of demonstration for the "theoretical basis of acupuncture-moxibustion techniques," as shown in Table 2.

3.4.3. Text Structure. For "from what aspects it should be stipulated in General Rules to ensure the uniformity of the text structure of English version," the result of investigation is shown in Table 3.

3.4.4. Safety Requirements. Safety is an important requirement that must be contained in all technical specifications [9].

Most experts agreed that, to enhance the safety guiding role of General Rules, the technical specifications of acupuncture and moxibustion should be specified mainly from five aspects, which were ranked from high to low according to experts' opinions, as shown in Table 4.

Some experts put forward that "the overall quality training of acupuncture professionals" and "the definition of the quality of needles" should also be included.

3.4.5. Common Technical Problems and Technical Manipulations to Be Specified in Details. Experts ranked the importance of the common problems covering various acupuncture techniques that need to be detailed in General Rules. The result is shown in Table 5.

For the specific contents of technical manipulations of acupuncture and moxibustion to be detailed, the ranking proposed by experts is shown in Table 6.

3.4.6. Practicability and Operability. Practicability and operability are the important characteristics that all the specifications must stress on. Most experts believed that General Rules should provide detailed descriptions on the practicability and operability of the "Technical Specifications of Acupuncture and Moxibustion" from six aspects, and the ranking from high to low according to experts' opinions is shown in Table 7.

Some experts put forward some additional opinions: (1) "whether the TCM theory could be well understood worldwide" should be considered; (2) to give explanations of origin and development of some acupuncture-moxibustion techniques; and (3) to give descriptions of the qualifications of practitioners using different manipulation methods.

3.4.7. International Applicability. The investigation result of "how to improve the international applicability of General Rules" is shown in Table 8.

4. Discussion

According to the result of the first-round investigation of the international demands [10], our research group determined the general scope of the key objective problems to be solved in General Rules and designed the investigation questionnaire accordingly. This investigation effectively collected experts' opinions on the target problems of General Rules and became a key step in the development process of General Rules. The analysis on the investigation result is as follows.

4.1. Determine the Target People and Structural Elements and Unify the Text Structure. The result of this investigation clarified that the target people of this General Rules mainly

Table 1: Investigation of structural elements to be included in General Rules.

Ordering	Options and suggestions	Selection frequency	Percentage (%)
1	Cover	48	94.12
2	Table of contents	48	94.12
3	Title of standards	48	94.12
4	Adverse reactions	48	94.12
5	Cautions	48	94.12
6	Contraindications	48	94.12
7	Terms and definitions	47	92.16
8	Scope	46	90.20
9	Manipulation procedure and rules	46	90.20
10	Manipulating methods	46	90.20
11	Management after manipulation	46	90.20
12	Forward	44	86.27
13	Normative references	44	86.27
14	Preparation before manipulation	44	86.27
15	Introduction	43	84.31
16	Annex	42	82.35
17	Qualifications of manipulators	40	78.43
18	Theoretical basis	38	74.51
19	Other	5	9.80

Table 2: Investigation on the contents of three structural elements to be added in General Rules.

Element	Ordering	Options and opinions	Selection frequency	Percentage (%)
"Adverse reactions"	1	Description of adverse reactions	48	94.12
	2	Corresponding measures or recommendations	48	94.12
	3	Definition of the degrees of adverse reactions	44	86.27
	4	Prognosis of adverse reactions	39	76.47
	5	Analysis of the causes of adverse reactions	38	74.51
	6	Other	5	9.80
"Diagnosis methods applicable to acupuncture-moxibustion techniques"	1	Acupoint examination	39	76.47
	2	Special diagnosis methods of special acupuncture-moxibustion methods	38	74.51
	3	Meridian examination	38	74.51
	4	Four diagnostic methods of conventional Chinese medicine	35	68.63
	5	Modern medical diagnostic methods	31	60.78
	6	None of the above choices	6	11.76
	7	Other	2	3.92
"Theoretical basis of acupuncture-moxibustion techniques"	1	From the perspective of traditional Chinese medicine	43	84.31
	2	From the perspective of clinical research	38	74.51
	3	From the perspective of modern medicine	35	68.63
	4	From the perspective of literature	28	54.90
	5	Other	6	11.76

included the global practitioners, compilers, and researchers of the Technical Specifications of Acupuncture and Moxibustion. The expert consensus formed in this survey was that, all the structural elements mentioned in the questionnaire should be included in the Technical Specifications of Acupuncture and Moxibustion; in addition, its text structures should be unified (including "text structure"; "scope and format of terms and definition"; "scope, format, and detailed requirements of the specific content of each part of General Rules"; "expressions of numbers, quantities, units, and values"; "scope and format of bibliography"; "symbols and signs"; "expressions of abbreviated terms"; "linguistic style").

4.2. Attach Great Importance to Safety and Put Forward Specific Requirements. The therapeutic effect and safety of acupuncture and moxibustion are the basis and foundation of its continuous development [11]. Especially for its international application, the safety of acupuncture is the focus of attention [12]. The result of this investigation showed that the structural element "adverse reactions" added in General

Table 3: The English text structure to be stipulated in General Rules.

Ordering	Options and opinions	Selection frequency	Percentage (%)
1	Text structure	49	96.08
2	Scope and format of terms and definition	48	94.12
3	Scope, format, and detailed requirements of the specific content of each part of General Rules	46	90.20
4	Expressions of numbers, quantities, units, and values	44	86.27
5	Scope and format of bibliography	43	84.31
6	Symbols and signs	43	84.31
7	Expressions of abbreviated terms	41	80.39
8	Linguistic style	33	64.71
9	Other	1	1.96

Table 4: Investigation of enhancing the safety guiding role of General Rules for the technical specifications of acupuncture and moxibustion.

Ordering	Options and opinions	Selection frequency	Percentage (%)
1	The scope of application of different acupuncture-moxibustion techniques	47	92.16
2	Adverse reactions that may occur to different acupuncture-moxibustion techniques	46	90.20
3	Contraindications of different acupuncture-moxibustion techniques	44	86.27
4	Specification of the operator's qualifications	36	70.59
5	Limitations of acupuncture-moxibustion application by laws and regulations in different countries/regions	32	62.75
6	Other	4	7.84

Table 5: Investigation on the common problems covering various acupuncture techniques that need to be detailed in General Rules.

Ordering	Options and opinions	Selection frequency	Percentage (%)
1	Contradictions	47	92.16
2	Indications	44	86.27
3	Taboo crowd	41	80.39
4	Target patients	39	76.47
5	Therapeutic effects	35	68.63
6	Other	2	3.92

Rules on the basis of the current China's national standard "Technical Specifications of Acupuncture and Moxibustion: General Rules for the Drafting" (GB/T 33416-2016) has been recognized by most experts. The result of the investigation on the composition of "structural elements" showed that the three structural elements ("adverse reactions," "precautions," and "contraindications") related to safety had the highest degree of expert consensus.

Further investigation into safety revealed that "General Rules" should provide guidance on security of all Technical Specifications of Acupuncture and Moxibustion from five aspects, including "the scope of application of different acupuncture-moxibustion techniques," "adverse reactions that may occur to different acupuncture-moxibustion techniques," "contraindications of different acupuncture-moxibustion techniques," "specification of the operator's qualifications," and "limitations of acupuncture-moxibustion application by laws and regulations in different countries/regions." The descriptions of the definition, treatment, prognosis, and causes of "adverse reactions" [13] and other factors can effectively improve the safety control for all technical specifications of acupuncture and moxibustion and avoid the delay of the best time for treatment due to the lack of correct understanding of the safety [14] of acupuncture and moxibustion and adverse reactions. By clearly stipulating the qualifications of practitioners, the clinical disorders caused by unqualified practitioners [15] can be avoided. To ensure the quality of the development of this series of standards, it is essential to emphasize the safety in General Rules, strengthen the security constraints on all the Technical Specifications of Acupuncture and Moxibustion, and promote the safe use of acupuncture and moxibustion in the international scope.

4.3. Define the Specific Details of Common Problems and Technical Manipulations of Acupuncture and Moxibustion and Detail the Practicability and Operability. The investigation result showed that most experts agreed to provide the details of the common problems covering multiple acupuncture techniques, including "contraindications," "indications," "taboo crowd," "target patients," and "therapeutic effects" in General Rules. This can promote the precise application of acupuncture and moxibustion techniques and avoid the side effects caused by improper operation. It coincides with the attention paid by doctors of all dynasties on the "contraindications" of acupuncture and moxibustion [16].

As for the specific content of the technical manipulations of acupuncture and moxibustion, most experts pointed out

Table 6: Specific contents of technical manipulations of acupuncture and moxibustion to be detailed in General Rules.

Ordering	Options and opinions	Selection frequency	Percentage (%)
1	Detailed manipulating techniques (including needle depth and needling sensation)	47	92.16
2	Selection of patient's body position and manipulating part	44	86.27
3	Disinfection and environmental requirements	44	86.27
4	Selection of acupuncture-moxibustion instruments	43	84.31
5	Cautions	42	82.35
6	Needle retention time	40	78.43
7	Treatment frequency	40	78.43
8	Precise location of the acupoints	39	76.47
9	Treatment course	39	76.47
10	Other	5	9.80

Table 7: Stipulated contents of practicability and operability.

Ordering	Options and opinions	Selection frequency	Percentage (%)
1	Descriptions of various acupuncture-moxibustion techniques' commonness and particularities based on their therapeutic effects	47	92.16
2	The reasons for choosing different acupuncture-moxibustion techniques	44	86.27
3	Manipulations of each technique and it is advisable to give specific examples	40	78.43
4	Key factors of the effectiveness for special acupuncture-moxibustion methods	39	76.47
5	Disinfection requirements of special acupuncture-moxibustion methods	37	72.55
6	Notification of special terms and definitions which are necessary for each part of the technical specification based on the characteristics of individual part	36	70.59
7	Other	6	11.76

Table 8: Investigation result of the factors to be considered in improving the international applicability of General Rules.

Ordering	Options and opinions	Selection frequency	Percentage (%)
1	Differences in laws and regulations of different countries	44	86.27
2	Differences in the qualifications of practitioners in different countries	43	84.31
3	Differences in demand of different countries	37	72.55
4	Differences in the policy of medical insurance payment systems in different countries on acupuncture-moxibustion	36	70.59
5	Differences in the understanding and positioning of acupuncture-moxibustion techniques in different countries	35	68.63
6	Differences in acupuncture-moxibustion techniques preferred by different countries	34	66.67
7	Differences in environments of different countries	34	66.67
8	Differences in the definition of tolerance in different countries	28	54.90
9	Other	3	5.88

that all options listed in the investigation should be detailed and explicit, including detailed manipulating techniques (including needle depth and needling sensation), selection of patient's body position and manipulating part, disinfection and environmental requirements, selection of acupuncture-moxibustion instruments, cautions, needle retention time, treatment frequency, precise location of the acupoints, treatment course, etc. In this way, the application of acupuncture and moxibustion techniques can be standardized and restrained more effectively, and the standard manipulation of acupuncture and moxibustion techniques can be guaranteed. At the same time, the specific requirements on the practicability and operability of the Technical Specifications of Acupuncture and Moxibustion should be detailed.

4.4. Emphasize the International Applicability. An important role of international standards is to eliminate communication barriers and promote the exchange and development of international technology. Therefore, in the compiling process of the Technical Specifications of Acupuncture and Moxibustion, the General Rules should detail the following contents with full consideration of the differences in the laws and regulations of different countries, requirements of the

qualifications of practitioners and their demands, the policy of medical insurance payment systems on acupuncture-moxibustion, the understanding and positioning of acupuncture-moxibustion techniques, preference of acupuncture-moxibustion techniques, and environments. At the same time, it is pointed out that General Rules should stipulate the text of this series of standards from 8 aspects to ensure its unity. All of these will help improve the international applicability of the series of WFAS Technical Specifications of Acupuncture and Moxibustion.

4.5. Less Consensus on "Theoretical Basis". The development and continuous improvement of any kind of technology cannot be separated from the theory guidance. Without the support of theory, technology will lose its foundation. Only by correctly interpreting and inheriting the theory can clinical practice be better guided and improved [17]. Explanation of the "theoretical basis" is helpful for the practitioners to understand the connotation of the applied technology, so as to better grasp its indications and manipulation essentials. It is beneficial to the accumulation and development of the manipulation experience of acupuncture and moxibustion. It is very necessary to add "theoretical basis" for the safe, effective, and standardized use of acupuncture and moxibustion technology. Therefore, the "theoretical basis" was added to the "structural elements" of General Rules. However, the investigation result showed that among all the 18 "structural elements," the "theoretical basis" was the least frequently selected, indicating that there still existed different viewpoints on whether the "theoretical basis" should be involved in the Technical Specifications of Acupuncture and Moxibustion. This also reflected the current situation of "attaching importance to technology and neglecting theory" in our clinical practice of acupuncture and moxibustion.

4.6. Shortcoming of This Investigation. In this investigation, the effective replies of 51 experts from 9 countries (China, Italy, Spain, Sweden, Norway, the Netherlands, the United States, Canada, and Korea)—three continents, Asia, Europe, and the America—were obtained. The sample size was relatively small, and its global representativeness was slightly insufficient. These are the limitations of this investigation. In the follow-up studies, our research group will consult more experts from various countries in order to achieve consensus in a wider range.

5. Conclusion

This investigation reveals that the key objectives of General Rules mainly include target people, structural elements, text structure, safety requirements, common technical problems of acupuncture-moxibustion, and specific details of technical manipulations. It provides a valuable reference for the formulation of specific Technical Specifications of Acupuncture and Moxibustion and guarantees the wide application and development of acupuncture and moxibustion technology in the world.

Data Availability

The data were taken from the published studies.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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Research Article

Effects of Traditional Chinese Acupuncture Compared with Sham Acupuncture on the Explosive Force Production by the Forearm Muscles in Female: A Randomized Controlled Trial

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Background. Acupuncture can effectively enhance musculoskeletal rehabilitation, with effects such as reduced pain intensity and muscle tension and decreased disability. *Objective*. The purpose of this study was to determine the efficacy of traditional Chinese acupuncture (TCA) compared with sham acupuncture (SA) in explosive force production by the forearm muscles in females. *Methods*. A total of 32 subjects were included and randomly assigned to two groups: TCA group ($n = 16$): stimulated specific acupoints including Quchi (LI11), Shousanli (LI10), Hegu (LI4), Xiaohai (SI8), Tianjing (SJ10), and Waiguan (SJ5) for 15 minutes; SA group ($n = 16$): using superficial needle insertion at nonacupoints without stimulation. The subjects warmed up for the 3-time isokinetic test with an angular velocity of 30°/s and then performed a set of 15 full flexion (Flex) and extension (Ext) with an angular velocity of 180°/s using the CON-TREX isokinetic test training system recorded as the pretest. After acupuncture for 15 min, perform a set of the same isokinetic movement isokinetic records as the posttest. The average max torque, average work, average power, average peak power, average max speed, and total work were collected to evaluate the forearm explosive force changes. Use two-way repeated measures ANOVA to compare the difference before and after acupuncture between two groups. *Results*. The results showed that acupuncture conditions (sham acupuncture as well as true acupuncture) and the intervention times (not acupuncture or acupuncture for 15 min) have a significant interaction effect on forearm explosive force and joint stiffness ($P < 0.05$). The simple main effect showed that the selected parameters of the TCA group increased significantly after acupuncture ($P < 0.05$), while the SA group did not ($P > 0.05$). We speculate that the activation of muscle may be related to the selected acupuncture points. *Conclusion*. Acupuncture can produce excitation in motor nerves and muscles, and nerve stimulation increases the recruitment of motor units, thus improving the muscle explosive force.

1. Introduction

Acupuncture originated approximately 2,500 years ago in China and is most familiar to Western medicine as a complementary or alternative therapy. This treatment modality is still applied in its original form [1] and can be used in the clinical treatment of diseases. In addition to having a therapeutic effect, acupuncture can effectively enhance musculoskeletal rehabilitation, with effects such as reduced pain intensity and muscle tension and decreased disability. The addition of acupuncture to a treatment regimen may facilitate and enhance physiotherapy performance in musculoskeletal rehabilitation for tension neck syndrome. Furthermore, acupuncture increases alertness and promotes energetic feelings in patients with multiple sclerosis [2]. Thus, acupuncture is effective in reducing pain and rehabilitating various musculoskeletal conditions. Acupuncture has been applied as an enhancer of sports performance. The use of acupuncture in resistance and endurance sports activities has demonstrated the association of traditional acupuncture protocols with increased muscular strength and power [3]. Needling of specific acupuncture points produced an increase in physical performance capacity and improved regulation of heart rate and blood pressure [4]. Thus,

acupuncture treatment not only improves athletic performance but also improves hemodynamics during endurance activities.

Acupuncture is able to produce the same excitatory characteristics within the motor nerves and muscles as exercise does [5]. Needles are inserted into specific points with nervous tissue. By stimulating these points with needles, the acupuncturist causes B-endorphins to be released for pain control [6]. Accordingly, acupuncture has been shown to reduce perceived pain arising from exercise-induced delayed-onset muscle soreness [7]. Acupuncture may enhance exercise performance and postexercise recovery. Unilateral electroacupuncture at selected acupoints even improved the muscle strength of both limbs [8]. Past research has found that overtraining and heavy sporting activity stress the immune status and neuroendocrine response of endurance athletes and lead to a decrease in overall sports performance [9]. However, acupuncture improved the subjective rating of muscle tension and fatigue and inhibited both the decrease in salivary secretory immunoglobulin and the increase in salivary cortisol after exercise stress [10]. Acupuncture has emerged as an alternative medical enhancer of human physical performance [3]. In a rat model, acupuncture can protect cells from acute sports injury, maintain the function of mitochondria to delay fatigue, prolong the working time of muscles, and delay muscle damage [11]. Therefore, this modality of treatment can increase physical function in both animals and humans. Electrical acupoint stimulation can enhance athletes' explosive strength. Specifically, the maximal peak moment of force, force, moment accelerating energy, and average power were increased [12]. In another study, transcutaneous electrical acupoint stimulation at selected acupoints enhanced the rate of muscle force recovery [13]. The treatment might also elevate the muscular pain threshold and change the perception of local muscle fatigue. The mechanism by which transcutaneous electrical acupoint stimulation influences recovery after exercise might be related to pain control [13]. Therefore, stimulation of the correct acupoints helps to delay exercise fatigue and improve muscle strength.

Isokinetic testing is considered the criterion standard for strength assessment. This method can be applied to the rehabilitation and prevention of throwing-related injuries and is important in determining return-to-play criteria [14]. Rehabilitation in medical clinics is also widely used. The energy efficiency of patients with chronic stroke is tested by measuring the isokinetic and isometric muscle strength of the upper limbs through the isokinetic test to achieve the effect of rehabilitation [15]. Acupuncture can achieve effective pain relief and improve the range of motion of joints [16]. Stimulating acupuncture points can improve athletic performance and improve biomechanical indexes, including maximum peak moment of force, force moment accelerating energy, and average power [12]. Combining acupuncture and isokinetic exercise training can be an effective rehabilitation therapy and may improve athletes' physical performance. At present, research on acupuncture extends beyond its use for rehabilitation therapy, but there is a lack of research regarding the effect of acupuncture on explosive force production by forearm muscles. Therefore, this study aims to explore the effect of acupuncture in explosive force production by the forearm muscles in females. Previous studies have found that acupuncture therapy has a direct effect on effectively improving the quadriceps muscle strength scale of recreational athletes [17]. The purpose of this study was to investigate the immediate effect of a single acupuncture session on strength improvement. None of the subjects in this study had previously received acupuncture with cumulative benefits.

2. Materials and Methods

2.1. Study Design. A randomised control trial (registration number: ChiCTR1900025407) was carried out to assess whether acupuncture has effects on explosive force production by the forearm muscles in females. The experiments were conducted at the Jilin Sport University Biomechanics Laboratory of the Health Technology College, Changchun, China, and was approved by the Joint Institutional Review Board of Jilin Sport University (JLSU; Changchun, China; JLSU-IRB no. 2018004). Volunteers were informed of the potential risks of acupuncture and were then asked to give written consent.

2.2. Subjects. In this study, the participants included 32 healthy female students (age = 25.4 ± 1.2 years, height = 161.6 ± 3.6 cm, body mass = 51.4 ± 5.4 kg, body mass index (BMI) = 19.6 ± 2.2 kg/m^2) from Jilin Sport University volunteered to participate in this study (September 1, 2019, to September 30, 2019), and all subjects were randomly divided into the TCA group and SA group. There was no significant difference between subjects in the TCA group and the SA group in terms of height, weight, and age. Participants were recruited through a recruitment announcement. The exclusion criteria were as follows: stroke, severe heart disease, diabetes, neuromuscular disorders, inability to participate in physical activity, arrhythmia, use of antiarrhythmic drugs or a pacemaker, severe cardiovascular disease, current use of drugs that affect muscle mass or muscle performance, strenuous exercise or muscle soreness during the first 24 hours, obesity (BMI >22), and body weight of more than 90 kg.

2.3. Protocol. The elbow Flex/Ext isokinetic measurements were performed using the CON-TREX isokinetic test training system (ConTrex MJ; CMV AG, Dübendorf, Switzerland). Subjects were tested separately. During the test, the angle between the seat back and the seat was adjusted to 85°; the seat was rotated 15° to the right, and the force measuring shaft was rotated to 15° to be parallel to the chair orientation. The subject sat in a comfortable position and was firmly fixed to the seat with the chest and waist straps and with the right elbow aligned with the axis of the dynamometer. The constant-velocity adapter was installed. The center of rotation of the elbow was carefully aligned with the center of rotation of the dynamometer's lever arm. Prior to isokinetic assessment, the subjects were performed a 3-time isokinetic test with an angular velocity of 30°/s for warm-up and to get familiar with the device. Then, the

subject performed elbow joint Flex/Ext 15 times at 180°/s recorded as the pretest. After that, all subjects received 15-minute TCA or SA. After acupuncture, the subjects performed 15 times of full Flex/Ext with an angular velocity of 180°/s recorded as the posttest. A flow diagram of the protocol is shown in Figure 1.

2.4. Traditional Chinese Acupuncture and Sham Acupuncture. Huatuo brand disposable sterile steel needles (size: 0.25 mm × 40 mm; manufactured by Suzhou Medical Appliance, Jiangsu, China) were used to stimulate the following acupuncture points: LI11, LI10, LI4, SI8, SJ10, and SJ5 (Figure 2). The needles were left in place for 15 min [18]. The acupuncturist placed his/her left index finger immediately above the acupoint, held the needle between his/her right thumb and index finger, and quickly pierced the skin of the patient. The slow inward pressure and twisting of the needle introduced a sensation of De Qi, radiating numb sensation, and acid bilge feeling. The acupuncturist then twisted the needle repeatedly at a speed of 3–5 r/s without the use of an electric or laser instrument. Each needle was rotated at 2 minutes, 5 minutes, and 10 minutes after insertion. While the needle was pulled out, sterilized cotton was pressed to the cheek for 5 s. The needle depths were approximately 50 mm [19].

A new sham acupuncture needle has been developed. Sham acupuncture points were used in a protocol similar to the traditional Chinese acupuncture points. For the sham condition, the same certified dental acupuncturist inserted the needles, which were left in place for 15 minutes, and during this period, use lifted, inserted, and twirled to stimulate the acupoints to generate the sense of "De Qi." The needling experience for the SA group was the same as the TCA group, except the needles were in a nonacupuncture point and penetrated the skin only 2–4 mm.

2.5. Reasons for Acupoints Selection. The triceps are divided into the long head of the triceps, the lateral head, and the medial head, which are the main muscles for extension the elbow joint [20]. Brachioradial muscle can make forearm supination and flexion [21]. Studies have showed that acupuncture may increase the explosive forces generated by acupoint-related muscles by stimulating nerves [22]. In our study, SI8 and SJ10 are located in the triceps muscle and LI11 is located in the brachioradial muscle. Acupuncture points are selected to stimulate and may have similar effects.

2.6. Data Collection. The stiffness of the elbow joint was calculated using the following formula:

$$E_{\text{joint}} = \frac{\Delta M_{\text{joint}}}{\Delta \theta_{\text{joint}}}, \tag{1}$$

where ΔM_{joint} is the change in the joint moment between the maximum elbow Ext and maximum elbow Flex, and $\Delta \theta_{\text{joint}}$ is the angular displacement of the joint between maximum elbow Ext and maximum elbow Flex. The joint moment was normalized to the participant's body weight.

2.7. Data Analysis. The max torque values were identified in association with the max voluntary isometric contraction for the elbow Ext and at 180°/s isokinetic contraction for the elbow Flex. These values were used for the normalization of the other data. The max torque values were computed as the mean torque obtained in a 1 s window centered at the peak value. For each isokinetic set, the repetition showing the greatest torque was chosen for the analysis.

The data are reported as mean ± standard deviation (SD) and were analyzed with a mixed design two-way analysis of variance (ANOVA) with repeated measures. There were the between-subject factor group (sham acupuncture vs. true acupuncture) and within-subject factor (pretest and posttest). This design allowed for testing the main effect of groups, the main effect of time, and the interaction of groups by time. In case of significant interaction, simple effects were examined, i.e., the effects of one factor holding the other factor fixed. MATLAB software (version R2019a; MathWorks, Inc., Natick, MA) was used for the statistical analysis. $P < 0.05$ was defined as statistically significant. Calculate effect estimates (effect size: ES) to summarize the effects of acupuncture on each outcome by recalculation with a change score (e.g., posttest minus pretest) as the numerator and sample variability (e.g., standard deviation) as the denominator:

$$\text{ES} = M(\text{post}) - \frac{M(\text{pre})}{\text{SD}}. \tag{2}$$

Cohen categorized ES values as small (ES: 0.2 – 0.5), moderate (ES: 0.5 – 0.8), and large (ES: >0.8) [23].

3. Results

To detect differences between the intervention times (not acupuncture or acupuncture for 15 minutes) and both acupuncture conditions (sham acupuncture as well as true acupuncture), we performed a two-way ANOVA with the between-subject factor group (sham acupuncture vs. true acupuncture) and within-subject factor (pretest and posttest). Tables 1 and 2 summarize the results before and after acupuncture. For the intervention time and acupuncture conditions, a statistically significant interaction effect was shown in terms of all parameters ($P < 0.05$). Therefore, the results suggest that intervention time and acupuncture conditions did significantly affect the elbow joint explosive force and joint stiffness.

At the end of the 15 min acupuncture, further analysis of the simple main effect revealed a significant difference between the SA and TCA groups across time. There was significant difference in the TCA groups before and after intervention, respectively, that significantly increased after acupuncture, including the average max torque Flex/Ext ($+\Delta = 0.41$, ES = 0.58, $P = 0.035$ and $+\Delta = 0.13$, ES = 1.21, $P < 0.001$), average work Flex/Ext ($+\Delta = 0.88$, ES = 0.50, $P = 0.003$ and $+\Delta = 0.41$, ES = 0.88, $P = 0.003$), average power Flex/Ext ($+\Delta = 0.31$, ES = 0.87, $P = 0.003$ and $+\Delta = 0.24$, ES = 0.77, $P = 0.007$), average peak power Flex/Ext ($+\Delta = 0.09$, ES = 0.86, $P = 0.004$ and $+\Delta = 0.32$, ES = 0.78, $P = 0.007$), average max speed Flex/Ext ($+\Delta = 0.13$,

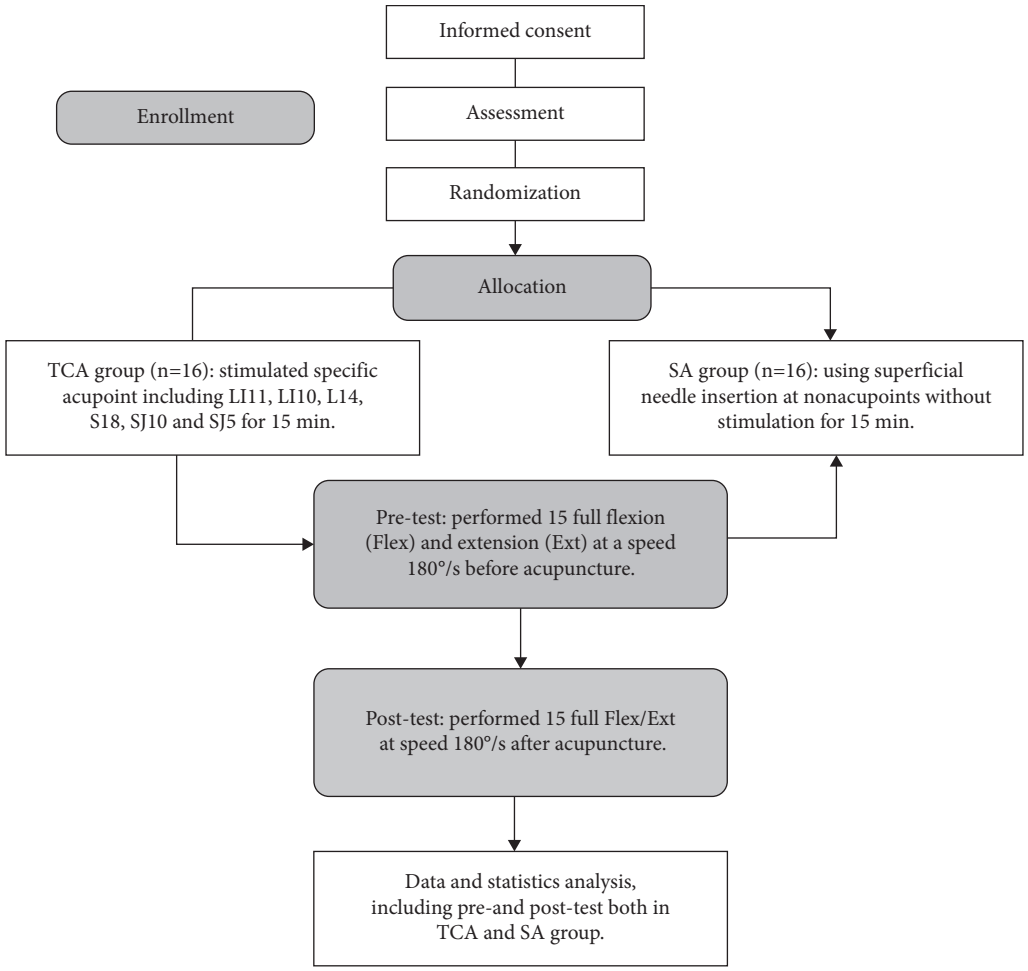

Figure 1: Flow diagram of the study.

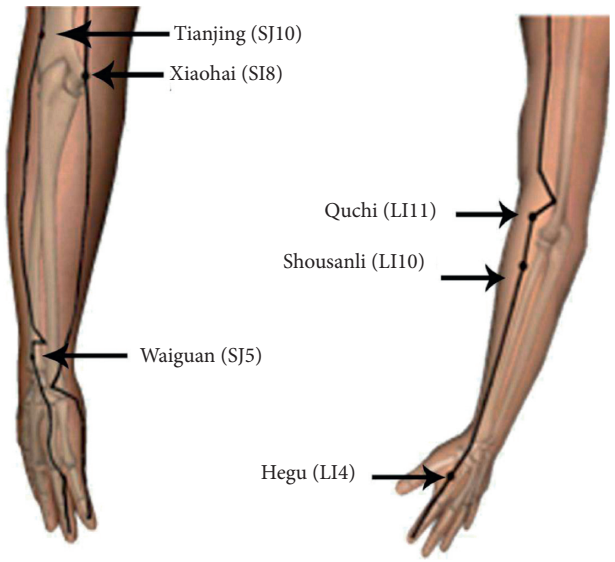

Figure 2: Acupuncture points.

Table 1: Mean ± SD of the elbow joint flexion (Flex) muscles before and after acupuncture for each variable.

Characteristic	Treatment	Pre	Post	Delta	P values		
					Main effects (time)	Main effects (group)	Interaction (time * group)
Average max torque Flex/kg (nm/kg)	SA	0.35 ± 0.10	0.32 ± 0.08	−0.09	0.469	0.058	0.033*
	TCA	0.34 ± 0.07	0.41 ± 0.09	0.41			
Average work Flex/kg (J/kg)	SA	0.34 ± 0.11	0.36 ± 0.08	0.06	0.004	0.058	0.021*
	TCA	0.33 ± 0.13	0.49 ± 0.15	0.48			
Average power Flex/kg (J/kg)	SA	0.19 ± 0.09	0.20 ± 0.08	0.05	0.012	0.018	0.040*
	TCA	0.26 ± 0.15	0.34 ± 0.16	0.31			
Average peak power Flex/kg (W/kg)	SA	0.31 ± 0.16	0.31 ± 0.15	0.00	0.007	0.031	0.001*
	TCA	0.47 ± 0.27	0.51 ± 0.28	0.09			
Average max speed Flex/kg (deg/s*kg)	SA	1.02 ± 0.29	0.97 ± 0.21	−0.05	0.238	0.009	0.023*
	TCA	1.16 ± 0.27	1.13 ± 0.29	0.13			
Total work Flex (J)	SA	236.94 ± 61.23	253.96 ± 56.19	0.07	0.001	0.087	0.019*
	TCA	234.32 ± 58.00	315.49 ± 62.46	0.35			
Stiffness Flex (Nm/kg*deg)	SA	0.008 ± 0.003	0.007 ± 0.002	−0.13	0.763	0.005	0.027*
	TCA	0.009 ± 0.003	0.011 ± 0.004	0.11			

Note: values are mean ± SD. TCA, traditional Chinese acupuncture; SA, sham acupuncture. *There are significant differences in the interaction between group and time ($P < 0.05$).

Table 2: Mean ± SD of the elbow joint extension (Ext) muscles before and after acupuncture for each variable.

Characteristic	Treatment	Pre	Post	Delta	P values		
					Main effects (time)	Main effects (group)	Interaction (time * group)
Average max torque Ext/kg (nm/kg)	SA	0.37 ± 0.10	0.37 ± 0.09	0.00	0.034	0.004	0.005*
	TCA	0.47 ± 0.14	0.53 ± 0.14	0.13			
Average work Ext/kg (J/kg)	SA	0.31 ± 0.08	0.34 ± 0.10	0.10	0.001	0.047	0.028*
	TCA	0.32 ± 0.09	0.45 ± 0.11	0.41			
Average power Ext/kg (J/kg)	SA	0.22 ± 0.10	0.20 ± 0.07	−0.09	0.229	0.060	0.032*
	TCA	0.25 ± 0.13	0.31 ± 0.13	0.24			
Average peak power Ext/kg (W/kg)	SA	0.34 ± 0.22	0.31 ± 0.21	−0.09	0.071	0.084	0.002*
	TCA	0.37 ± 0.43	0.49 ± 0.49	0.32			
Average max speed Ext/kg (deg/s*kg)	SA	1.00 ± 0.25	0.96 ± 0.22	−0.12	0.178	0.005	0.030*
	TCA	1.21 ± 0.36	1.36 ± 0.38	0.34			
Total work Ext (J)	SA	250.18 ± 67.47	280.10 ± 70.81	0.12	<0.001	0.073	0.037*
	TCA	264.75 ± 84.25	355.35 ± 90.63	0.34			
Stiffness Ext (Nm/kg*deg)	SA	0.011 ± 0.009	0.008 ± 0.004	−0.27	0.334	0.261	0.009
	TCA	0.010 ± 0.006	0.015 ± 0.011	0.50			

Note: values are mean ± SD. TCA, traditional Chinese acupuncture; SA, sham acupuncture. *There are significant differences in the interaction between group and time ($P < 0.05$).

ES = 0.62, $P = 0.026$ and +Δ = 0.34, ES = 1.48, $P < 0.001$), and total work Flex/Ext (+Δ = 0.35, ES = 1.04, $P = 0.001$ and +Δ = 0.34, ES = 0.99, $P = 0.002$). However, in the SA group, there was no significant difference in those same parameters before and after acupuncture or after acupuncture compared with the TA group (all $P > 0.05$) (Figure 3(a)).

Additionally, there were significant differences between the TA group and the SA group after acupuncture. Specifically, significant differences were found between the TCA group and the SA group after acupuncture, including the average max torque Flex/Ext (+Δ = 0.28, ES = 1.07, $P = 0.005$ and +Δ = 0.34, ES = 01.34, $P = 0.001$), average work Flex/Ext (+Δ= 0.36, ES = 1.11, $P = 0.004$ and +Δ= 0.32, ES = 0.99, $P = 0.009$), average power Flex/Ext (+Δ= 0.70, ES = 1.08, $P = 0.005$ and +Δ = 0.55, ES = 1.03, $P = 0.007$), average peak power Flex/Ext (+Δ= 0.65, ES = 0.89, $P = 0.017$ and +Δ= 0.58, ES = 1.10, $P = 0.004$), average max speed Flex/Ext (+Δ= 0.35, ES = 1.34, $P = 0.001$ and +Δ= 0.42, ES = 1.29, $P = 0.001$), and total work Flex/Ext (+Δ= 0.24, ES = 1.34, $P = 0.006$ and +Δ= 0.27, ES = 0.93, $P = 0.014$) (Figure 3(b)).

Figure 4 shows the joint stiffness of elbow joint Flex/Ext for the TCA group and SA group as assessed before and after intervention. There were significant differences before and after acupuncture at the TCA group, that is, stiffness Flex (+Δ= 0.11, ES = 0.70, $P = 0.013$) and stiffness Ext (+Δ= 0.50, ES = 0.75, $P = 0.009$) (Figure 4(a)). After acupuncture, the stiffness Flex/Ext also significantly and respectively increased compared with the SA group (+Δ= 0.43, ES = 1.38, $P < 0.001$ and+Δ= 0.80, ES = 0.53, $P = 0.031$) (Figure 4(b)).

Accordingly, after 15-min acupuncture at LI11, LI10, LI4, SI8, SJ10, and SJ5 can immediately improve the explosive force and joint stiffness of the elbow joint Flex/Ext compared to sham acupuncture.

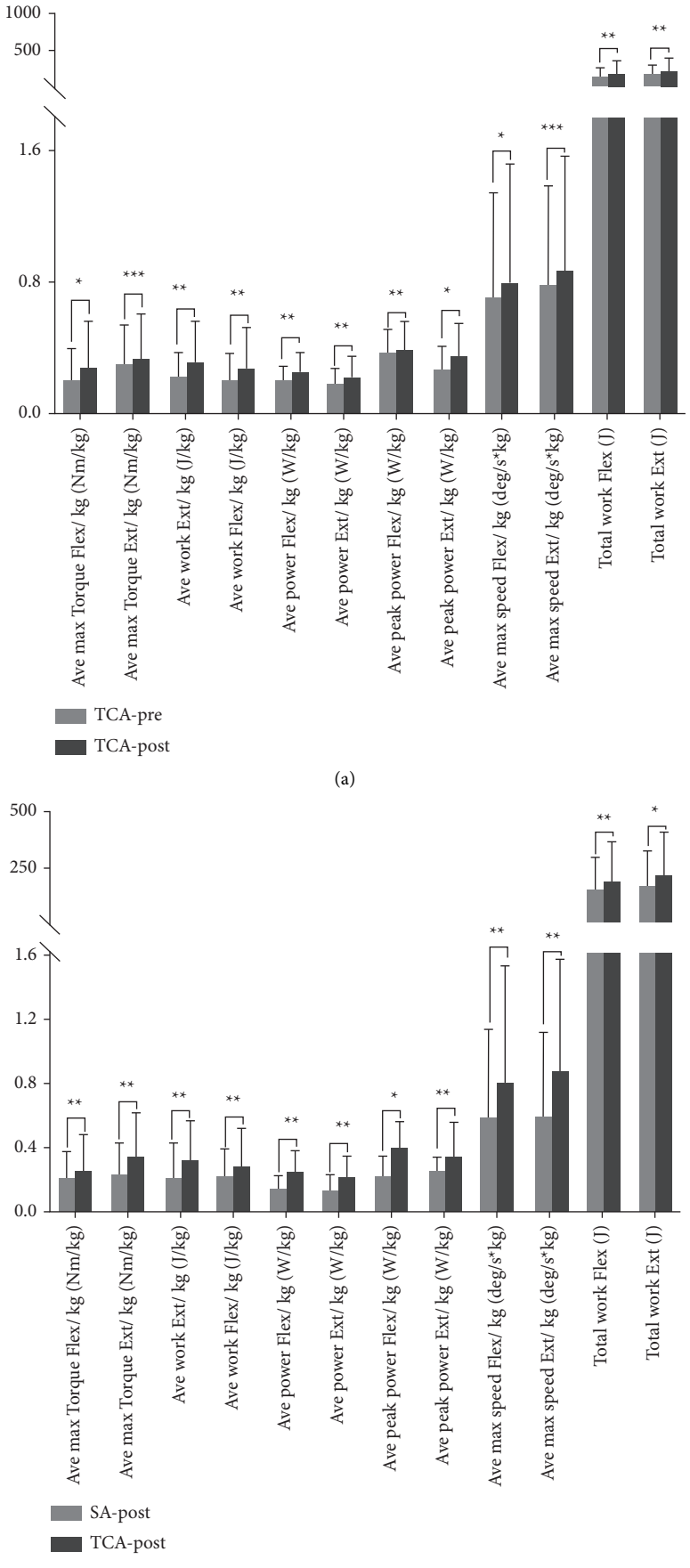

Figure 3: The differences of the elbow joint Flex/Ext for each isokinetic parameter within-subject factor (pretest and posttest) at the TCA group (a) and between-subject factor (TCA vs. the SA group) at the posttest (b). Note: values are mean ± SD. TCA, traditional Chinese acupuncture; SA, sham acupuncture. $^{*}P < 0.05$. $^{**}P < 0.01$. $^{***}P < 0.001$.

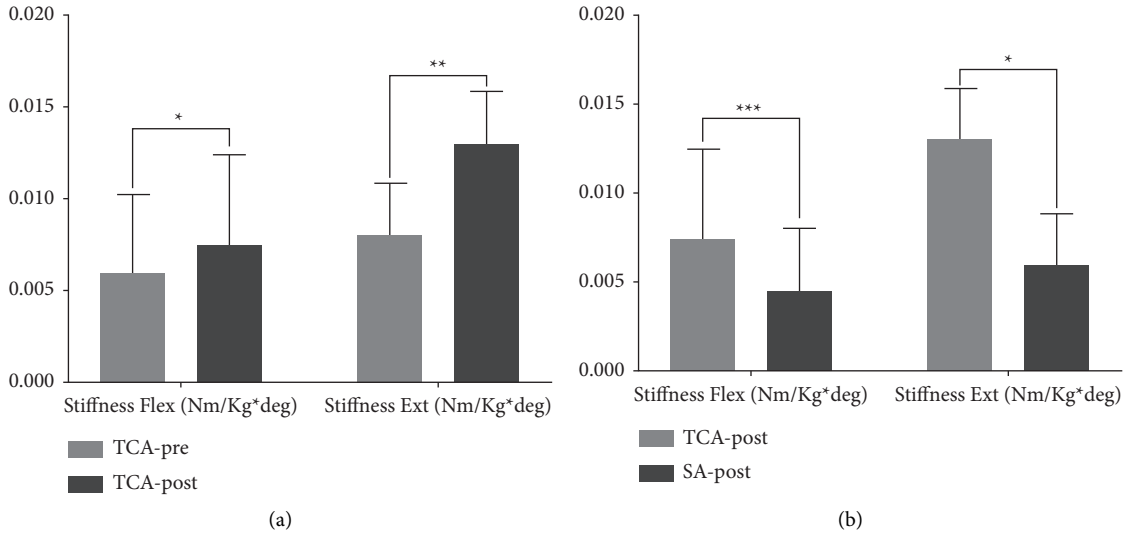

Figure 4: The differences of elbow joint stiffness Flex/Ext within-subject factor (pretest and posttest) at the TCA group (a) and between-subject factor (TCA vs. the SA group) at the posttest (b). Note: values are mean ± SD. TCA, traditional Chinese acupuncture; SA, sham acupuncture. $^{*}P < 0.05$. $^{**}P < 0.01$. $^{***}P < 0.001$.

4. Discussion

After acupuncture, the average max torque Flex/Ext value was greater than the pretest values, which may be due to increased muscle strength and increased explosive power. Neural factors are an important determinant of torque gain in this training protocol [24]. The neural effects of acupuncture may stimulate muscle contraction, increasing the muscles explosive force during training or competition. The mild tonic somatosensory stimulation produced by acupuncture produces long-term plastic changes in the excitability of very distant nervous structures that exert motor control over remote muscles [25]. Enhanced muscle fiber conduction velocity increases torque [26, 27]. Therefore, it is possible to increase the speed of muscle fiber conduction velocity through acupuncture, causing the average max torque Flex/Ext to improve. The phenomenon of related physiological reactions after acupuncture is called "De Qi" and is widely considered necessary for the therapeutic effect of acupuncture. "De Qi" can increase the torque and muscle mass of the upper and lower extremities [28]. Transcutaneous electrical acupoint stimulation of muscles that have developed fatigue has been found to increase the rate of muscle force recovery and peak torque. Past research has shown that acupoint stimulation (true ST 36) in young football players leads to increased knee extension and flexion strength and increased peak torque [29]. After stimulation of the LI11, LI10, LI4, SI8, SJ10, and SJ5 acupoints, the average max torque Flex/Ext was improved compared with the pretest, suggesting that acupuncture can stimulate the muscles and increase the torque.

In our study, we found that the average max speed Ext and the max speed Ext were significantly increased after acupuncture. Additionally, the total work Ext increased compared with the pretest value. Past research has found that electrical acupoint stimulation increases the maximal peak moment, force, moment accelerating energy, and average power [12]. In a study of astronauts, stimulating the antagonist muscle to resist the volitional contraction of the agonist increased the extension torque, average power, and total work output of the elbow joint [30]. Therefore, acupuncture may increase the speed of muscle contraction and increase work output. Acupuncture in rats can increase Ca^{2+}-ATPase (adenosine triphosphate, ATP) activity and Ca^{2+} content in the sarcoplasmic reticulum of skeletal muscle cells in a state of motor fatigue, which may protect cells from acute sport injury and maintain the function of mitochondria to delay fatigue, prolong the working time of muscles, and protect muscles from damage; this effect on Ca^{2+} transport may contribute to the beneficial effect of acupuncture on motor ability [11,31]. Therefore, the increase in total work Ext in this study may occur because acupuncture stimulates muscles to increase their sarcoplasmic reticulum Ca^{2+} content, stimulates Ca^{2+}-actin interactions, and increases the work output of the muscles during contraction.

In this study, we found that elbow joint stiffness was greater after acupuncture. Acupuncture is able to induce long-term plastic changes in the central nervous system [25]. Increasing the conduction velocity of muscle fibers increases the torque at the corresponding joint [26,27]. Past research has shown that acupuncture stimulation enhances stiffness and improves the viscoelasticity of tendon structures [32]. Muscle contractions preceding an activity can result in increased force generation through PAP. Isometric muscular contractions may affect subsequent strength and power performance. Therefore, acupuncture may improve neuromuscular control and generate nervous system excitation, thus increasing joint stiffness.

5. Conclusion

This study focused on the changes in explosive force production by the forearm muscles in females before and after acupuncture both in TCA and SA groups. Our data show that 15-minute acupuncture is beneficial to the production of forearm muscles by inducing the PAP effect. The choice of acupoints also affects the difference in elbow joint stiffness upon extension and flexion. Acupuncture can produce excitation in motor nerves and muscles, and nerve stimulation increases the recruitment of motor units, thus improving the muscle explosive force (such as torque, power, and work). This experiment is a clinically valuable contribution to motor neuromuscular therapy, providing a reference to a method for coaches to improve athletic performance.

This study has several limitations to consider. First, only articles written in English were included, which may limit the scope of some of the acupuncture literature. In addition, the present study included only young healthy females; therefore, compared with athletes using the same research methods, the results may not be the same. Furthermore, future research will explore the timeliness and gender differences of acupuncture that improve the explosive force of the forearm muscles of the candidates.

Data Availability

The datasets used and analyzed to support the findings of this study are included within the article.

Ethical Approval

This trial was approved by the Ethics Committee of Joint Institutional Review Board of Jilin Sport University (JLSU; Changchun, China; JLSU-IRB no. 2018004). The protocol used with the subjects was reviewed and approved by the Jilin Sport University Joint Institutional Review Board.

Disclosure

Shu Zhou and I-Lin Wang are the co-first authors.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

ILW and JW designed the experiments. RH, JYS, and SZ involved in preparation and laboratory experiments. SZ and YMC contributed reagents, materials, and analysis platforms. ILW and JW supervised and reviewed and edited the article. Shu Zhou and I-Lin Wang contributed to the work equally.

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Review Article

Electroacupuncture as an Adjuvant Approach to Rehabilitation during Postacute Phase after Total Knee Arthroplasty: A Systematic Review and Meta-Analysis of Randomized Controlled Trials

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Background. Increasing attention has been paid to electroacupuncture (EA) for promoting postoperative rehabilitation, but the effectiveness of EA for rehabilitation after total knee arthroplasty (TKA) remains obscure. *Objective*. To examine the effect of EA on rehabilitation after TKA. *Methods*. Database searches on PubMed, CINAHL, Embase, and China National Knowledge Infrastructure (CNKI) were carried out to obtain articles, from inception to 15 October 2020. All identified articles were screened, and data from each included study were extracted independently by two investigators. Meta-analysis was conducted to assess the effects of acupuncture on pain, range of knee motion, and postoperative vomiting after TKA. *Results*. In the current study, a total of ten randomized clinical trials were included according to the inclusion and exclusion criteria. Compared to basic treatment, EA combined with basic treatment showed a significantly greater pain reduction on 3, 7, and 14 days postoperatively after TKA. However, we found that EA had no significant improvement in enhancing the range of knee motion and decreasing the percentage of vomiting. Subgroup analysis suggested that a combination of EA and rehabilitation training was superior to rehabilitation training in pain relief, while EA combined with celecoxib capsules showed no significant difference in improving pain compared to celecoxib capsules alone. *Conclusions*. In the postacute phase after TKA, EA, as a supplementary treatment, could reduce postoperative pain, but no evidence supported the benefits of EA for improving ROM of knee and decreasing the ratio of vomiting. Additional high-quality and large-scale RCTs are warranted.

1. Introduction

Knee osteoarthritis (KOA) is characterized by the degeneration of joint cartilage, which leads to pain, swelling, dysfunction, and even joint deformity in middle-aged and elderly patients [1]. With the growing population of aging, KOA has become a global public health concern. Total knee arthroplasty (TKA) is considered as the final treatment for KOA, which is widely used to alleviate pain in the patients with advanced KOA due to the high degree of patient satisfaction. Despite its beneficial effects, most patients continue experiencing persistent moderate/severe pain and functional limitations after TKA [2]. As is reported, patients with high postoperative pain present poorer outcomes including quality of life and function during the rehabilitation process after TKA [3].

In order to allow accelerated postoperative rehabilitation, many therapeutic methods focusing on pain reduction and function improvement have been developed. As the first-line methods, pharmacological therapies such as steroids [4], opioids [5], and nonsteroidal anti-inflammatory drugs [6] are preferred in clinic practice. However, drug-related side effects like nausea, vomiting, and retention of urine [7] are frequently reported, which require prescribers to remain vigilant when prescribing the relevant drugs. Therefore, it is critical and urgent to explore a safe, effective, and feasible nonpharmacological therapy for postoperative rehabilitation to reduce the consumption of medications and related adverse effects in TKA patients [8].

Electroacupuncture (EA), as a pain management technique, has been utilized worldwide to treat acute and chronic pain. It is suggested that EA can activate various bioactive chemicals via peripheral, spinal, and supraspinal mechanisms [9]; inhibit the induction and transmission of pain signals; regulate the interactions of neuro-immune-endocrine; and consequently improve pain and inflammatory [10]. In recent years, it is proposed to be applied for rehabilitation in individuals undergoing TKA. Until now, only one meta-analysis [11] including two RCTs reported the inexplicit effect of EA on postoperative pain after TKA. However, an increasing number of studies report the impacts of EA on rehabilitation in TKA patients, and some randomized controlled trials (RCTs) aiming to evaluate the efficacy of EA for rehabilitation after TKA are being carried out [12, 13]. In this study, we undertook a systematic review and meta-analysis by gathering evidence from the available RCTs on EA to assess its effectiveness in rehabilitation for patients receiving TKA.

2. Methods

In this study, ethical approval was not required because all the analyses were performed according to data published in previous studies. And this meta-analysis was conducted following the Preferred Reporting Items for Systematic Review and Meta-Analyses [14].

2.1. Search Strategy. In order to identify relevant studies, we searched electronic databases, including Cumulative Index to Nursing and Allied Health Literature (CINAHL), PubMed, Embase, and China National Knowledge Infrastructure (CNKI) database, from inception to 15 October 2020. Keywords such as "electroacupuncture", "total knee arthroplasty", "total knee replacement", "total knee*", "randomized controlled trial", "controlled clinical trial", "randomly", "randomized", "placebo", "trial", and so on were utilized to search without restrictions. The search strategy was recorded in detail in Supplementary eFigure 1. Two researchers (WJ Chen and ZH Chen) independently screened titles and abstracts. Subsequently, the remaining literatures were screened strictly by reading full texts, and all eligible studies were included according to the inclusion and exclusion criteria. Finally, the materials and data in the included studies were extracted. During the period of screening and data extraction, the discrepancy would be resolved through discussion or consultation with the primary reviewer.

2.2. Selection Criteria. In the current study, the PICO (patients, interventions, comparators, and outcomes) question was taken into consideration at our primary search [15]. The inclusion criteria were as follows: (1) study design: clinical randomized controlled study; (2) patients: patients receiving primary TKA; (3) intervention: EA; (4) comparators: EA versus other treatments, EA + other treatments versus other treatments, and EA versus placebo or sham EA; (5) outcomes: postoperative rehabilitation, at least one efficacy index; and (6) languages: Chinese and English. Studies would be excluded if they met any of the following criteria: (1) conference abstracts, full-text unavailable articles, or unpublished literatures and (2) repeated publications, revision TKA, unicompartmental knee arthroplasty, animal experimental studies, manual acupuncture, transcutaneous electrical stimulation, transcutaneous neuromuscular electrical stimulation, meta-analysis, or reviews.

2.3. Data Extraction. The two researchers who screened the literatures independently extracted the following information from the included articles: authors' names, publication year, countries involved, age and gender of patients, study design, sample size, intervention type and control characteristics, acupuncture points, needle retaining time, intervention dose, and main outcomes.

2.4. Quality Assessment. The quality of the included RCTs was qualitatively assessed using the risk of bias table according to 5.1.0 [16] of the Cochrane manual. The risk of bias is structured into seven aspects: sequence generation, allocation concealment, blind of participants and personnel, blind of outcome, incomplete outcome data, selective reporting, and other biases. The risk of each item is categorized into three levels: high, unclear, and low.

2.5. Statistical Analysis. This meta-analysis was conducted using the Review Manager 5.3 software to examine the effects of EA on postoperative rehabilitation after TKA using the reported indicators in the included literatures, and the corresponding results were depicted by the forest map intuitively. The continuous variables were pooled by standard mean differences (SMDs) or mean differences (MDs) with 95% confidence intervals (95% CI), while the odds ratios (OR) were used to estimate the enumeration data. Heterogeneity assessment was performed using Cochran's Q-test and the I^2 index [17]. When I^2 was statistically greater than 50%, a random-effects model would be utilized. According to the *Cochrane Handbook for Systematic Reviews of Interventions* [18], sensitivity analyses or subgroup analyses would be applied when substantially heterogeneous was detected among more than 5 studies. Begg's and Egger's tests were selected to evaluate publication bias [19]. P values <0.05 were viewed as statistically significant differences.

3. Results

3.1. Study Selection. A total of 94 potentially relevant records were yielded by searching Chinese and English databases. After removing 37 duplicates, eliminating 46 articles by screening titles and reading summary and full text, and excluding one study without full text (Supplementary eTable 1), 10 RCTs [20–29] were included, and 484 TKA patients with the experimental group ($n = 241$) and the control group ($n = 243$) were enrolled. The flowchart for the selection process was depicted in Figure 1, and the characteristics of each included RCT are summarized in Table 1.

3.2. Risk of Bias. All the included studies were described as random generation, and 8 articles [19–24, 27, 28] documented the methods of randomization in detail. Five of the 10 included studies recorded blind methods in detail [19, 20, 22, 24, 28]. As shown in Figure 2, most of the included RCTs were defined as low risk of bias; we could conclude that the methodological quality of the included studies was fair to middling.

3.3. Meta-Analysis

3.3.1. Postoperative Pain. From the fixed-effects model, the meta-analysis of 4 studies [20, 27–29] suggested no statistically significant improvement in the electroacupuncture group (EG) versus the control group, with mean differences of −0.18 (95% CI: −0.46, 0.09; $P = 0.19$; $I^2 = 39\%$) on pain evaluated on the first day after surgery (Figure 3(a)). However, a significantly greater pain reduction was observed in the EG when compared to the CG on postoperative day 3 (MD = −0.75; 95% CI: −1.01, −0.48; $P < 0.00001$; $I^2 = 0\%$; Figure 3(b)). Meta-analysis of 4 pain relief studies [22, 26–28] was performed using a fixed-effects model because of substantial heterogeneity, the result of which revealed a significant pain improvement in EG in comparison to the CG at 7-day follow-up (MD = −0.43; 95% CI: −0.82, −0.04; $P = 0.03$; $I^2 = 55\%$; Figure 3(c)). Moreover, the EG showed a smaller VAS score than the CG on postoperative day 14 (MD = −0.97; 95% CI: −1.74, −0.21; $P = 0.01$; $I^2 = 81\%$; Figure 3(d)).

3.3.2. Range of Motion (ROM) and Nausea/Vomiting (an Analgesia-Related Adverse Effect). ROM of knee was recorded in 3 studies [23, 24, 26], while sufficient data was not provided in 1 study [23]. We attempted to contact authors but received no response. The analysis result (Figure 4) suggested that there was no significant improvement in both flexion and extension deficit of knee between EG (MD = 2.11; 95% CI: −1.26, 5.48; $P = 0.22$; $I^2 = 62\%$) and CG (MD = 0.43; 95% CI: −0.00, 0.86; $P = 0.05$; $I^2 = 47\%$) at 2-week follow-up. Meanwhile, meta-analysis of 3 studies [21, 23, 29] showed there was a closely similar percentage of nausea/vomiting in the 2 groups (OR = 0.83; 95% CI: 0.37, 1.87; $P = 0.006$; $I^2 = 0\%$; Figure 5).

3.4. Sensitivity Analysis. In this study, considering the substantial heterogeneity in postoperative pain at 7-day follow-up, we conducted sensitivity subgroup analyses to detect the source of heterogeneity. The subgroup analysis was performed according to the different comparisons, and we found that there was a significantly greater pain reduction improvement in the EG treated with a combination of EA and rehabilitation training when compared to the CG treated with rehabilitation training only (MD = −0.71; 95% CI: −1.34, −0.09; $P = 0.03$; $I^2 = 6\%$). However, no significant improvement in pain between the EG treated with EA and celecoxib capsules and the CG receiving celecoxib capsules (MD = −0.33; 95% CI: −0.82, −0.44; $P = 0.21$; $I^2 = 75\%$; Supplementary eFigure 2).

3.5. Publication Bias. In this study, we examined publication bias using Begg's and Egger's tests. As shown in Supplementary eTable 2, we found there was no evidence for significant publication bias among the included studies.

3.6. Adverse Events. No serious adverse event was reported in the included studies.

4. Discussion

EA has been proved to be beneficial for pain relief, and its unique advantages in alleviating pain are increasingly taken seriously due to few side effects. Considering its advantages, it has been commonly used for the treatment of KOA, and there are some evidence supporting the efficacy in increasing knee ROM, reducing pain, and improving function for KOA patients [30]. Postoperative pain always attracted worldwide attention, especially major orthopedic surgery, such as TKA. A substantial number of TKA patients suffer from persistent pain, and the ratio of patients with moderate-to-severe pain is up to 28%, which can strongly influence the success of postoperative rehabilitation [31]. It is crucial and urgent to find an effective method to achieve excellent pain management after surgery. It was previously reported that EA could exhibit greater analgesic effects during the treatment of different types of pain [32]. Gradually, EA began to be used to try solving this problem in the clinic. However, even though several studies reported the effect of EA on pain, ROM, and function during the recovery period, the conclusion is inconsistent. In the previous meta-analysis including 2 RCTs, there was no evidence whether EA should be recommended explicitly or not. In our study, we pooled more RCTs published before to estimate the effects of EA on rehabilitation for patients receiving TKA.

Overall, regarding postoperative pain, the results of this study revealed that EA exerted a significantly positive impact on pain control in the early postoperative phase after TKA. We found that patients treated with EA combined with the basic therapy revealed a significant pain reduction when compared to patients receiving the basic therapy alone on postoperative day 3, 7, and 14. However, EA displayed no significant improvement in pain on the first postoperative day. It might be the reason that patients receiving

Table 1: Characteristics of studies.

First author, year	Country	Age (years)		Sample size	Male/female		Intervention		Study design	Intervention parameters	Intervention dose	Main outcome
		EG	CG	EG/CG	EG	CG	EG	CG				
Chen, 2019 [19]	China	67 ± 7	67 ± 6	20/20	2/18	0/20	EA + multimodal analgesia	Multimodal analgesia	RCT	Acupoints: Liangqiu (ST 34), Xuehai (SP 10), Yinlingquan (SP 9), Zusanli (ST 36), Fenglong (ST 40), and Qiuxu (GB 40) on the operation side; Frequency: 2 Hz/ 100 Hz; Intensity: 2~5 mA	30 min, once/day, on the first 7 days after operation	VAS, prostaglandin E2, and β-endorphin
Tzeng et al., 2015 [21]	China (Taiwan)	69.6 ± 5.6	70.1 ± 6.9	16/17	4/12	3/14	EA + PCA	PCA	RCT	Acupoints: ST36 and GB34 on contralateral to the operated leg; Frequency: 2 Hz/; Intensity: 2 mA	30 min on the first two postoperative days	The time of first demand for PCA, total dosage of PCA solution, and rate of vomiting
Tao et al. 2019 [22]	China	71 ± 11	68 ± 10	40/40	23/17	21/19	EA + routine treatment	Routine treatment	RCT	Acupoints: Hegu (LI 4), Qihai (CV 6), Zhongwan (CV 12), Pishu (BL 20), and Shenshu (BL 23) on the operation side; Frequency: 2 Hz/ 100 Hz; Intensity: 4 mA	15 min, once every other day; 1 month	Insulin resistance index , fasting insulin
Chen et al., 2018 [23]	China	67.1 ± 7.0	66.7 ± 6.3	20/20	2/18	0/20	EA + basic treatment	Basic treatment	RCT	Acupoints: Liangqiu (ST34), Xuehai (SP10), Yinlingquan (SP9), Zusanli (ST36), Fenglong (ST40), Qiuxu (GB40) on the operation side; Frequency: 2 Hz/ 100 Hz; Intensity: 4 mA	30 min, once daily for 7 days after operation	VAS, rate of vomiting , and range of motion
Chen et al., 2012 [24]	China	67.4 ± 5.1	65.6 ± 5.2	35/35	13/22	12/23	EA + rehabilitation training	Rehabilitation training	RCT	Acupoints: Xuehai (SP10), Liangqiu (ST34), Dubi (ST35), Neixiyan (EX-LE4), and Yanglingquan (GB34) on the operation side; Frequency: 2 Hz	30 min, once daily on the first 14 days, once/week from the 3rd to 12th week after operation	VAS, HSS, range of motion

Table 1: Continued.

First author, year	Country	Age (years)		Sample size	Male/female		Intervention		Study design	Intervention parameters	Intervention dose	Main outcome
		EG	CG	EG/CG	EG	CG	EG	CG				
Zhao et al., 2018 [25]	China	65.23 ± 4.03	66.70 ± 3.84	30/30	12/18	14/16	EA	Placebo-EA	RCT	Acupoints: Sishencong (EX-HN1), Shenting (GV24), and bilateral Benshen (GB13) as the main acupoints, while Baihui (GV20), bilateral Hegu(LI4), and bilateral Taichong (LR3); Frequency: 2/100 HZ; Intensity: 3 mA	30 min, 5 days before the surgery, once daily, and continued for total 5 days	Incidence of POCD, levels of serum IL-1β, TNF-α, and S-100β
Sun et al., 2018 [26]	China	60 ~ 81		30/30		22/38	EA + basic treatment	Basic treatment	RCT	Acupoints: Xuehai (SP10), Yanlingquan (GB34), Weizhong (BL40), and Qiuxu (GB40) on the operation side	20 min, once daily for 14 days after operation	Range of motion, VAS
Yang et al., 2017 [27]	China	59.6 ~ 78.4	58.4 ~ 80.3	30/30	6/24	7/23	EA + celecoxib capsules	Celecoxib capsules	RCT	Acupoints: Fenglong (ST40), Sanyinjiao (SP6), Jimen (SP11), and Biguan (ST31) on the operation side; on the 3rd to 7th day: Xuehai (SP10), Liangqiu (ST34), Zusanli (ST36), and Diji (SP8); Frequency: 2/100 HZ; Intensity: 6 ~ 21 mA	30 min, once/day, from the 1st day before operation to 7th day after operation	Range of motion, VAS
Chen et al., 2015 [28]	China	51 ~ 73	55 ~ 81	20/20	11/9	8/12	EA + rehabilitation training	Rehabilitation training	RCT	Acupoints: Xuehai (SP10), Yanlingquan (GB34), Fenglong (ST40), Sanyinjiao (SP6), Zusanli (ST36), and Xiyangguan (GB33) on the operation side	30 min, once/day, for 7 days after operation	Swelling degree, VAS
Chen et al., 2015 [29]	China (Taiwan)	71.7 ± 4.5	72.9 ± 3.6	30/31	Not available		EA + PCA	PCA	RCT	Acupoints: Yanlingquan (GB34) and Yinlingquan (SP9) on the nonoperation side; Frequency: 100 HZ; Intensity: 4 ~ 7 mA	30 min for once, once/day	VAS, rate of vomiting

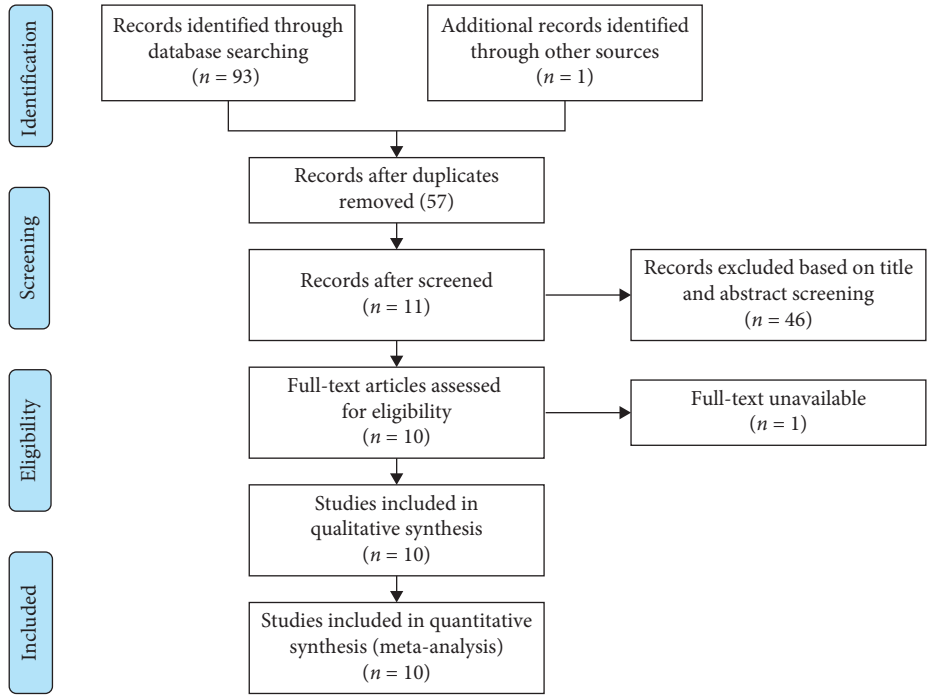
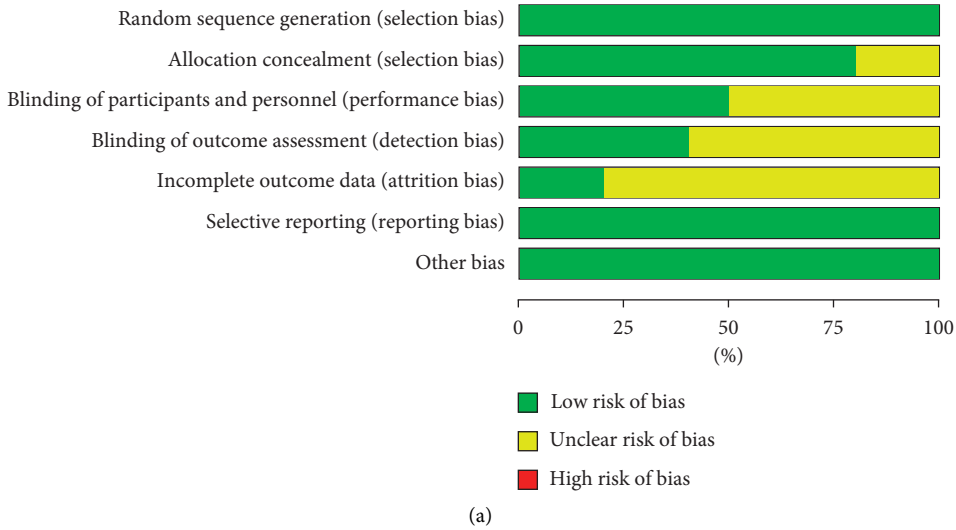
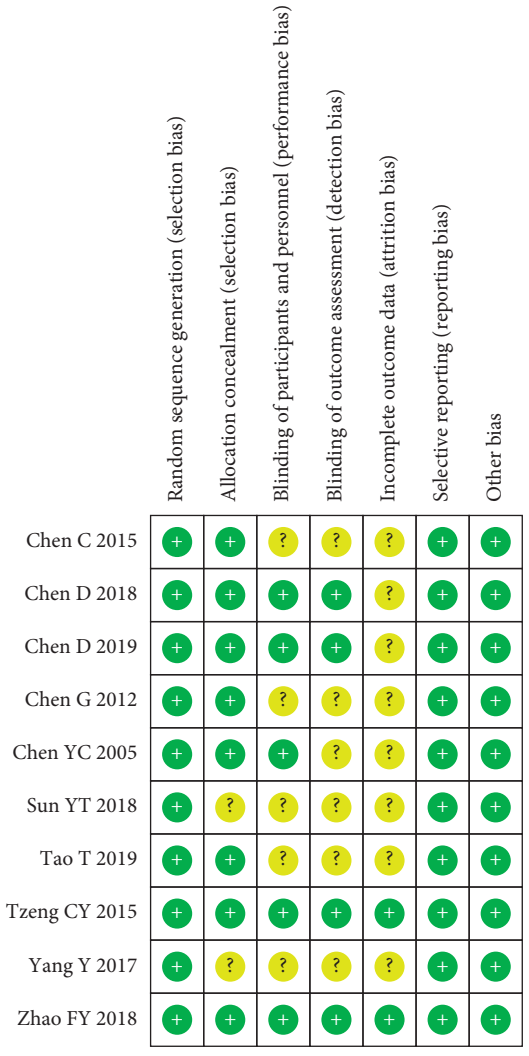


Figure 1: Flowchart of study selection.



(a)

Figure 2: Continued.



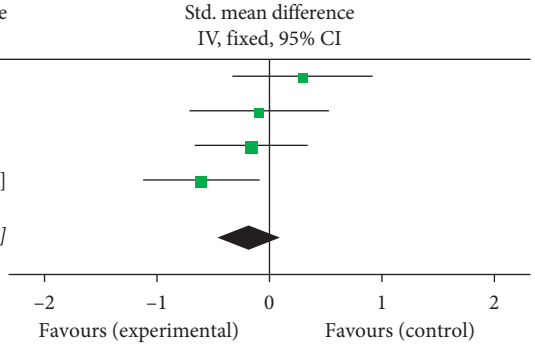
(b)

Figure 2: Risk of bias graph.

Study or subgroup	Experimental Mean	SD	Total	Control Mean	SD	Total	Weight (%)	Std. mean difference IV, fixed, 95% CI
Chen C 2015	4.73	1.53	20	4.13	2.38	20	20.0	0.29 [−0.33, 0.92]
Chen D 2019	5.79	1.26	20	5.91	1.31	20	20.2	−0.09 [−0.71, 0.53]
Chen YC 2005	0.33	1.24	30	0.6	1.96	31	30.8	−0.16 [−0.66, 0.34]
Yang Y 2017	4.91	0.93	30	5.45	0.83	30	29.0	−0.60 [−1.12, −0.09]
Total (95% CI)			*100*			*101*	*100.0*	*−0.18 [−0.46, −0.09]*

Heterogeneity: chi^2 = 4.88, df = 3 (P = 0.18); I^2 = 39%

Test for overall effect: Z = 1.30 (P = 0.19)



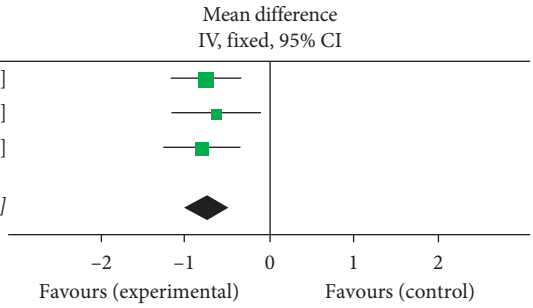
(a)

Figure 3: Continued.

Study or subgroup	Experimental Mean	SD	Total	Control Mean	SD	Total	Weight (%)	Mean difference IV, fixed, 95% CI
Chen D 2019	4.14	0.65	20	4.9	0.7	20	40.7	−0.76 [−1.18, −0.34]
Sun YT 2018	6.03	1.07	30	6.67	1.03	30	25.3	−0.64 [−1.17, −0.11]
Yang Y 2017	4.35	0.88	30	5.16	0.93	30	34.0	−0.81 [−1.27, −0.35]
Total (95% CI)			*80*			*80*	*100.0*	*−0.75 [−1.01, −0.48]*

Heterogeneity: chi^2 = 0.23, df = 2 (P = 0.89); I^2 = 0%

Test for overall effect: Z = 5.48 (P < 0.00001)

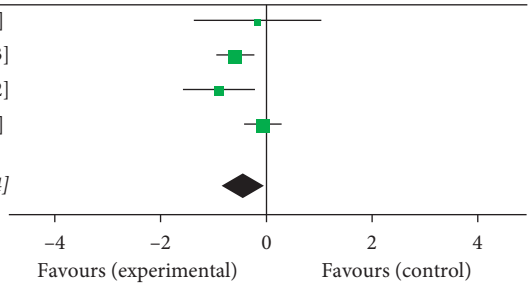


(b)

Study or subgroup	Experimental Mean	SD	Total	Control Mean	SD	Total	Weight (%)	Mean difference IV, random, 95% CI
Chen C 2015	1.87	1.96	20	2.04	1.93	20	8.8	−0.17 [−1.38, 1.04]
Chen D 2019	2.08	0.56	20	2.67	0.6	20	35.4	−0.59 [−0.95, −0.23]
Sun YT 2018	4.8	1.35	30	5.7	1.34	30	20.1	−0.90 [−1.58, −0.22]
Yang Y 2017	3.05	0.67	30	3.12	0.73	30	35.7	−0.07 [−0.42, 0.28]
Total (95% CI)			*100*			*100*	*100.0*	*−0.43 [−0.82, −0.04]*

Heterogeneity: tau^2 = 0.08; chi^2 = 6.60, df = 3 (P = 0.09); I^2 = 55%

Test for overall effect: Z = 2.14 (P = 0.03)

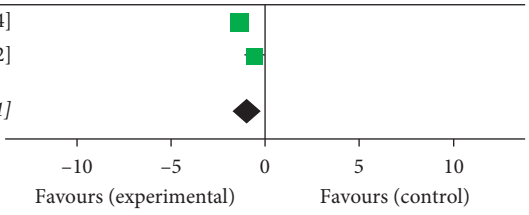


(c)

Study or subgroup	Experimental Mean	SD	Total	Control Mean	SD	Total	Weight (%)	Mean difference IV, random, 95% CI
Chen G 2012	4.78	0.99	35	6.12	0.69	35	52.9	−1.34 [−1.74, −0.94]
Sun YT 2018	3.87	1.04	30	4.43	1.1	30	47.1	−0.56 [−1.10, −0.02]
Total (95% CI)			*65*			*65*	*100.0*	*−0.97 [−1.74, −0.21]*

Heterogeneity: tau^2 = 0.25; chi^2 = 5.16, df = 1 (P = 0.02); I^2 = 81%

Test for overall effect: Z = 2.50 (P = 0.01)



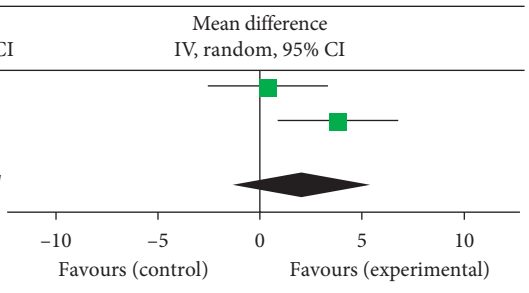
(d)

Figure 3: Meta-analysis and forest plot for postoperative pain at different periods. (a–d) Pain on postoperative day 1, 3, 7, and 14, respectively.

Study or subgroup	Experimental Mean	SD	Total	Control Mean	SD	Total	Weight (%)	Mean difference IV, random, 95% CI
Chen G 2012	92.16	6.6	35	91.77	5.97	35	50.1	0.39 [−2.56, 3.34]
Sun YT 2018	95	6.43	30	91.17	5.2	30	49.9	3.83 [0.87, 6.79]
Total (95% CI)			*65*			*65*	*100.0*	*2.11 [−1.26, 5.48]*

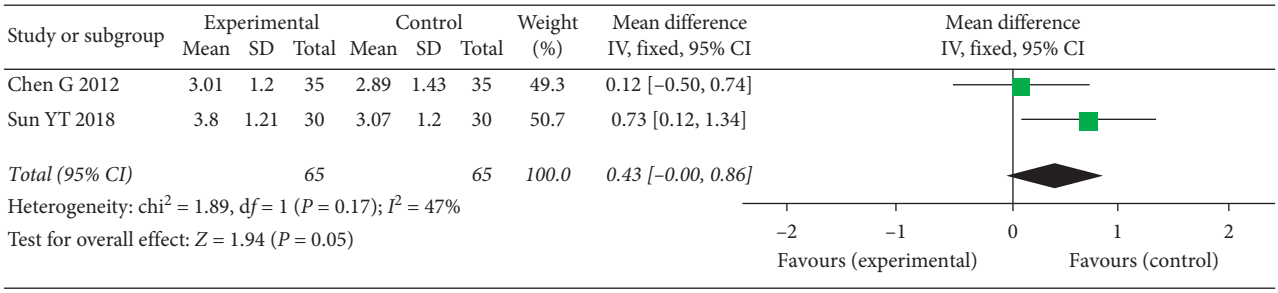
Heterogeneity: tau^2 = 3.65; chi^2 = 2.61, df = 1 (P = 0.11); I^2 = 62%

Test for overall effect: Z = 1.23 (P = 0.22)



(a)

Figure 4: Continued.



(b)

Figure 4: Meta-analysis and forest plot for ROM of knee. (a) Maximum flexion angle of knee; (b) active range of movement in knee extension (extension deficit).

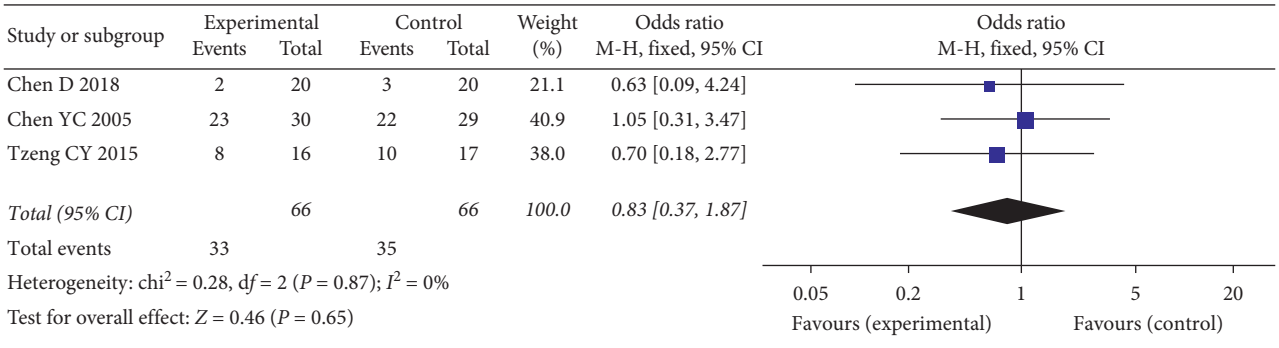


Figure 5: Meta-analysis and forest plot for nausea/vomiting.

continuous regional anesthesia would show a lower pain within postoperative 24 hours, which resulted in an undetectable difference in pain improvement due to interferences of anesthesia resuscitation period. In our subgroup analysis of pain on postoperative day 7, our findings suggested that a combination of EA and rehabilitation training was superior to rehabilitation training alone in pain relief, while EA combined with celecoxib capsules seemed to be equal to celecoxib capsules alone in improving pain. It could be explained that celecoxib capsules, as a specific cyclooxygenase-2 inhibitor, could reach excellent pain management on day 7 after TKA so that the effect of EA on pain could not be reflected adequately. By contrast, the benefit of EA in pain improvement was more explicit when eliminating the masking effect of analgesic.

In addition, the results derived from this study revealed that EA was ineffective for improving ROM of the knee and reducing the percentage of vomiting after the operation. ROM represented joint flexion activity, which was closely associated with joint function and mobility. Previously, many studies investigated the effect of EA on ROM of various joints, while different results varied with different assessed joints. Many studies highlighted that EA could improve ROM of cervical vertebra [33], knee [34, 35], and shoulder [36], while it was proved that EA would reduce quadriceps strength, which was not good for improving knee ROM because of concomitant impaired quadriceps strength in TKA patients.

Postoperative nausea/vomiting was one of the analgesia-related adverse effects, which reduced the patient's satisfaction and consequently influenced the recovery confidence after the operation. As was known to us, EA was effective in the prevention of postoperative nausea and vomiting, the mechanism of which was considered to be related to reducing the content of 5-hydroxytryptamine and dopamine [37]. Moreover, it was reported that therapeutic outcome could be affected by acupoints, intervention point [38,39], and EA frequency [40]. In the previous studies, Neiguan (PC6) [40, 41] and 2Hz/100Hz frequency [41] were recommended to be applied in clinical practice.However, in the included studies, those intervention parameters of EA had substantial heterogeneity and were not selected as recommended. It could be the reason why the effect of EA on postoperative vomiting was not satisfactory.

There are several limitations in this study. Firstly, blind methods were not recorded in detail in most of the included studies. Secondly, the acupoints, intervention dose, and frequency selected for treatment in the 10 included studies are not consistent, which may influence the reported effects. Thirdly, we could only include a small number of studies in the analysis for several outcomes because some studies lack sufficient data. Fourthly, we can't assess the long-run effect because of the lack of RCTs with long-term follow-up. Therefore, in the future, RCTs with the long-term following and focusing on the comparison of EA combined with analgesia and analgesia used alone should be conducted to identify further the efficacy of EA on pain after TKA. Meanwhile, considering the effect of acupoint selection for outcomes, RCTs with the same acupoint

selection could be more reasonable to obtain a persuasive conclusion.

5. Conclusion

In this paper, we systematically reviewed and quantified the effect of EA on postoperative rehabilitation for patients receiving TKA. Overall, EA, as a supplementary treatment, could reduce pain on day 3 to 14 after TKA. However, more than 7 days after TKA, this positive efficacy might not be significant when EA combined with analgesic was applied to treat postoperative pain in comparison to analgesic only. Notably, EA was found to be ineffective for improving ROM of the knee and decreasing the ratio of vomiting after surgery. But given the limitation in this study, additional high-quality and large-scale RCTs and systemic reviews are needed to confirm these findings.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Authors' Contributions

Weijian Chen, Zehua Chen, and Jiao Li have contributed equally to this work. XM-X and ZH-C designed the study. ZH-C and WJ-C did the literature searches and designed the data-extraction form. WJ-C and ZH-C selected studies. WJ-C and ZH-C extracted the data. ZH-C, J-L, and Z-S did statistical analyses. WG-L and XM-X supervised the study. WJ-C, JX-S, and JY-Z wrote the draft. GQ-C and T-J revised this manuscript. H-W, WG-L, and ZG-W did the language editing. All authors read and approved the submitted version.

Acknowledgments

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Supplementary Materials

Supplementary eFigure 1: the search strategy in this review. Supplementary eFigure 2: subgroup analysis and forest plot for postoperative pain at 7-day follow-up. Supplementary eTable 1: excluded studies found from search strategy and reason. Supplementary eTable 2: assessment of publication bias. (*Supplementary Materials*)

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Review Article

Acupuncture Combined with Three-Step Analgesic Drug Therapy for Treatment of Cancer Pain: A Systematic Review and Meta-Analysis of Randomised Clinical Trials

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Objective. The purpose of this study was to systematically evaluate the efficacy and safety of acupuncture combined with the WHO three-step analgesic drug ladder for cancer pain. *Methods*. The Cochrane Library, PubMed, and CNKI Database of Systematic Reviews were searched. Using the Cochrane Register for Randomized Controlled Trials, the quality of the included literature was evaluated, and the meta-analysis was carried out with RevMan 5.3 software. *Results*. Compared with three-step analgesia alone, acupuncture combined with three-step analgesia for cancer pain increased pain relief response rates (RR = 1.12, 95% CI: 1.08~1.17, $P < 0.00001$), reduced NRS score (SMD = −1.10, 95% CI: −1.86~−0.35, $P = 0.004$), reduced the rate of side effects (RR = 0.45, 95% CI: 0.38~0.53, $P < 0.00001$), including nausea ($P < 0.00001$), vomiting ($P = 0.008$), constipation ($P < 0.00001$), and dizziness ($P = 0.010$), reduced the burst pain rate (SMD = −1.38; 95% CI: −2.44~−0.32, $P = 0.01$), shortened analgesia effect onset time ($P = 0.004$), and extended the duration of response ($P < 0.0001$). *Conclusion*. For the treatment of cancer pain, acupuncture combined with three-step analgesic drugs is better than using only three-step analgesic drugs.

1. Introduction

Pain is one of the most debilitating symptoms experienced by patients with advanced cancer. According to WHO statistics, 70% of cancer patients worldwide have some degree of pain in the advanced stages of cancer [1]. Because the pain is intense and easily aggravated, it directly affects the appetite, sleep, psychological status, and treatment effect of patients, reduces their quality of life, and increases their psychological stress [2, 3]. Cancer pain has become a medical, psychological, and social issue of great concern. At present, the treatment of cancer pain mostly utilizes the three-step "ladder" treatment principle proposed by the WHO, where mild, moderate, and severe pain are treated with nonsteroidal anti-inflammatory drugs (NSAIDs), weak opioids, and strong opioids, respectively [4]. Although the analgesic effect of three-step drugs is good, their side effects, such as liver and kidney function damage, risk of dependency and addiction, respiratory inhibition, and gastrointestinal side effects, limit their clinical application [5]. One primary reason why cancer pain is difficult to control is because cancer pain patients cannot tolerate the side effects of analgesics. Therefore, there is a consensus to seek other effective and safe analgesic methods [6]. Acupuncture is an important part of TCM. Acupuncture is to insert a needle at one of the patient's acupoints and use a specific manipulation to stimulate the patient's acupoints to achieve an effect (DE QI). Acupuncture has outstanding performance in the treatment of all kinds of pain through its principle of moving qi, dredging qi channels and collaterals, and activating blood. Various acupuncture treatments can be combined, with no risk of addiction, no side effects, convenient application and at a low cost, demonstrating the unique advantages of TCM in the treatment of cancer pain [7]. In the

last 20 years, there have been many clinical reports on the utility and safety of acupuncture for the treatment of cancer pain, and acupuncture therapy is a widely recognised alternative measure for the treatment of cancer pain. Therefore, it is necessary to use a systematic evaluation method to rigorously evaluate the randomised controlled study of acupuncture combined with three-step analgesic drugs to treat cancer pain, to assess its exact effect in the treatment of cancer pain.

2. Methods

2.1. Data Sources. The following databases were searched from their inception to January 10, 2021: the Cochrane Library, PubMed, Embase, CNKI, China Biology Medicine disc (CBMdisc), Chinese Journal of Science and Technology database (VIP), and Wanfang database. We searched MeSH (Medical Subject Headings) term trees for "acupuncture" and "pain" in PubMed, and the keywords searched included "acupuncture", "needling", "tumour", "cancer", "neoplasm", "ache", "pain", and "randomised controlled trial". The keywords were translated into Chinese and searched in the above-mentioned Chinese databases. Search terms were combined with the Boolean "AND" and "OR" terms in search strategies, for example, ("acupuncture" OR "needling") AND ("cancer" OR "tumour" OR "neoplasm" OR "ache" OR "pain") AND ("randomised controlled trial"). Comprehensive retrieval was carried out according to the characteristics of different databases. Then, the literature mentioning "randomised controlled" and "randomised grouped" was screened. In addition, we manually searched our own personal literature files. After reading the full text of the included literature and related articles, we collected the documents together in hard copy format for preservation.

2.2. Inclusion Criteria

2.2.1. Types of Studies. The included studies were all randomised controlled clinical trials. The published experiments included were mainly in the form of theses and abstracts. There were no restrictions on the language of publication.

2.2.2. Types of Participants. The subjects were patients with malignant tumours confirmed by cytology or histopathology, and all patients had cancer pain. There were no limits on age, gender, race, and nationality of the patients; however, patients had to be able to clearly describe their pain to medical staff.

2.2.3. Types of Interventions. In the literature, the intervention treatment group was treated with acupuncture augmented by three-step analgesia, including traditional acupuncture or other acupuncture methods, such as ear acupuncture and electroacupuncture. Acupuncture points included traditional acupuncture points and pain points. The control groups only received three-step analgesic treatment.

2.2.4. Types of Outcome Evaluations. The included materials had a clear evaluation standard for curative effects and at least one clinical index related to cancer pain, including the effective rate of pain relief after treatment, quality of life score, side effect rates, burst pain rate, onset time to analgesic effect, and duration of response.

2.3. Exclusion Criteria. The exclusion criteria were as follows: if patients had one or more other type(s) of pain in addition to cancer pain; if the study used moxibustion, percutaneous electrical stimulation of nerves, acupoint injection, laser irradiation, cupping, massage, herbal medicines, or other intervention measures; if the experiments were carried out on patients during or a few days after surgical therapy, radiotherapy, chemotherapy, or hyperthermia-therapy on their malignant tumours; if the trial design was not rigorous; if inappropriate statistical methods were used; if the paper was only an abstract, review, or summary of previously published literature; if the study has no result indicators; if the experimental design was unreasonable; or if the literature could not be obtained by contacting the author.

2.4. Data Extraction and Bias Risk Assessment. Two researchers independently evaluated the quality of each study meeting the inclusion criteria and extracted the data, including the baseline situation, intervention measures, and efficacy results, and cross-checked the data. Any disagreements were resolved through discussion or assessment by a third researcher. We used a "Modification of Cochrane Tool to assess the risk of bias in randomised trials," where a decision regarding bias must be made, categorised into "probably no" or "probably yes," for items that are thought to be of unclear risk [8]. We judged trials with more than 2 and more than 4 high-risk components as moderate risk and high risk, respectively [9]. The following criteria were used to assess the risk of bias: whether the study was randomised; how allocation concealment was conducted; whether the study was double-blind or triple-blind; whether the results data were complete; and whether there was selective reporting or other types of bias. The authors categorised studies into "low risk," "unclear risk," and "high risk" categories. For dropout patients, we contacted the authors of the studies twice over four weeks via e-mail for missing or unclear data. If missing data could not be found, they were recorded as high risk; if no response was received, the data were marked as unclear risk. All authors reached a consensus on the results of bias risk assessment.

2.5. Data Synthesis. The effect of acupuncture combined with three-step analgesic drug therapy for treatment of cancer pain was analyzed in terms of response rate, numerical rating scale (NRS), side effect rates, times of burst pain, onset time, and duration of response (DOR). If the information included in the study was insufficient, we communicated with the main author to obtain accurate data. RevMan 5.3 software provided by the Cochrane Collaboration Network was used for the

meta-analysis. The relative risk (RR) was used for the enumeration data, the mean difference (MD) was used for the measurement data, and the 95% confidence interval (CI) was used for each effect quantity. When the heterogeneity of test results was not statistically significant ($P > 0.05$), a fixed effects model was selected; when the heterogeneity of test results was statistically significant ($P < 0.05$), a random effects model was selected. A funnel plot was used to analyse and detect publication bias.

3. Results

3.1. Study Description. The first search found 115 potentially relevant articles. After reading and screening, 19 articles met our inclusion criteria (Figure 1). The critical data from all the included RCTs are shown in Table 1 [10–28]. In total, 1502 cancer pain cases were included. The numbers of cases of acupuncture combined with three-step analgesic drug therapy (treatment group) and three-step analgesic drug therapy (control group) were 751 and 751, respectively. All patients' cancers were confirmed by cell histology or pathology, and pain was their main symptom. The baseline was comparable between the two groups. Almost all of the research was on the use of manual acupuncture (AT), which is guided by the theory of TCM for acupuncture interventions. Two studies used electroacupuncture (EA) [11, 28]. One study used floating acupuncture (FA) [12]. Two studies used fire needle (FN) [13, 14]. Three studies used wrist-ankle acupuncture (WA) [17–19]. Among them, the two acupuncture methods were all included in Fu Yang et al.'s report [17], in which morphine hydrochloride sustained-release tablets and acupuncture or wrist-ankle acupuncture were used in the treatment of cancer pain. All studies provided patients with a semistandardised acupuncture programme, that is, the use of a predefined set of acupoints combined with a set of acupoints according to the location of the tumour. The Ashi point, Zusanli (ST36), Hegu (LI4), Sanyinjiao (SP6), and Taichong (LR3) points were most frequently used. For most studies, patients received acupuncture treatment for 1 to 3 weeks, for durations of 20 to 60 min per session. The evaluation criteria for the curative effect were similar across studies. The objective outcome measures were treatment response rate, NRS, side effect rates (nausea, vomiting, constipation, hiccups, dizziness, itching, palpitation, and abdominal distention), times of burst pain, onset time to analgesic effect (min), DOR (h), quality of life (QOL), Karnofsky performance status (KPS), and quality of life questionnaires (QLQ-C30). The minimal important difference (MID) refers to the change in the score of the smallest efficacy evaluation questionnaire recognised by the patient. MID indicates an important improvement in symptoms and signs; the intervention has achieved the minimal important difference.

3.2. Risk of Bias. Most included RCTs had a high risk of bias. Nineteen RCTs [10–28] described their randomisation methods. Among them, 9 RCTs [10, 12–14, 16–18, 22, 24] used a random number table, 1 study [15] used a computer-generated random number sequence for randomisation, and 3 RCTs [20, 21, 26] randomly numbered cases according to the order of hospitalization. Three RCTs [10, 15, 17] described incomplete outcome methods, and these three studies had cases of dropouts. Two studies reported details about allocation concealment [15, 16]. Fourteen RCTs described adverse events from acupuncture combined with three-step analgesic drugs [10, 11, 13–23, 26]. Table 2 presents the Cochrane risk of bias assessment of the included articles. There were 2 trials with high risks of bias [10, 17], 5 trials with moderate risk of bias [12, 15, 18, 23, 25], and 12 trials with low risk of bias [11, 13, 14, 16, 19–22, 24, 26–28]. A high risk of bias resulted from lack of blinding of participants and personnel and lack of blinding among outcome assessors. A moderate risk resulted from selective reporting bias and incomplete outcome data, and a low risk of bias resulted from randomisation sequence generation and allocation concealment (see Figure 2).

3.3. Response Rates. Eighteen studies reported the response rates to pain relief after treatment [10–14, 16–28]. In the treatment group, 621 out of the 679 cases had effective responses; in the control group, among the 672 cases, 548 had effective responses. The heterogeneity test in the meta-analysis showed that $\chi^2 = 22.19$, $P = 0.22$, $I^2 = 19\%$, and there was no significant difference between the studies, so a fixed effects model was used. The total response rate of the treatment group was better than that of the control group, and the difference was statistically significant ($n = 1351$, RR = 1.12; 95% CI: 1.08~1.17, $P < 0.00001$; see Figure 3).

3.4. NRS Score. Seven studies reported NRS scores after treatment [10, 11, 14, 17, 19, 22, 24]. Overall, 282 cases were in the treatment group, and 281 were in the control group. The heterogeneity test of the meta-analysis showed that $\chi^2 = 158.90$, $P < 0.00001$, $I^2 = 96\%$, and the differences between the studies were statistically significant, so a random effects model was used. The NRS score of the treatment group was lower than that of the control group, and the difference was statistically significant ($n = 563$, SMD = −1.10, 95% CI: −1.86~−0.35, $Z = 2.87$, $P = 0.004$; see Figure 4).

3.5. Side Effect Rates. Side effects mainly included nausea, vomiting, constipation, and dizziness. Eight studies reported the number of cases of nausea [10, 13, 16, 18, 21–23, 26], 7 studies reported the number of cases of vomiting [10, 13, 16, 18, 21, 22, 26], 11 studies reported the number of cases of constipation [10, 11, 13, 16–18, 20–23, 26], and 5 studies reported the number of cases of dizziness [11, 13, 17, 20, 22]. The consolidated statistics results demonstrated that, compared to the control group, in the treatment group, the incidence of nausea ($n = 659$, RR = 0.48, 95% CI: 0.34~0.66, $P < 0.00001$), vomiting ($n = 452$, RR = 0.56, 95% CI: 0.37~0.86, $P = 0.008$), constipation ($n = 843$, RR = 0.38, 95% CI: 0.29~0.49, $P < 0.00001$), and

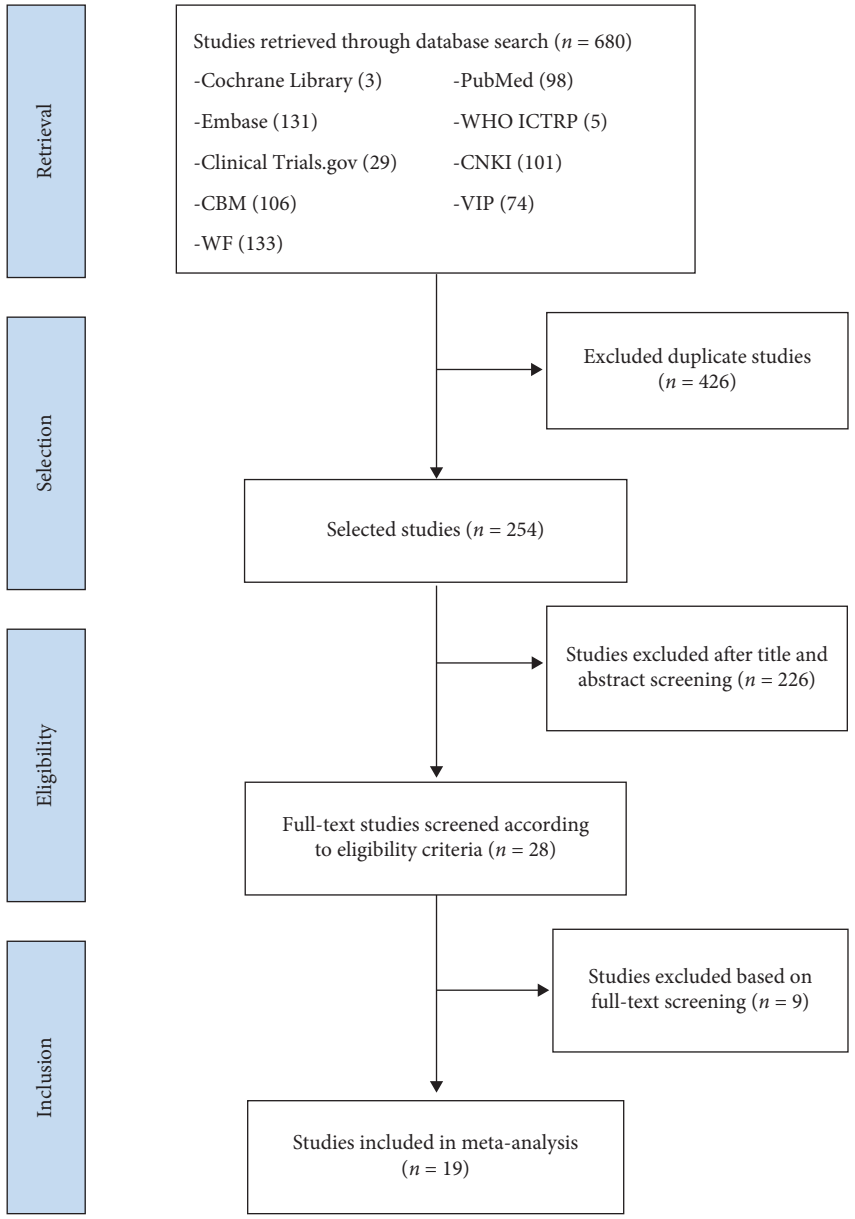

Figure 1: Flowchart of the literature review and selection process.

Table 1: Summary of randomised clinical studies of acupuncture combined with three-step analgesic drug therapy for the treatment of cancer pain.

Study (year)	Type of cancer	Sample sizes		Interventions		Acupuncture point selection	Session frequency and duration	Main outcomes and assessment of pain
		T	C	T	C			
Wang (2018) [10]	Various	35	35	AT + C	Drug (three-step analgesic ladder)	LI4, LR3, and Ashi point	30 min qd 6 weeks	Response rate, NRS, and side effect rate
Wang (2016) [11]	Lung cancer	30	30	EA + C	Oxycodone sustained-release tablets	LI4, PC6, ST36, and SP6	30 min qd 14 days	Response rate, NRS, side effect rate, burst pain, onset time, and DOR
Zhong (2016) [12]	Various	30	30	FA + C	Morphine sulfate sustained-release tablets	Ashi point	Once a day 14 days	Response rate, QOL, and burst pain

Table 1: Continued.

Study (year)	Type of cancer	Sample sizes		Interventions		Acupuncture point selection	Session frequency and duration	Main outcomes and assessment of pain
		T	C	T	C			
Mi (2010) [13]	Gastric cancer	32	30	FN + AT + C	Drug (three-step analgesic ladder)	FN: BL21, BL18, and BL17. AT: CV12, ST25, and St36	30 min qod 4 weeks	Response rate and side effect rate
Bai (2019) [14]	Various	50	50	FN + C	Drug (three-step analgesic ladder)	Ashi point, ST36, and SP6	qod 14 days	Response rate, NRS, and side effect rate
Liu (2018) [15]	Various	72	75	TEAS + C	Drug (three-step analgesic ladder)	LI4, PC6, ST36, and SP6	30 min bid 3 weeks	Response rate, BPI-S, KPS, and side effect rate
Liu (2011) [16]	Liver cancer	30	30	AT + C	Tramadol hydrochloride sustained-release tablets	SP4, PC6, GB41, TE5, SI3, BL62, LU7, KI6, LR3, and LR14	qd 14 days	Response rate, NRS, QOL, side effect rate, onset time, and DOR
Fu (2019) [17]	Various	16/16	16	AT + C WA + C	Morphine hydrochloride sustained-release tablets	PC6 and SP6	1 h qd	Response rate, NRS, KPS, side effect rate, burst pain, onset time, and DOR
Wu (2019) [18]	Various	30	30	WA + C	Drug (three-step analgesic ladder)	Based on syndrome differentiation and disease differentiation	12 h qd 10 days	Response rate, VAS, burst pain, and side effect rate
Dong (2018) [19]	Various	60	60	WA + C	Drug (three-step analgesic ladder)	Based on syndrome differentiation and disease differentiation	10–12 h qd 7 days	Response rate, NRS, QLQ-C30, and side effect rate
Sun (2016) [20]	Various	30	30	AT + C	Oxycodone	LI4, PC6, ST36, SP6, Ashi point, and others	30 min qd 14 days	Response rate, NRS, KPS, QOL, and side effect rate
Zhang (2014) [21]	Various	30	30	AT + C	Drug (three-step analgesic ladder)	LI4 and ST36. Lung cancer: PC6 and LU6. Liver cancer: GB34, LR6, and LR3. Colorectal cancer: PC6, CV12, and TE6	30 min qd 7 days	Response rate, QOL, side effect rate, onset time, and DOR
Hui (2019) [22]	Various	40	40	AT + C	Drug (three-step analgesic ladder)	Ashi point, LI4, GV14, BL11, GB34, and LR3	30 min qd 14 days	Response rate, side effect rate, onset time, and DOR
Tan (2012) [23]	Various	106	101	AT + C	Drug (three-step analgesic ladder)	LI4 and PC6. Lung cancer: LU6. Liver cancer: GB34 and LR6. Colorectal cancer: CV12, ST36, and TE6	0.5–1 h qd 3 weeks	Response rate and side effect rate
Fan (2017) [24]	Lung cancer	35	34	AT + C	Drug (three-step analgesic ladder)	PC6, LI4, ST36, GB34, and SP6	20 min qd 20 days	Response rate, NRS, onset time, and DOR
Jiang (2016) [25]	Various	25	25	AT + C	Drug (three-step analgesic ladder)	Ashi point, LR3, and LI4	30 min qd 7 days	Response rate and NRS
Li (2017) [26]	Gastric cancer	30	30	AT + C	Drug (three-step analgesic ladder)	ST36, LR3, and LI4	30 min qd 7 days	Response rate, NRS, QOL, and side effect rate
Huang (2018) [27]	Various	31	31	AT + C	Drug (three-step analgesic ladder)	PC6. Lung cancer: LI4, LU4, LU6, and ST36. Liver cancer: GB34 and LR3. Breast cancer: LI4, STI8, and CV9. Gastric cancer: CV12, ST36, and TE6	30 min qd 7 days	Response rate
Peng (2012) [28]	Various	23	24	EA + C	Drug (three-step analgesic ladder)	LI4, PC6, ST36, and SP6	30 min qd 7 days	Response rate, onset time, and DOR

T: treatment group, C: control group, AT: acupuncture, EA: electroacupuncture, FA: floating acupuncture, FN: fire needle, WA: wrist-ankle acupuncture, DOR: duration of response, NRS: numerical rating scale, BPI-S: brief pain inventory-severity, QOL: quality of life, and KPS: Karnofsky performance status.

Table 2: Risk of bias for the 19 included studies using a modified approach to the Cochrane risk of bias tool.

	Risk of bias							Trial characteristics		
Source	Random sequence generation	Allocation concealment	Blinding of participants and personnel	Blinding of outcome assessors	Infrequent loss to follow-up	Free of selective outcome reporting	Free of other types of bias	Statistical analysis (per protocol, intention to treat, etc.)	How is loss to follow-up handled?	Adverse event
Wang (2018) [10]	Definitely yes	Definitely yes	Probably no	Probably no	Definitely no	Definitely no	Definitely no	Not mentioned	Ignored	Yes
Wang (2016) [11]	Probably yes	Probably yes	Probably no	Probably no	Definitely yes	Definitely yes	Probably yes	Not mentioned	Not mentioned	Yes
Zhong (2016) [12]	Definitely yes	Definitely yes	Probably no	Probably no	Definitely yes	Definitely no	Probably yes	Per protocol	Not mentioned	No
Mi (2010) [13]	Definitely yes	Definitely yes	Probably no	Probably no	Definitely yes	Definitely yes	Probably yes	Not mentioned	Not mentioned	Yes
Bai (2019) [14]	Definitely yes	Definitely yes	Probably no	Probably no	Definitely yes	Definitely yes	Probably yes	Not mentioned	Not mentioned	Yes
Liu (2018) [15]	Definitely yes	Definitely yes	Probably no	Probably no	Definitely no	Definitely yes	Probably yes	Not mentioned	Ignored	Yes
Liu (2011) [16]	Definitely yes	Definitely yes	Probably no	Probably no	Definitely yes	Definitely yes	Probably yes	Not mentioned	Not mentioned	Yes
Fu (2019) [17]	Definitely yes	Definitely yes	Probably no	Probably no	Definitely no	Definitely no	Definitely no	Per protocol	Ignored	Yes
Wu (2019) [18]	Definitely yes	Definitely yes	Probably no	Probably no	Definitely yes	Definitely no	Probably yes	Not mentioned	Not mentioned	Yes
Dong (2018) [19]	Probably yes	Probably yes	Probably no	Probably no	Definitely yes	Definitely yes	Probably yes	Not mentioned	Not mentioned	Yes
Sun (2016) [20]	Probably yes	Probably yes	Probably no	Probably no	Definitely yes	Definitely yes	Probably yes	Not mentioned	Not mentioned	Yes
Zhang (2014) [21]	Probably yes	Probably yes	Probably no	Probably no	Definitely yes	Definitely yes	Probably yes	Per protocol	Not mentioned	Yes
Hui (2019) [22]	Definitely yes	Definitely yes	Probably no	Probably no	Definitely yes	Definitely yes	Probably yes	Not mentioned	Not mentioned	Yes
Tan (2012) [23]	Probably yes	Probably yes	Probably no	Probably no	Definitely yes	Definitely no	Probably yes	Not mentioned	Not mentioned	Yes
Fan (2017) [24]	Definitely yes	Definitely yes	Probably no	Probably no	Definitely yes	Definitely yes	Probably yes	Per protocol	Not mentioned	No
Jiang (2016) [25]	Probably yes	Probably yes	Probably no	Probably no	Definitely yes	Definitely no	Probably yes	Per protocol	Not mentioned	No
Li (2017) [26]	Probably yes	Probably yes	Probably no	Probably no	Definitely yes	Definitely yes	Probably yes	Not mentioned	Not mentioned	Yes
Huang (2018) [27]	Probably yes	Probably yes	Probably no	Probably no	Definitely yes	Definitely yes	Probably yes	Not mentioned	Not mentioned	No
Peng (2012) [28]	Probably yes	Probably yes	Probably no	Probably no	Definitely yes	Definitely yes	Probably yes	Per protocol	Not mentioned	No

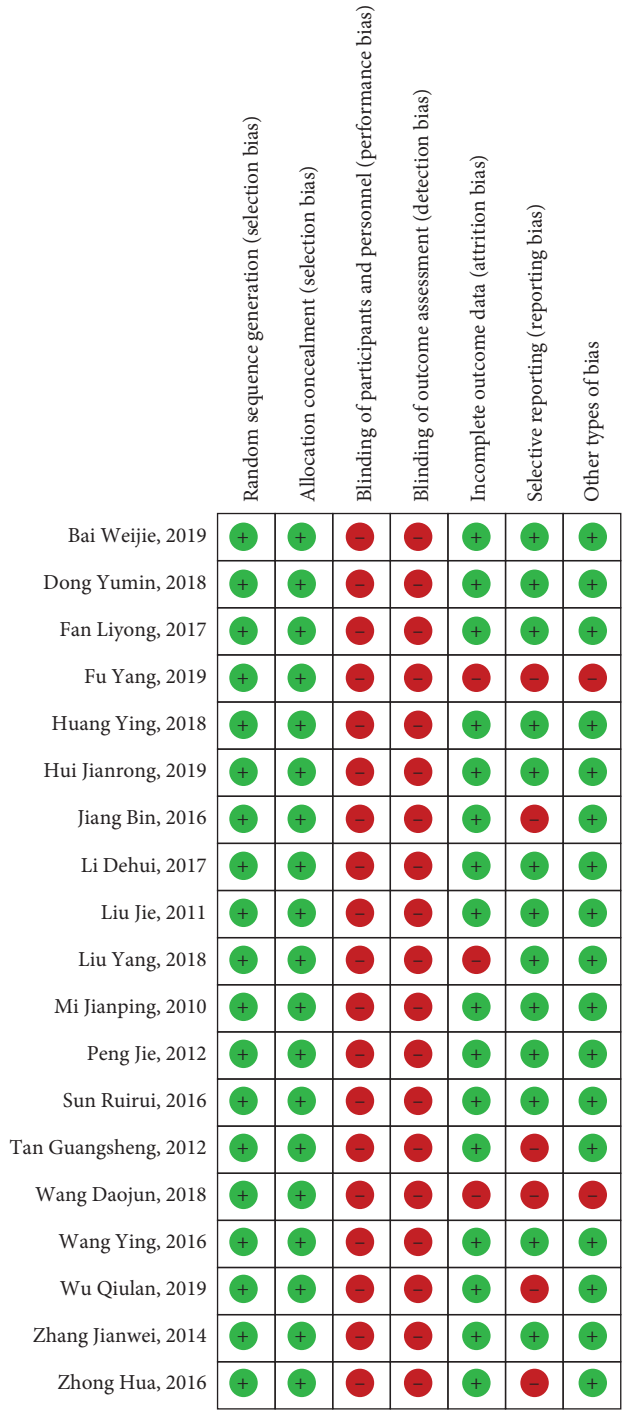

Figure 2: Cochrane risk of bias by trial.

dizziness ($n = 326$, RR = 0.53, 95% CI: 0.33~0.86, $P = 0.010$) decreased (see Figure 5).

3.6. Burst Pain. Four studies reported the mean number of burst pain events [11, 12, 17, 18]. The heterogeneity test in the meta-analysis showed that $\chi^2 = 78.30$, $P < 0.00001$, $I^2 = 95\%$, and the differences between the studies were statistically significant, so a random effects model was used. The combined statistical results showed that the incidence of burst pain in the treatment group was lower than that in the control group ($n = 244$, SMD = −1.38, 95% CI: −2.44~−0.32, $P = 0.01$; see Figure 6).

3.7. Onset Time to Analgesic Effect and Duration of Response. Five studies reported the mean onset time [11, 17, 19, 24, 28]. The combined statistical results showed that the onset time in the treatment group was shorter than that in the control group ($n = 360$, SMD = −20.11, 95% CI: −33.90~−6.33,

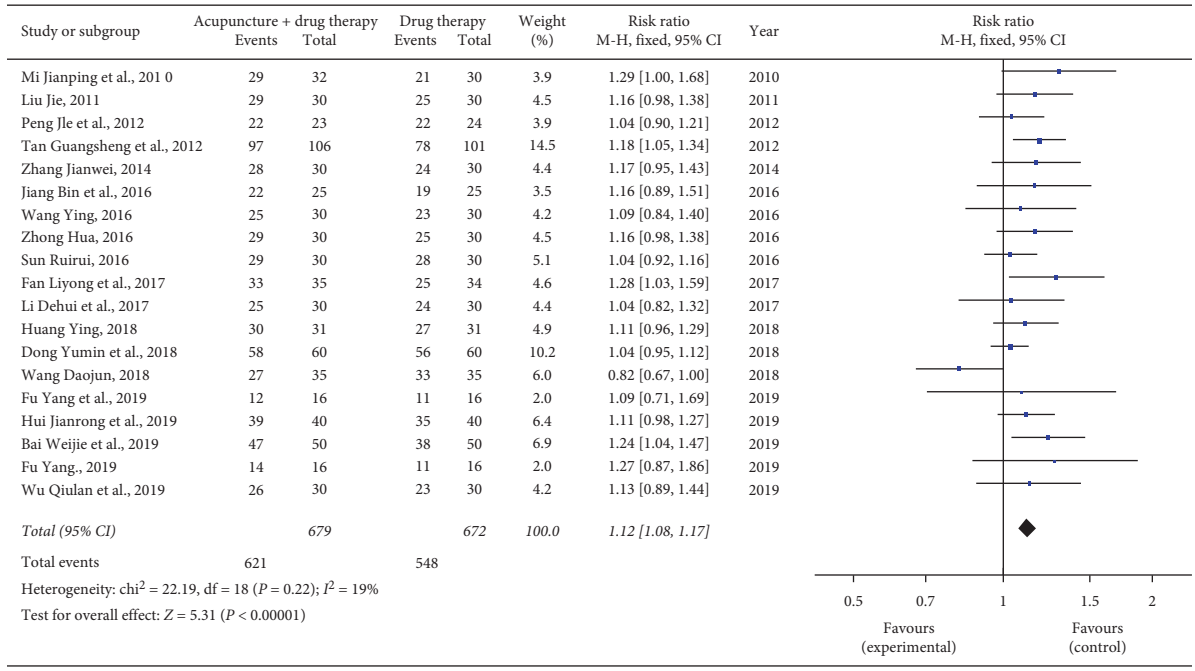

Figure 3: Forest plot of the total response rates of acupuncture combined with three-step analgesic drugs versus three-step analgesic drugs alone for cancer pain.

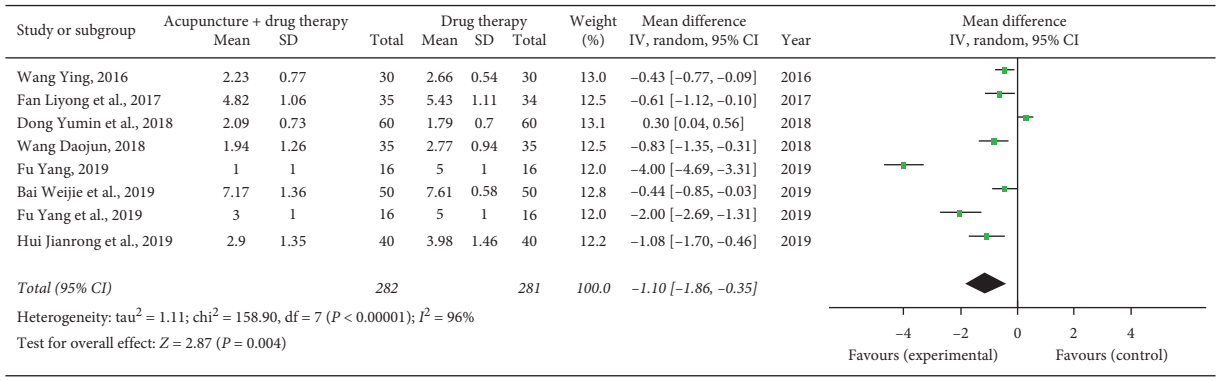

Figure 4: Forest plot of NRS score of acupuncture combined with three-step analgesic drugs versus three-step analgesic drugs alone for cancer pain.

$P = 0.004$). Six studies reported the mean duration of response [11, 17, 19, 22, 24, 28]. The combined statistical results showed that the duration of response in the treatment group was longer than that in the control group ($n = 440$, SMD = 3.22, 95% CI: 1.63~4.80, $P < 0.0001$); see Figures 7 and 8.

3.8. Publication Bias. Publication bias, which has always been a problem in meta-analysis, refers to the fact that research with positive results is easier to publish than research with negative results. The funnel chart analysis results of the main outcome indicators of the response rates of pain relief suggested that publication bias might exist and exaggerate the efficacy of acupuncture combined with three-step analgesic drugs in the treatment of cancer pain; see Figure 9.

4. Discussion

Cancer is a significant global public health issue, and the disease burden is growing. Globally, there are 18.1 million new cancer cases and 9.6 million cancer deaths each year; cancer deaths are expected to exceed 13 million by 2030, and 70% of cancer deaths globally occur in low-income and middle-income countries. In China in 2018, nearly 24% (4.3 million) of global new cases and 30% (2.9 million) of deaths occurred [29, 30]. China is the largest developing country. Chinese doctors need to pay attention to promoting cancer prevention for people and treating cancer patients. Cancer pain is severe, intolerable, and intractable pain, and such pain is a main symptom in the advanced stages of malignant tumours. When the tumour body markedly enlarges, tissue necrosis, erosion, and so on result in severe compression of, damage to, and irritation of the nerve sheath, nerve fibres,

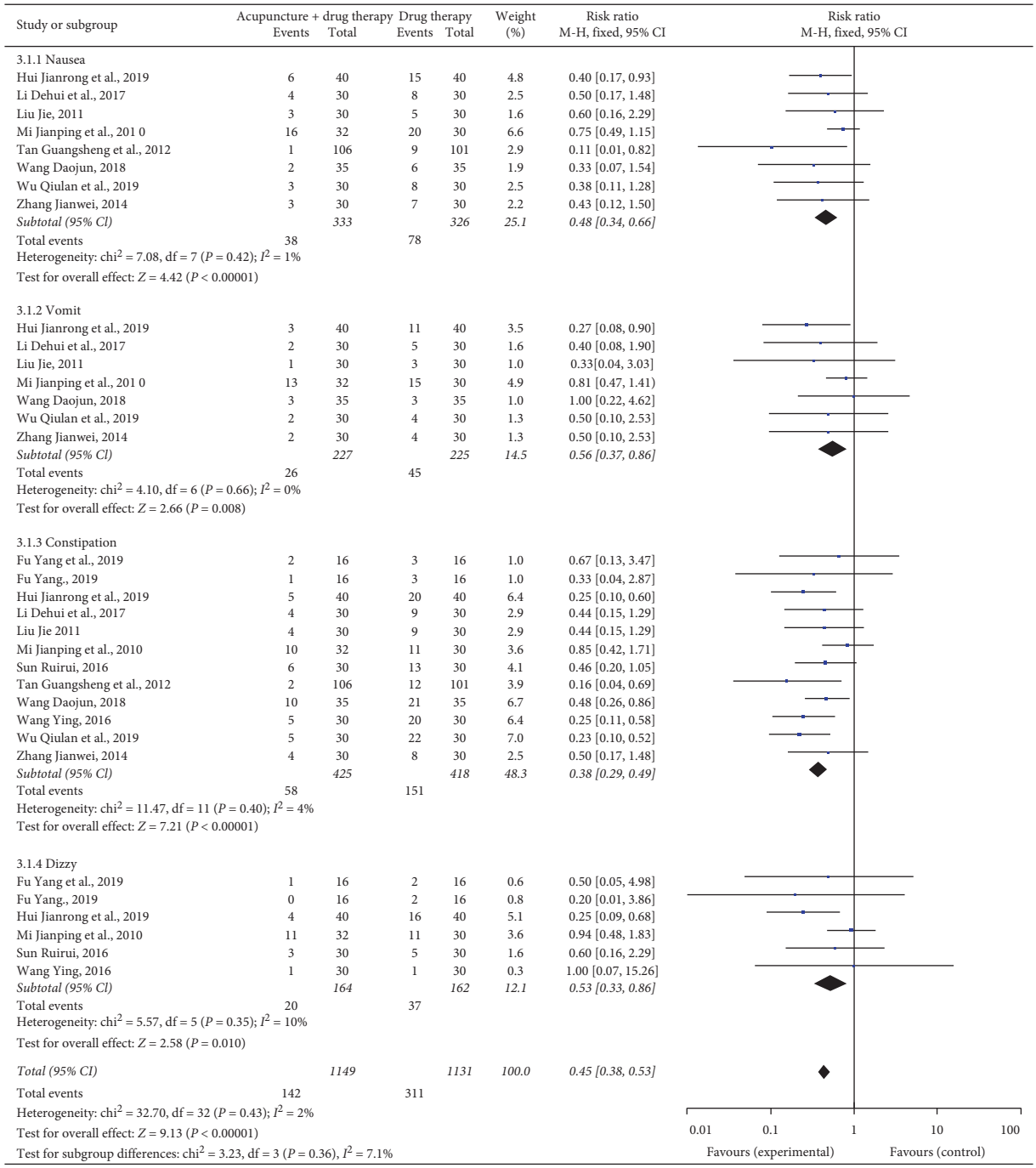

Figure 5: Forest plot of side effect rates of acupuncture combined with three-step analgesic drugs versus three-step analgesic drugs alone for cancer pain.

and blood vessels. Although there are many ways to treat cancer pain, many years of clinical experience at home and abroad indicate that providers believe that drug therapy is still the most common and effective way to control cancer pain. The WHO three-step cancer pain treatment programme has become an internationally accepted cancer pain drug treatment method that can control most cancer pain; however, three-step pain drugs, especially opioids, are often accompanied by side effects such as nausea, vomiting, constipation, drowsiness, dizziness, and respiratory depression [31].

There are many ways to treat cancer, but in recent years, TCM has played an increasingly important role in cancer prevention and treatment. As an integral part of TCM, acupuncture has been used to treat pain for thousands of years. The complications of acupuncture in the treatment of pain diseases are fewer than those of drug treatment [32]. Wang Limei et al. reported that [33] complications such as

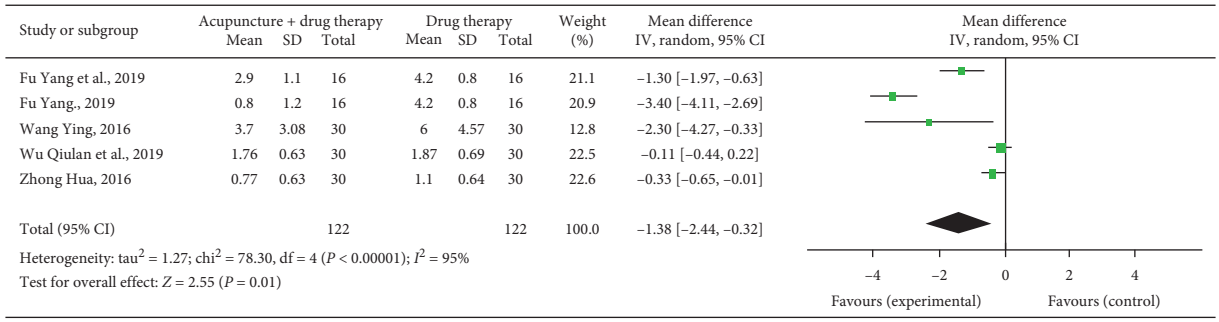

Figure 6: Forest plot of times of burst pain of acupuncture combined with three-step analgesic drugs versus three-step analgesic drugs alone for cancer pain.

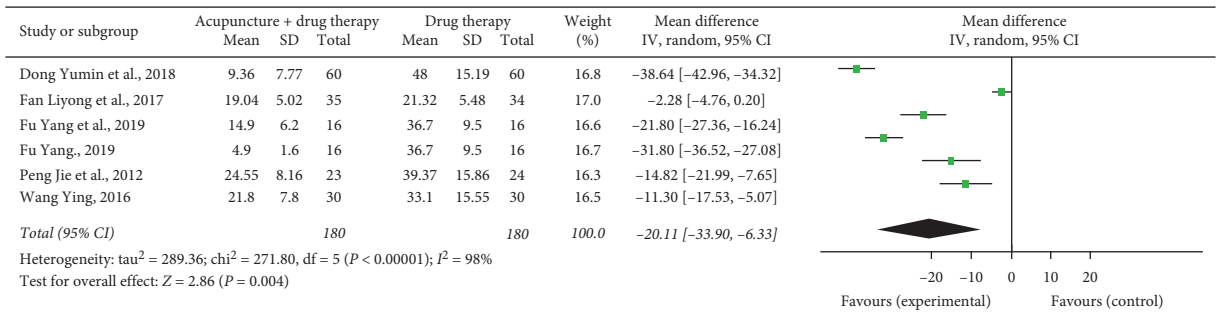

Figure 7: Forest plot of the onset time of acupuncture combined with three-step analgesic drugs versus three-step analgesic drugs alone for cancer pain.

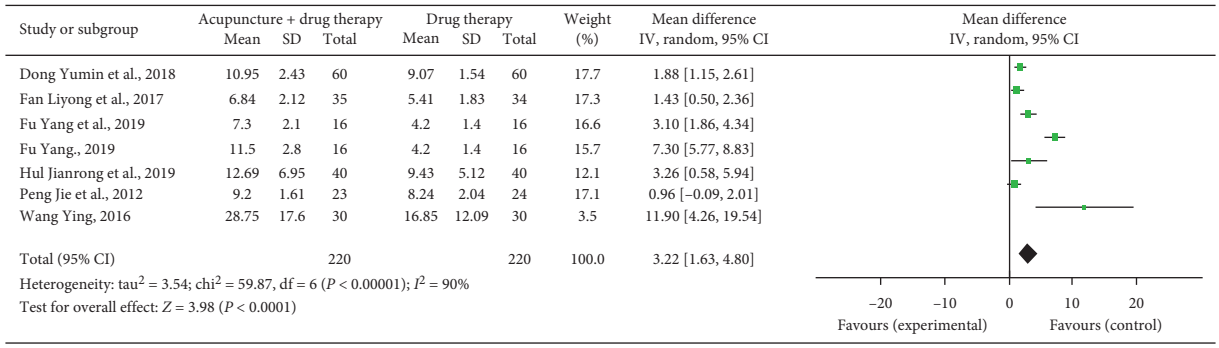

Figure 8: Forest plot of the duration of response of acupuncture combined with three-step analgesic drugs versus three-step analgesic drugs alone for cancer pain.

pneumothorax, dizziness, pain, needle syncope, infection, and visceral puncture can occur due to improper acupuncture manipulation; however, when doctors master anatomical knowledge, perform acupuncture correctly, and sterilise needles strictly, complications are further reduced. Modern research shows that the mechanism of acupuncture analgesia may be related to regulating the self-healing of the body, changing patients' perceptions of pain, and affecting the conduction of the central nervous system [34]. Another possible acupuncture mechanism is stimulation/excitation of the endogenous pain modulation system, which induces the secretion of endogenous opioids, blocks the transmission of neurotransmitters, and regulates the perception of pain to achieve analgesia [35]. A third possibility is that the pain signals from acupuncture are modulated in the pain receptor areas, and the dorsal root ganglion cells of the outgoing primary neurons transmit the signal to the near end of the secondary neurons. The pain signal produced by acupuncture may then induce the secretion of endogenous opioids and analgesia in the periaqueductal grey matter of the midbrain, or it may induce the penetration of electric ions, stimulate neurons, and exert an inhibitory effect in the intercellular area of the periaqueductal grey matter of the midbrain [36]. The exact mechanism of acupuncture's analgesic effect has not yet been elucidated. However, this review of acupuncture treatment of cancer pain with a large number of RCT experiments demonstrated that acupuncture treatment of cancer has fewer adverse reactions such as

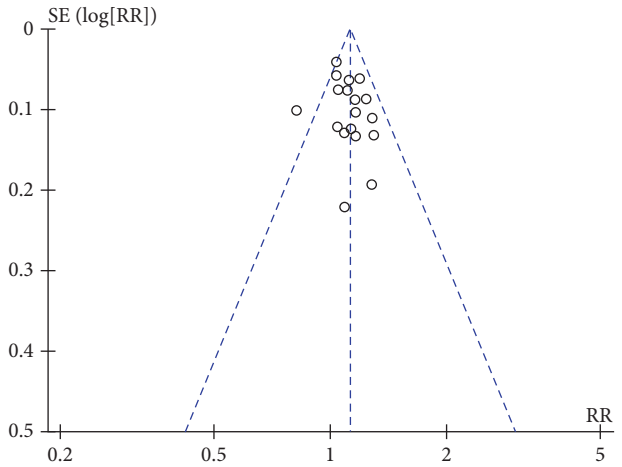

Figure 9: Total efficiency funnel.

nausea and vomiting than analgesic drug treatment alone. Acupuncture treatment for cancer pain is considered to have sufficient evidence to determine its effectiveness [37]; these results are encouraging and support further research on acupuncture treatment for cancer.

5. Conclusion

Based on the meta-analysis of 19 studies, compared with the treatment of cancer pain with three-step analgesic drug treatment alone, the response rates of pain relief from acupuncture combined with three-step analgesic drug treatment were higher, the NRS scores were lower, the incidence of adverse reactions such as nausea and vomiting was less frequent, the incidence of times of burst pain was also less frequent, the onset time to analgesic effect was shorter, and the duration of pain response was longer.

There were several limitations in this study. The lack of high-quality studies in the literature may limit the validity of the results. Meta-analyses generally face methodological challenges such as insufficient literature retrieval, potential selection bias for which studies are included, and inappropriate evaluation of the quality of the original research. This study only included published literature and did not search for unpublished literature; in addition, there may be publication bias in the literature.

In conclusion, this study shows that acupuncture combined with three-step analgesic drugs has specific advantages over three-step analgesic drugs alone in the treatment of cancer pain. It is hoped that, in the future, rigorous randomised controlled trials will be carried out with multicentre and large-sample studies to determine acupuncture's exact curative effect and further demonstrate the superiority of acupuncture combined with three-step analgesic drugs over the use of such drugs alone to treat cancer pain.

Data Availability

The data can be obtained from the author upon reasonable request.

Conflicts of Interest

All the authors declare no conflicts of interest.

Authors' Contributions

De-hui Li conceived and designed the study, analysed and interpreted the data, and drafted the manuscript. Yi-fan Su and Na Guo contributed to the literature searches, study selection, data extraction, and data synthesis. Huan-fang Fan and Chun-xia Sun assisted in the development of search strategies and critically reviewed the manuscript. All the authors read and approved the final version of the manuscript.

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