Randomized Trial

Ashi Acupuncture Versus Local Anesthetic Trigger Point Injections in the Treatment of Abdominal Myofascial Pain Syndrome: A Randomized Clinical Trial

Andréia Moreira de Souza Mitidieri, Pht, PhD, Maria Carolina Dalla Vecchia Baltazar, Pht, Ana Paula Moreira da Silva, Pht, PhD, Maria Beatriz Ferreira Gurian, Pht, PhD, Omero Benedicto Poli-Neto, MD, PhD, Francisco José Cândido-dos-Reis, MD, PhD, Antônio Alberto Nogueira, MD, PhD, and Júlio César Rosa-e-Silva, MD, PhD

From: Department of Gynecology and Obstetrics Ribeirao Preto Medical School University of Sao Paulo

Address Correspondence: Júlio Cesar Rosa e Silva, MD, PhD Department of Gynecology and Obstetrics Av. Bandeirantes 3900, 8°andar,14049-900. E-mail address: juliocrs@usp.br

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Background: Chronic pelvic pain (CPP) is defined as recurrent or continuous pain in the lower abdomen or pelvis, non-menstrual or non-cyclic, lasting at least 6 months. There is strong evidence that up to 85% of patients with CPP have serious dysfunction of the musculoskeletal system, including abdominal myofascial syndrome (AMPS). AMPS is characterized as deep abdominal pain, originating from hyperirritable trigger points, usually located within a musculoskeletal range or its fascia of coating. In the literature, there are few studies that address AMPS.

Objective: This study aimed to compare the responses of ashi acupuncture treatment and local anesthetic injection in the treatment of chronic pelvic pain secondary to abdominal myofascial pain syndrome in women.

Study Design: Randomized controlled clinical trial.

Setting: Tertiary University Hospital.

Methods: Women with a clinical diagnosis of CPP secondary to AMPS were randomized and evaluated using instruments to assess clinical pain, namely, the visual analogue scale (VAS), numerical categorial scale (NCS), and the McGill Questionnaire, after receiving treatment with ashi acupuncture (group A, n = 16) or local anesthetic injections (group B, n = 19). They were reevaluated after one week and one, 3, and 6 months after each treatment, in addition to assessments of pain and adverse events performed during the sessions.

Results: Ashi acupuncture and local anesthetic injections were both effective in reducing clinical pain assessed through the analyzed variables among study participants. There was no difference between the groups and there was a strong correlation between these pain assessment instruments.

Limitations: The absence of blinding to the different forms of treatment among the patients and the researcher directly involved in the treatment, the absence of a placebo group, the selective exclusion of women with comorbidities and other causes of CPP, and the difference between the number of sessions used for each technique.

Conclusion: Treatments with ashi acupuncture and local anesthetic injections were effective in reducing clinical pain in women with abdominal myofascial pain syndrome.

Key words: Chronic pelvic pain, abdominal myofascial pain syndrome, trigger points, acupuncture, topical injectable anesthetic

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Chronic pelvic pain (CPP) is defined as recurrent or continuous pain that occurs in the hypogastric region (lower abdomen or pelvis), is non-menstrual or non-cyclic, lasts at least 6 months, and is severe enough to interfere with the patient's usual activities and affect the quality of life, necessitating surgical or pharmacological treatment (1-3). Among the musculoskeletal disorders related to CPP, abdominal myofascial pain syndrome (AMPS) appears to be a major condition. AMPS usually affects women more than men (54% and 45%, respectively) and is more frequent among women aged 30 to 40 years (4-7). Its estimated prevalence ranges from 30% in primary care centers to 85% – 93% in specialized centers for the treatment of pain (8).

AMPS is characterized as intense and superficial abdominal pain caused by hyperirritable trigger points (MTrP), usually located within a musculoskeletal range or its coating fascia, (9-11) and may be classified as active or latent (8). Active MTrP may cause resting pain, and the stimulation of these active points may promote the activation of other latent trigger points, which are sometimes located in regions distant from the active trigger point being stimulated. While latent MTrPs do not cause spontaneous pain, they may restrict movement or cause muscle weakness, and only become painful if they receive direct pressure. They can also produce muscle spasms and autonomic phenomena such as piloerection, vasoconstriction, hyperhidrosis, temperature changes, and a variety of somatovisceral reflexes (8,12).

It is important to distinguish AMPS from abdominal neuropathic pain. The latter is often described as a burning pain, and characteristically presents irradiation to the dermatome corresponding to the affected nerve, either by surgical procedures, radiculopathies, or abdominal traumas. The nerves most affected are the ilioinguinal nerves, i.e., the iliohypogastric and genitofemoral nerves (13,14). However, AMPS and abdominal neuropathy are very similar, and there is a lack of consensus in the literature on the differential diagnosis of these 2 conditions (15). The combination of criteria most used for the diagnosis of myofascial syndrome is a hypertonic point in a set of muscular fibers, recognition of pain on point palpation, referred pain pattern; muscle contracture as the local response to point palpation, and limited range of motion (9,16,17).

In addition to systemic pharmacological treatments including pain killers, myorelaxation, and non-steroidal antidepressants and anti-inflammatory drugs (12), specific therapies have been proposed, such as ischemic compression (12,18,19), electrotherapy (12), and anesthetic injections at the trigger point (14,20-22). Nevertheless, there is a need for studies to verify the effectiveness of non-pharmacological therapeutic options such as ashi acupuncture, an ancient Chinese technique that uses acupuncture needles in pain points (23-28), that will allow directed treatment at the physical, emotional, and systemic levels in patients with AMPS. Acupuncturists follow a Traditional Chinese approach in which they consider MTrP as ashi points (26). The term "ashi" was proposed almost 2000 years ago (28), one of the theories employed for this term would be from the Wu dialect, which divided ashi into "A" as the cry of the patients and "shi" (yes), such as the confirmation of the painful site or palpation point, prior to treatment (27-30). Thus the ashi acupuncture method could be done in the following way: The acupuncturist would press the place where there is pain in search of painful points, so that he could then insert the needle or deal with other acupuncture resources, regardless of whether the pain was an acupuncture point (meridian) or not (31). Essentially, ashi points are local points or points around local lesions (28). Therefore, it is essential for the successful outcome of acupuncture treatment in myofascial syndrome (SMF) to identify active MTrPs and to properly shape points in both the Western Acupuncture or ashi approach (local points) and in the Eastern acupuncture approach (meridian points) in order to resolve the conditions that involve myofascial pain.

This study aimed to evaluate the efficacy of ashi acupuncture in comparison with local anesthetic injections in the treatment of clinical pain in women presenting with CPP secondary to AMPS.

Methods

Design

An experimental study was conducted by performing a randomized controlled trial including 35 women, with 16 patients in the ashi acupuncture group and 19 patients in the local anesthetic injection group. The study followed the ethical principles established by the Declaration of Helsinki and was approved by the Research Ethics Committee of the Hospital das Clínicas of the Ribeirao Preto Medical School at the University of Sao Paulo on March 28, 2011, according to the HCRP process no. 14301/2010. Informed consent forms were signed by all patients. This study was enrolled in Rebec under the number RBR-4Y8VD2.

Patients

Eligibility criteria

Women meeting the diagnostic criteria for CPP secondary to AMPS were included based on the studies by Carnett (16); Tough et al (17), and Ferraz (32), with only one active trigger point present and who had not undergone previous topical injectable anesthetic blockade or acupuncture, over 18 years of age and premenopausal.

Women with anticoagulation or hemorrhagic disorders, local or systemic infections, allergy to anesthetics, acute muscle trauma, extreme fear of needles, history of complaints of chronic musculoskeletal pain due to conditions such as fibromyalgia, chronic fatigue, or diabetes were excluded. Also excluded were those who had ingested aspirin within 3 days prior to initiation of treatment and patients with clinical suspicion of endometriosis, interstitial cystitis, irritable bowel syndrome, or other diseases justifying or contributing to CPP. Patients with endometrioma or hernia, evidenced by ultrasound of the abdominal wall, those with abdominal wall infections, and those who missed sessions after the beginning of the treatment were also excluded.

Settings and locations for data collection

The patients were recruited and treated at the Chronic Pelvic Pain Outpatient Clinic of a tertiary university hospital. After confirmation of AMPS, following the criteria of the authors Simons et al (9), Carnett (16), by physicians (JCRS and OBPN), patients who met the inclusion criteria were invited to participate in the study. The active trigger point was measured using a tape measure, and to determine its exact location, the distance from the point to the various abdominal anatomical structures was measured. The clinical pain threshold was then evaluated using the numerical categorical scale (NCS) (33). and visual analog scale (VAS) and the McGill questionnaire (33-35). After completion of the treatments, participants were re-evaluated with the same parameters at one week and one, 3, and 6 months after the interventions, and the patients in both groups were instructed not to use central analgesics or nonsteroidal anti-inflammatory drugs for 72 hours before the re-evaluations. These evaluation procedures were performed by a second researcher (MCDVB).

Interventions

Ashi acupuncture

Participants underwent palpation at the active and latent trigger points so that the needles were then inserted into the specific sites of pain and then the local acupuncture treatment was performed (ashi points in the abdominal region) (26-31,36). Palpation for location of the trigger points and insertion of the needles were performed by a researcher with professional qualification and specialization in acupuncture (AMSM). Each session was performed once a week for a total of 10 consecutive weeks, and the needles remained in situ for 25 minutes, without manual stimuli, during each session (37). Cylindrical filiform high-grade stainless steel needles with low nickel content (to minimize possible allergic reactions) and thickness between 0.25 and 0.40 mm were used (DBC Brand; Dong Bang Acupuncture, Inc., Republic of Korea, 2014; Importer: XU LI Import and Export Trade Ltd., Sao Caetano do Sul - SP, Brazil). For the application, disposable conductors were used to facilitate needle introduction and allow application free of contamination and direct manual contact. Sterile (ethylene oxide) and disposable needles were used according to the World Health Organization (WHO) guidelines. The skin was previously disinfected using 70% alcohol (Ciplan).

Injection of local anesthetic

At each session, the patients were submitted to the active trigger point palpation examination on the abdominal wall, to locate the pain site and to administer lidocaine at the site referred by the participant. Patients were administered 2 mL of 1% lidocaine without a vasoconstrictor (12,14,21) using a 22-gauge needle measuring 0.70 mm × 0.25 mm (Injex Indústrias Cirúrgicas Ltda, Ourinhos-SP, Brazil, 2014) directly and perpendicularly to the active trigger point. At the end of the session, direct compression with sterile cotton was applied for at least 2 minutes to avoid local hematoma formation (8,12). The treatment was performed once a week for 4 consecutive weeks, as standardized by the outpatient clinic (14).

Sample size

The sample size was calculated to test 2 experimental conditions with samples of the same size (treatment with lidocaine injection versus treatment with ashi acupuncture) using the following expression:

$$n = \frac{[z_{\alpha}\,(p1 \times q1 + p2 \times q2)^{1/2} + z_{1-\beta}\,(p1 \times q1 + p2 \times q2)^{1/2}]^2}{(p_2 - p_1)^2}$$

Based on the literature (Kamanli et al [21]), the following considerations were made:

- p2 = 60%. A result less than 30% would be considered unsatisfactory;
- $Z\alpha = 1.645$, considering $\alpha = 5\%$ and unilateral test;
- $z1\text{-}\beta = 1.2815$, considering the power of the test $(1\text{-}\beta) = 90\%$

p1: Proportion of improvement in the acupuncture group
p2: Proportion of improvement in the local anesthetic injection group
q1: Proportion of no improvement in the acupuncture group (1-p1)
q2: Proportion of no improvement in the local anesthetic injection group (1-p2)
z: Critical value of the normal distribution.

Thus, the sample size was determined to be equal to 16 individuals for each group, so that it can be pointed out that the response rate is different between groups, with the conditions of significance and test power considered, 5% and 80%, respectively.

Randomization

Patients were randomly assigned to the 2 experimental groups according to a randomized list generated using a website built for this purpose (random.org).

Blinding

This study was blinded to the researchers who conducted the data analysis and statistics, as well as for the researchers who applied the questionnaires and assessments before and after treatments.

Statistical methods

An exploratory data analysis was carried out by assessing measures of central position and dispersion. A univariate analysis was performed using the chi-squared and Student's t-tests. A significance level of 5% was considered. A linear regression model of mixed effects was considered to verify the effect of time in relation to the study groups on the variables of interest. The residuals of the model were checked to verify if the data were well adjusted to the model. The templates were implemented in the PROC MIXED of the SAS program version 9.3.

Results

Fifty-five women diagnosed with AMPS who had not received local anesthetic injections or ashi acupuncture treatments previously were recruited. Eight of these women were excluded because they did not fulfill all the inclusion criteria, and one was excluded because of difficulty in understanding the questionnaires, making the analysis impossible without the evaluator's interference. All excluded patients were treated with the local anesthetic injection and underwent medical follow-up at an outpatient clinic outside the study. Four other recruited patients refused to participate in the study because of extreme fear of needles and issues related to their work hours. Thus, we performed evaluations and treatments on 42 patients with AMPS. During the sessions, 7 patients left the study: 2 patients left for work-related reasons (one each from the ashi acupuncture group and the local anesthetic injection group), one left to undergo surgery that she had been waiting to undergo before the study (Group B), and 4 (A = 3 and B = 1) withdrew from the treatment (Group B) without any explanation. They were absent after the beginning of the sessions, and attempts by the researchers to contact them via telephone and e-mail were unsuccessful. Thus, we conducted this study with 35 patients, who were randomly divided into Group A (n = 16) and Group B (n = 19) (Fig. 1). Table 1 shows all the analyzed variables, with no statistical differences observed between the groups. Thus, the sample could be considered homogeneous ($P > 0.05$).

The acupuncture treatment proved to be as effective as the local anesthetic injection treatment with respect to reducing clinical pain (Figs. 2 and 3) at all reevaluation points ($P < 0.001$), with no significant difference observed between the intervention groups. An intergroup difference ($P = 0.01$) was observed only in the McGill pain questionnaire (Fig. 4) at the one-week time interval, where acupuncture was rated not effective ($P = 0.17$) in the multidimensional assessment of clinical pain perception, while injection of local anesthetic was considered effective. This increased the total score value, which would indicate worsening of clinical pain after one week ($P < 0.001$). The Wilcoxon statistical test was used to determine the mean, standard deviation, and *P* value and for comparisons between groups. In a comparison of the NCS and VAS scores between treatment sessions, it was possible to observe a significant improvement during all phases of treatment with both ashi acupuncture and local anesthetic injection (Tables 2 and 3).

Published reports have shown that acupuncture is not risk-free (38); in the assessments of adverse events

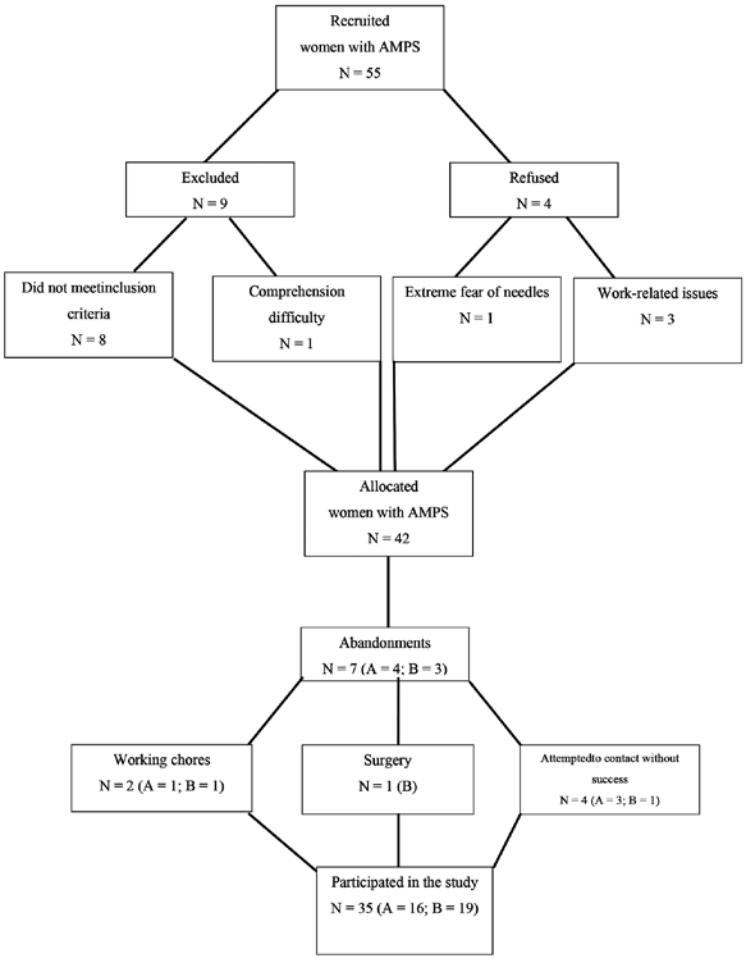

Fig. 1. *Recruitment and allocation of patients.*

in the ashi acupuncture group, 8 patients (50%) had ecchymosis episodes at treatment points. In the present study, the authors concluded that bruising, as well as other situations (local light bleeding, numbness, local weight sensation, pain or discomfort) as easily resolved adverse effects (38-40) and corroborates with our data because they appeared the week after the application of the needles and then, there was spontaneous resolution, without intercurrences and without other associated complaints. Headache was also reported in one patient (6%), and another patient experienced abdominal bloating (6%) after the first application, with no repetition of these symptoms noted in the following sessions. In contrast, in the group treated with lidocaine injection, 7 episodes (37%) of ecchymosis at the active trigger points, with no associated complaints, occurred in the week following the local injection. Three patients reported headache (16%), one reported a sensation of dormancy in the abdominal region corresponding to the active trigger point (5%) after the third application, and 4 (21%) reported dizziness after the anesthetic injection. All of these conditions were considered as minor and possibly treatment-related. Other events such as nausea, sweating, fainting, or even more rare and serious conditions such as needle shedding, procedural infections, or insult to the viscera were not reported or observed in either group.

Table 1. *Sample characterization in both intervention groups.*

Variables	Group A (N = 16) Mean (SD)	Group B (N = 19) Mean (SD)	*P**
Age	45.69 (± 8.27)	41.58 (± 11.35)	0.23
BMI	32.09 (± 7.61)	30.27 (± 5.44)	0.41
Marital Status	**N (%)**	**N (%)**	
Married/cohabitating	14 (88%)	14 (74%)	0.41a
Single/divorced/widow	1 (6%)	2 (10%)	
Education	**N (%)**	**N (%)**	
Elementary and middle school incomplete	3 (19%)	3 (16%)	
Elementary and middle school complete	0%	4 (21%)	
High school incomplete	4 (25%)	2 (10%)	0.32aa
High school complete	6 (38%)	6 (32%)	
College or university incomplete	1 (6%)	3 (16%)	
College or university complete	2 (12%)	1 (5%)	
Parity			
Gestations	2.38 (± 1.36)	2.89 (± 1.97)	0.74
Caesarians	1.31 (± 1.08)	2.0 (± 1.11)	0.08
Normal Deliveries	0.38 (± 0.81)	0.32 (± 0.67)	0.84
Abortions	0.69 (± 0.87)	0.58 (± 1.64)	0.13
Physical activity	**N (%)**	**N (%)**	
Active	3 (19%)	2 (11%)	0.64a
Sedentary	13 (81%)	17 (89%)	

Student's t test, Fisher'saExact, and Chi-squareaa Test
N – sample size; P*– *P* value; SD – standard deviation; BMI – body mass index.

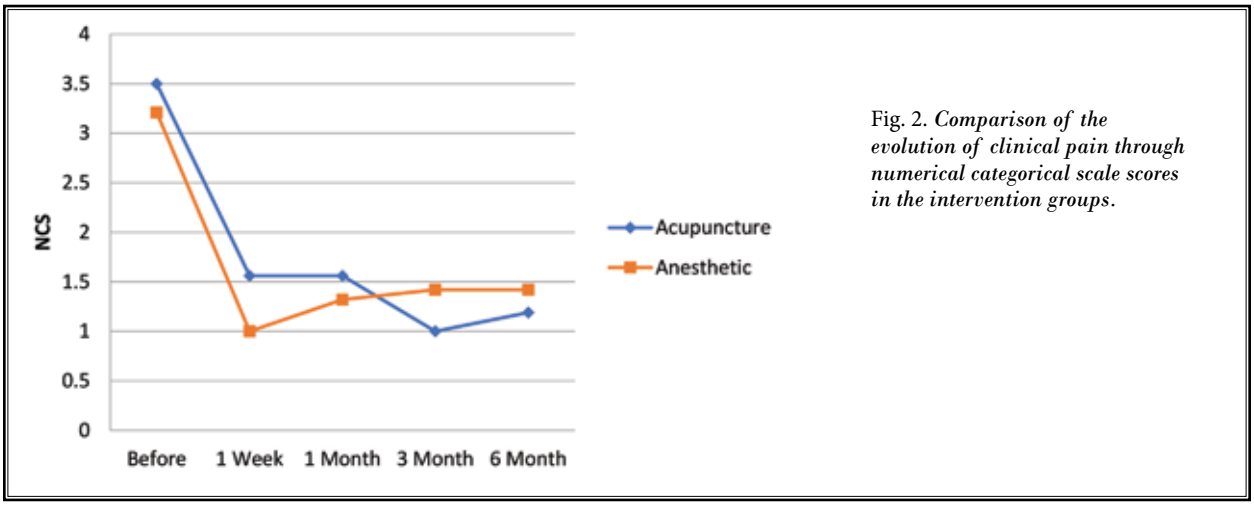

Fig. 2. *Comparison of the evolution of clinical pain through numerical categorical scale scores in the intervention groups.*

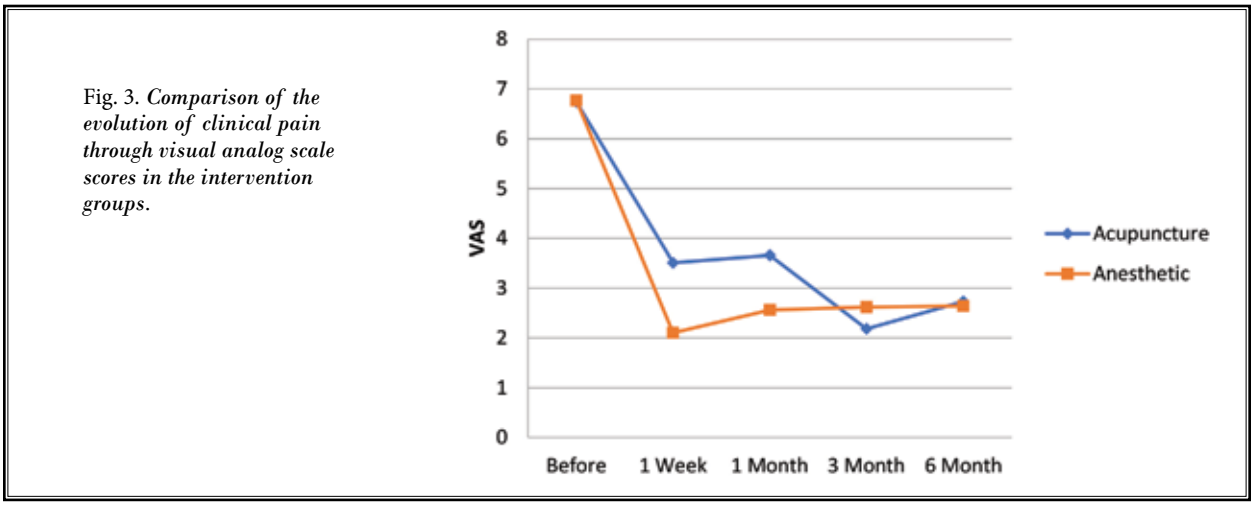

Fig. 3. *Comparison of the evolution of clinical pain through visual analog scale scores in the intervention groups.*

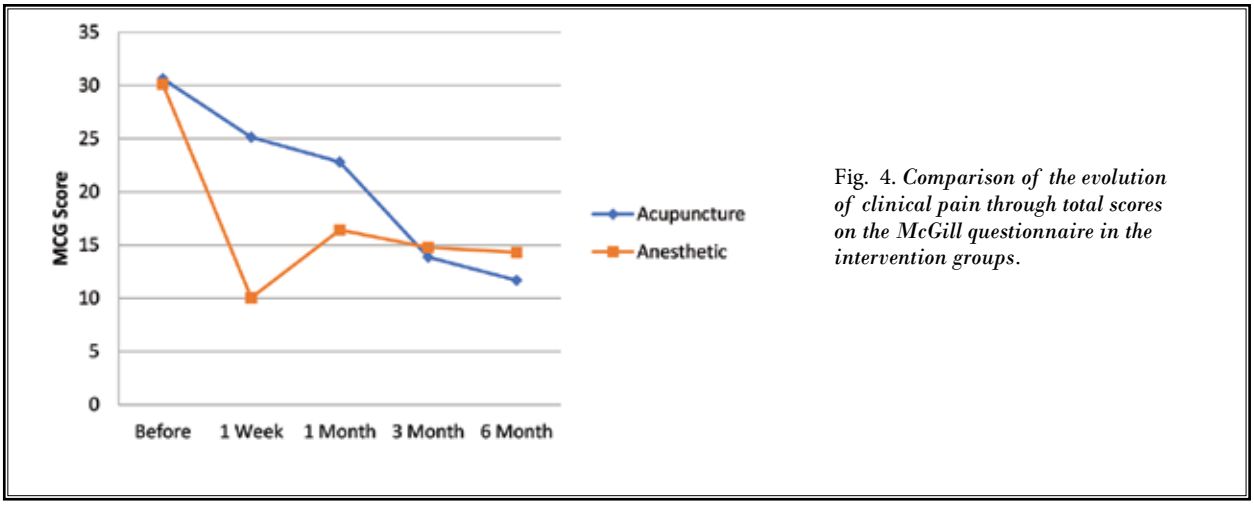

Fig. 4. *Comparison of the evolution of clinical pain through total scores on the McGill questionnaire in the intervention groups.*

Table 2. *Evolution of clinical pain over each session in the ashi acupuncture group.*

Acupuncture Ashi	NCS	*P* value*	VAS	*P* value*
1st session	3.4 ± 1.0	-	6.7 ± 2.0	-
2nd session	1.9 ± 1.4	0.00	4.4 ± 2.9	0.00
3rd session	2.6 ± 2.1	0.09	5.1 ± 2.9	0.07
4th session	2.0 ± 1.8	0.00	4.7 ± 3.5	0.02
5th session	2.3 ± 2.0	0.02	4.6 ± 2.6	0.01
6th session	2.6 ± 2.0	0.09	3.8 ± 3.0	0.00
7th session	2.0 ± 2.0	0.00	3.8 ± 3.0	0.00
8th session	2.0 ± 1.9	0.00	4.0 ± 3.2	0.00
9th session	1.7 ± 1.8	0.00	3.2 ± 2.7	< 0001
10th session	1.4 ± 1.4	< 0001	2.9 ± 2.8	< 0001

P Wilcoxon Test
P* – value of "P"; NCS – numerical categorical scale; VAS – visual analog scale.

Table 3. *Evolution of clinical pain over each session in the group receiving injections of local anesthetic.*

Local anesthetic	NCS	*P** value	VAS	*P** value
1st session	3.4 ± 0.8	-	6.7±1.9	-
2nd session	2.7 ± 1.5	0.05	5.2±2.9	0.03
3rd session	1.7 ± 1.6	< 0001	3.4±3.6	< 0001
4th session	2.0 ± 1.6	0.00	3.9±3.2	< 0001

P Wilcoxon Test
P* – value of "P"; NCS – numerical categorical scale; VAS – visual analog scale.

Discussion

Main findings

Ashi acupuncture was as effective as local anesthetic injections in reducing secondary clinical pain associated with AMPS.

Interpretation of Results

The study by Rivera et al (41) aimed to evaluate the efficacy of 2 invasive techniques in the treatment of myofascial pain through puncture with acupuncture needle and infiltration of 1% lidocaine in trigger points. They found that both acupuncture and lidocaine infiltration at trigger points were effective in reducing pain. Mitidieri et al (42) studied the efficacy of ashi acupuncture in women with CPP secondary to myofascial syndrome that was not responsive to treatment with lidocaine injection at MTrP and found that acupuncture could reduce pain and improve the quality of life in these patients. These data are consistent with the findings of our study, in which the patients showed a significant improvement in the perception of clinical pain via NCS, VAS, and McGill questionnaire assessments.

A study by Montenegro et al (43) concluded the superiority of the local anesthetic for the treatment of trigger points in the lower abdominal wall of women with CPP when compared to the non-invasive technique of ischemic trigger point compression. It is believed that the effects promoted by local anesthetics occur through interruption of nerve excitation and conduction by direct interaction with sodium channels, which promote reduction of inflammation and activation of acetylcholine at the neuromuscular junction (44).

Dry needling, a relatively new and widespread technique for the treatment of MTrP by physiotherapists, involves a minimally invasive procedure wherein insertion of an unmedicated needle into an MTrP (45) is performed. The difference between the dry needling technique and ashi acupuncture is that acupuncture follows a Traditional Chinese approach in that it considers the MTrP as ashi point that can be active or latent (26). Essentially, ashi points are local points or points around local lesions (36). Ashi acupuncture can reduce nociceptive responses, yielding pain modulating effects (pain gate theory) (46,47). Hong (48) reported that the stimulation of strong pressure caused by needling in the MTrP may provide more intense neural impulses to the dorsal horn cells of the spinal cord and thereby help make the MTrP latent. In addition, Longbottom (26) and Shah and Gilliams (49) demonstrated that acupuncture in MTrP is intended to stimulate hyperirritability in neuromuscular junction foci causing a specific twitch response, thus altering inflammatory extracellular mediators around the trigger point, thus suggesting local pain reduction. In the peripheral mechanism, the insertion of the needle produces lesions in the pain tissues, and activates neuroendocrine, immunological and cardiovascular reactions around the punctures (5), local synthesis and release of growth factors, cytokines, vasoactive substances, degradation enzymes and structural matrix elements occurs. Thus, the mechanical signals produced by the manipulations of the needle, generate cascades of physiological effects (50). Needle insertion in the acupoint provokes acute local inflammatory defensive response through the somatic afferent fibers of neurons (A-delta and C fibers) and sympathetic neurons (for control of sweat glands and fine blood vessels); fine arterial and venous blood vessels (nutrition supply and temperature regulation); lymphatic tissue, mast cell (immune function), and connective tissues (structural and functional support). A local blush occurs, due to vasodilatation of the autonomic system (ANS) mediated by substance P secreted by cutaneous nociceptive sensory nerves. Then, the immune reaction is triggered by mast cells that produce histamine, platelet activating factor (FAP), and leukotrienes (51). At the site of the needle insertion, a cutaneous microcurrent circuit is formed which produces a current of the lesion (about 10 mA), which stimulates tissue growth (52) and does not generate tolerance like morphine. This means that repetitive needle insertion does not reduce its therapeutic effects.

In some perspectives, acupuncture treatments are generally viewed with suspicion, and it has been proposed that the efficacy of alternative therapies can be attributed primarily to the placebo effect (53). Considering the clinical effects of acupuncture, it is inevitable that psychological factors are involved in acupuncture analgesia, mainly in cases involving chronic pain, and this is a focus of discussion/argument for many researchers (54-58). Nevertheless, some authors highlight that acupuncture treatment can be explained by peripheral and central mechanisms (53,59-62). Even sham acupuncture treatment is not a totally inert treatment, or even considered to be of controversial effects (63-65). The authors noted in their studies that acupuncture analgesia was superior to placebo in patients (66,67) and in healthy volunteers (52,68).

Adverse events were noted with both interventions, but none of them caused serious harm to the patients in this study. Although the MTrP approach with multiple injections of lidocaine is more effective than a single injection (69), it is important to note that long-term application of anesthetics may be associated with neurotoxicity, myotoxicity, and could cause important alterations such as dysesthesia, paresthesia, or sensorimotor deficits. But clinically, serial trigger point injections have been very commonly practiced on a very large scale for many years and are easily well tolerated by patients; these adverse events are very rare in the clinical practice (70). On the other hand, the needles used in acupuncture are free of medications and can reduce pain through sensory stimuli and act via nociceptive modulation at the central and peripheral levels (53-55).

Limitations

The absence of blinding to the different forms of treatment among the patients and the researcher directly involved in the treatment was a limitation of the study. However, we minimized the impact of this by blinding those who conducted the analyses and the researchers who applied the questionnaires and evaluations. Unfortunately, our study design does not allow complete blinding. The absence of a placebo group was another limitation, but the ethical implications of not treating a patient with chronic pain prevented us from forming such a group. The third limitation is the selective exclusion of women with comorbidities and other causes of CPP. In considering this fact, we cannot confirm that both interventions work in the same way in a real scenario where most of the women followed up in a clinic specializing in chronic pain present with associated comorbidities and more than one painful region that can also be justified by the somatization process. However, these exclusions were essential to avoid biases in interpretation. The fourth limitation presented in this study can be considered the difference between the number of sessions used for each technique, influenced by the long interaction and link between doctor-patient in the acupuncture sessions. Although we have attempted to minimize this link through the reception, positioning, and demarcation of the points of pain being performed by a researcher, while the applications of both techniques were performed by specific professionals without communication with the patient, we cannot ignore the therapeutic effects which may have been influenced by this link.

Conclusion

Both ashi acupuncture treatment and local anesthetic injections were effective modalities for reducing clinical pain in women with CPP secondary to AMPS.

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Author Contributions: Dra. Andréia Moreira de Souza Mitidieri had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Drs. Omero Benedicto Poli-Neto, Antônio Alberto Nogueira, Maria Beatriz Ferreira Gurian, Francisco José Cândido dos Reis, and Ana Paula Moreira da Silva designed the study protocol. Maria Carolina Dalla Vecchia Baltazar managed the literature searches and summaries of previous related work and wrote the first draft of the manuscript. Dr. Júlio César Rosa e Silva designed the study protocol, provided revision for intellectual content, and provided final approval of the manuscript.

Conflict of Interest

The authors have no conflicts of interest to report. None of the authors of the manuscript received any remuneration. Further, the authors have not received any reimbursement or honorarium in any other manner.

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