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The existence of peripheral and central sensitization at acupoints in patients with unilateral shoulder pain

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Abstract

Objective—To investigate the pattern of experimental pain responses at acupoints in clinical patients with unilateral shoulder pain.

Design—A purely observational design.

Setting—Acupuncture and Moxibustion Department, Beijing Hospital of Traditional Chinese Medicine Affiliated to Capital Medical University.

Participants—Volunteer sample of 60 participants (30 clinical patients with unilateral shoulder pain, 30 healthy participants).

Interventions—Not applicable.

Main Outcome Measures—Pressure-pain thresholds (PPTs) were measured at four acupints, including Tianzong (SI 11), Jianliao (SJ 14), Jianyu (LI 15) and Jianzhen (SI 9) on the painful/non-painful side in patients with or without unilateral shoulder pain, respectively. The correlations between peripheral sensitization indexes (PSI) and central sensitization indexes (CSI) were compared in this study.

Results—The analysis revealed significantly lower PPT values at acupints on the painful side compared with non-painful side in clinical patients ($P < 0.05$). Meanwhile, PPTs on the non-painful side of clinical patients were diminished compared to ipsilateral side of the healthy participants ($P < 0.05$). No distinct differences of PPT values were found at non-acupoint among the painful/non-painful side in clinical patients and ipsilateral side of the healthy participants ($P > 0.05$). Additionally, it was observed that the pressure pain assessment acupoints have strong association between the PSI and CSI, and three acupoints of SJ 14, LI 15 and SI 9 showed correlation in PSI or CSI particularly.

Conclusion—The results suggest the presence of peripheral and central sensitization at acupoints in participants with unilateral shoulder pain. There exists obvious relationship among the three acupoints of SJ 14, LI 15 and SI 9, which are n usually chosen to treat the shoulder pain. The results provide the evidence for acupoints selection to treat shoulder pain by acupuncture.

Keywords: acupoints; peripheral sensitization; central sensitization; pressure pain threshold; shoulder pain

Strengths and limitations of this study

The strength of this study was to support the alteration in both peripheral and central sensitization at acupoints in patients with musculoskeletal pain.

The association between peripheral sensitization and central sensitization was observed at the pressure pain assessment acupoints.

Furthermore, three acupoints of Jianliao (SJ 14), Jianyu (LI 15) and Jianzhen (SI 9), which are usually chosen to treat the shoulder pain, exists obvious relationship.

And the peripheral and central sensitization at acupoints was seemed to occur correlatively with sex and independently from other baseline variable.

The limitation of this study was the only pressure pain assessment performed at acupoints, which might be inadequate to describe the complexity of pain perception.

INTRODUCTION

Acupuncture is one of the most widely-used complementary and alternative medicines. There are about 183 countries adopting acupuncture treatment according to a survey conducted by the World Federation of Acupuncture and Moxibustion Societies in 2013 [1]. The WHO reports that the acupuncture treatment can be beneficial for more than 40 disorders. Pain is one of those disorders, which is particularly sensitive to acupuncture. The evidence in reviews demonstrates that acupuncture treatment can reduce clinical pain effectively in multiple clinical trials [2-4]. Recently, a meta-analysis reported that shoulder pain, low back pain, neck pain, osteoarthritis and chronic headache can be alleviated significantly after acupuncture treatment [5]. Shoulder pain is the third most common musculoskeletal disorder, of which the prevalence rate varies from 6.9% to 26% for point prevalence and even increases to 66.7% for lifetime prevalence in the general population [6, 7]. In our previous study, we identified acupuncture treatment can alleviate shoulder pain, particularly in special acupoints (data unpublished).

Acupoints are some special sites, which distribute at precise locations and lie on ‘meridians’ without a physical structure. According to the theory of meridians in traditional Chinese medicine, it is well known that the acupoints reflect the disorder of visceral conditions. Many studies have observed visceral lesions result in changed pain perception of some special

acupoints on the body surface [8]. Ben et al assessed the phenomenon of experimental pain-sensitive points in patients with gastric ulcer or gastritis and found some special acupoints were more sensitive than other areas [9]. Nevertheless, whether acupoints behave more sensitively under musculoskeletal pain has not been described detailedly in previous studies.

We performed a multicenter, single blind, factorial randomized controlled clinical trial previously. The phenomenon that the pain threshold decreased at related acupoints of the patients with unilateral shoulder pain was widespread. Therefore, we chose some patients to detect whether acupoints are hypersensitivity in musculoskeletal pain disease.

Sensitization is a nervous system phenomenon that can occur in conjunction with pain [10]. When sensitization is present, there are two types of hypersensitivity including peripheral and central sensitization. Peripheral sensitization is defined as the broadening of nociception in pain perception during activities or movements, where would be not painful [11]. Central sensitization refers to hypersensitivity in non-affected tissues [12]. The primary aim of this study is to examine whether acupoints exist peripheral or central sensitization phenomenon in patients with unilateral shoulder pain. Index variables are used as the indicator of the sensitivity of peripheral and central sensitization in patients with chronic musculoskeletal pain [13]. The second aim of this study is to compute the peripheral and central sensitization index of acupoints on the surface of patients with unilateral shoulder pain.

MEDTHODS AND ANALYSIS

Participants

Clinical patients—This study recruited both of clinical and healthy participants. All participants provided informed consent before participation. The Research Ethical Committee of Beijing Hospital of Traditional Chinese Medicine Affiliated to Capital Medical University approved the trial. All participants need signed informed consent form.

The inclusion criteria included presenting with one-sided shoulder pain for at least six weeks and up to two years, reporting the pain intensity > 50 mm on a visual analogue scale (VAS), and the painful sites on affected side in clinical patients do not overlap the pressure pain assessment sites. The individuals were chosen from the 164 patients, who have not received acupuncture or other analgesic therapies in the preceding month.

The exclusion criteria are as follows: pain in bilateral shoulder; referred pain from the cervical spine; previous history of shoulder surgery, stoke or ipsilateral breast surgery; heart diseases and severe hypertension; osteoarthritis of the glenohumeral joint or systemic bone and joint disorder (rheumatoid arthritis); endocrine diseases such as hyperthyroidism; severe infection; undergoing current therapy involving analgesics and especially major illness depression.

Healthy participants—Healthy participants were age- and sex-matched to clinical patients. Healthy participants were recruited from the University of Capital Medical campus via posted flyers and general advertisements. Participants were eligible if they not currently performing resistance exercise for the upper extremity. Participants were excluded based on the following criteria: receiving acupuncture or other analgesic therapies in the preceding month, experiencing neck or shoulder pain, having the history of shoulder surgery or neurological impairments of the upper extremity, and taking pain medication currently.

Protocol

This study was a purely observational design. All participants completed a packet of questionnaires including demographic data (age, sex, race, hand dominance) and psychological information. Clinical patients were initially examined by a researcher who assessed the compliance using the inclusion and exclusion criteria. Beck Depression Inventory (BDI) is a multiple choice self-reported inventory for measuring the severity of depression [14]. If the BDI outcome is more than 4, the participants will be excluded. Clinical pain intensity was assessed by VAS, which consist line of 0–100 mm: 0 representing ‘no pain at all’ and 100 mm representing ‘the most intense pain imaginable’. If the VAS outcome is over 50 mm, the clinical patients will be included.

After finishing the questionnaires, both shoulders of participants were exposed and marked the measurement sites. An expertly acupuncturist was responsible for the operation. Five points were marked in unilateral in this study and shown in Figure 1 and Appendix Table 1. To assess the pain sensitivity of acupoints, four acupoints Jianliao (SJ 14), Jianyu (LI 15), Jianzhen (SI 9), and Tianzong (SI 11) were marked bilaterally with a maker pen drawing a circle (Figure 1. A. B). To assess the pain sensitive in the non-acupoint, 2 cm down to Tianzong (SI 11) was marked bilaterally in participants (Figure 1. B).

Measurement of pressure pain thresholds (PPT)

Both shoulders were exposed for examination. The participants were asked to take a prone position on the examination bed with a suitable pillow under the chest when Jianzhen (SI 9), Tianzong (SI 11) and non-acupoint were measured. Then the participants were required sitting on a chair with a researcher to keep the arm and shoulder in parallel when Jianliao (SJ 14) and Jianyu (LI 15) were measured.

PPT is widely used to evaluate the pain sensory threshold for patients with shoulder pain [15]. The lower value of PPT indicates the decreased nociceptive threshold of pain perception, and signifies the presence of sensitization [16]. A handheld electronic pressure algometer (Wagner Instruments, Greenwich, CT) mounted with a 1 cm2 rubber tipped plunger was used in this study. The probe was held perpendicular to marked points bilaterally, and the pressure was increased at a rate of 30kPa/s, in order to avoid potential skin penetration. All participants were instructed to indicate when the pressure became painful, at the same time the pressure was immediately stopped and the digits were recorded. PPT was calculated as the mean of three trials on each point. There was approximately 2 min interval between the repetitions. The operator should not to be told which side is the painful shoulder of the participants.

Data Analysis

All data were entered into SPSS 17.0 software. The distributed data were used parametric statistical test if it agreed with normal distribution. Otherwise, the data were used non-parametric statistical test. Data were presented as mean ± standard deviation (SD). Significance level was determined by $P< 0.05$ for all data.

Firstly, we compared the experimental pain responses between the painful side and non-painful side of clinical patients. The independent t-test was used to compare the difference between the painful side and non-painful side at the pressure pain assessment sites. Then, we performed the comparison of mechanical pain sensitivity on the non-painful side of clinical patients to the ipsilateral side of the healthy participants. Pair t-test was performed to analyze the data.

In addition, we used peripheral and central sensitization index to determine whether pain-sensitivity existed at the pressure pain assessment sites. We computed a standardized score (z-

score) using the different value of raw score (clinical patients) minus mean (healthy participants) and then dividing standrard deviation (healthy participants) for each clinical participant. We examined a clinical participant's ratio response for PPT in painful side fell below the 25th percentile of non-painful side which would indicate peripheral sensitization. The ratio responses were referred as the peripheral sensitization index (PSI). We computed a similar standardized (z-score) for each clinical patient by using the healthy participant's mean and standard deviation, which can indicate the central sensitization when clinical participant's averaged responses for PPT in non-painful side fell below the 25th percentile of the ipsilateral side of healthy participants. The ratio responses were named as the central sensitization indices (CSI). The 25th percentile was suggested as a lower limit reference value for enhanced sensitivity. Each index was examined by the Pearson's r correlation. We analyzed association between sensitization subgroups and the relevant baseline characteristics including demographic and clinical variables. Comparisons among the variables were examined using one way analysis of variance or Chi-square and assessment of 95% confidence intervals (CI).

RESULTS

Recruitment and baseline characteristics

Between January 2014 and September 2014, forty individuals were chosen from 164 patients who had experienced chronic unilateral shoulder pain, of which thirty (11 males and 19 females) were screened for eligibility criteria (Table 1). The average age of thirty patients who agreed to participate was 50.4 ± 12.3 years. The healthy participants included 11 males and 19 females with age ranging from 25 to 65 years with a mean age of 50.4 ± 12.3 years. All participants were right hand dominant.

Table 1. Baseline characteristics of the clinical patients and healthy participants.

	Clinical Patients (N = 30)	**Healthy Participants (N = 30)**
Age (years)	50.60±12.19	50.63±12.20
Sex (N of females)	19	19
Race (number of Han)	30	30
Pain duration (weeks)	19.07 ± 16.99	-
VAS	66.17±14.60	-

Values represented as N, mean ± SD. Abbreviations: N – Number, SD – Standard Deviation, VAS – Visual Analogue Scale, BDI – Beck Depression Inventory.

PPT comparison of related acupoints

For clinical patients, lower PPT level was detected at acupoints on the painful side compared with the non-painful side (Table 2). The differences were 94.9 (95% CI, 15.98 to 173.82; p = 0.02) at Tianzong (SI 11), 102.91 (95% CI, 5.20 to 200.62; p = 0.04) at Jianliao (SJ 14), 91.19 (95% CI, 13.35 to 169.03; p = 0.02) at Jianyu (LI 15), and 86.79 (95% CI, 170.11 to 3.46; p = 0.04) at Jianzhen (SI 9), respectively.

The PPT value difference between the non-painful side of clinical patients and the ipsilateral side of healthy participants was also compared. PPT at the acupoints on non-painful side of clinical patients was lower than on the ipsilateral side of healthy participants. The differences were 57.71 (95% CI, 24.72 to 90.70; p = 0.001) at Tianzong (SI 11), 81.03 (95% CI, 41.40 to 120.67; p < 0.001) at Jianliao (SJ 14), 72.2 (95% CI, 10.90 to 133.50; p = 0.02) at Jianyu (LI 15), and 88.09 (95% CI, 39.87 to 136.30; p = 0.001) at Jianzhen (SI 9), respectively.

Table 2. Pressure pain threshold values for the clinical patients and healthy participants.

	Clinical Patients		Painful side vs Non-painful Side		Health Participants	Non-painful Side vs Ipsilateral Side	
	Painful Side	**Non-painful Side**	**Mean (95% CI)**	**P value**	**Ipsilateral Side**	**Mean (95% CI)**	**P value**
Tianzong (SI 11)	414.83±135.61	509.73±168.05	94.9 (15.98, 173.82)	0.02	567.44±153.84	57.71 (24.72, 90.70)	0.001
Jianliao (SJ 14)	469.70±181.07	572.61±196.71	102.91(5.20, 200.62)	0.04	653.64±211.50	81.03 (41.40, 120.67)	<0.001
Jianyu (LI 15)	434.79±140.39	525.98±160.16	91.19 (13.35, 169.03)	0.02	598.18±181.76	72.2 (10.90, 133.50)	0.02
Jianzhen (SI 9)	453.08±154.54	539.87±167.59	86.79 (170.11, 3.46)	0.04	627.96±209.67	88.09 (39.87, 136.30)	0.001
Non-acupoint	521.34±147.02	538.67±153.89	17.33 (-60.46, 95.10)	0.66	549.18±143.66	10.51 (-18.87, 39.89)	0.47

Values represented as mean ± SD. The unit of values was Kpa. Abbreviations: SD – Standard Deviation, CI –Confidence Intervals.

PPT comparison of non-acupoint

Figure 2 showed PPT at the non-acupoint. For clinical patients, the analysis revealed no obvious difference of 17.33 (95% CI, -60.46 to 95.10; p = 0.66) in PPT value on the painful side compared to the non-painful side. Meanwhile, no significant difference of PPT level, which behaved 10.51 (95% CI, -18.87 to 39.89; p = 0.47), was found between the non-painful side of clinical patients and the ipsilateral side of healthy participants.

Peripheral Sensitization Index

All measured acupoints demonstrated side-to-side difference in clinical patients. Then they were used to compute peripheral sensitization index (PSI). PPT values at acupoints on painful side below the 25th percentile of the non-painful side would indicate peripheral sensitization. The ratios of clinical patients with peripheral sensitization were 77% at Tianzong (SI 11), 37% at Jianliao (SJ 14), 13% at Jianyu (LI 15) and 60% at Jianzhen (SI 9) (Table 3). Significant correlation (Appendix Table 2) was observed in PSI among Jianliao (SJ 14), Jianyu (LI 15) and Jianzhen (SI 9) ($p < 0.01$). Correlation between PSI and sex at all the pressure pain assessment acupoints approached statistical significance ($p < 0.01$), and there was no significant association between PSI and other baseline variables (Appendix Table 3).

Table 3. Frequencies meeting the peripheral sensitization index (PSI) and central sensitization index (CSI) based on PPT response.

Sites	PSI	CSI	PSI×CSI
Tianzong (SI11)	23 (77%)	13 (43%)	13 (43%)
Jianliao (SJ 14)	11 (37%)	17 (57%)	9 (30%)
Jianyu (LI 15)	13 (43%)	19 (63%)	12 (40%)
Jianzhen (SI 9)	18 (60%)	18 (60%)	15 (50%)

Values are individual counts (percentages). Abbreviations: PPT – Pressure-pain threshold.

Central Sensitization Index

Four measured acupoints demonstrated difference between the painful side of clinical patients and the ipsilateral side of healthy participants. Therefore, they used to compute central

sensitization index (CSI). The responses of clinical patients were 43% at Tianzong (SI 11), 57% at Jianliao (SJ 14), 63% at Jianyu (LI 15) and 60% at Jianzhen (SI 9) (Table 3). Meanwhile, distinct significant association (Appendix Table 4) was observed among Jianliao (SJ 14), Jianyu (LI 15) and Jianzhen (SI 9) in CSI ($p < 0.01$). Appendix Table 5 showed that correlations between CSI and sex at Tianzong (SI 11), Jianliao (SJ 14), Jianyu (LI 15), and Jianzhen (SI 9) approached statistical significance ($p < 0.01$).

Comparison of Peripheral and Central Sensitization Index

The frequencies of clinical patients who had both PSI and CSI were 43% at Tianzong (SI 11), 30% at Jianliao (SJ 14), 40% at Jianyu (LI 15) and 50% at Jianzhen (SI 9), respectively (Table 3). Significant correlation was observed between PSI and CSI in all the pressure pain assessment acupoints (Appendix Table 6). Table 4 showed that association between subgroups and sex approached statistical significance at the assessment acupoints ($p < 0.001$). No significant association was observed between subgroups and other baseline variable ($p > 0.05$).

Table. 4 Demographic, clinical, and psychological characteristics of sensitization groups.

	Peripheral Sensitization	Central Sensitization	Peripheral and Central Sensitization	No Sensitization	P value
Tianzong (SI11)					
Age (years)	48.78 (43.45, 54.21)	50.38 (44.02, 56.75)	50.38 (44.02, 56.75)	56.57 (48.00, 65.15)	0.47
Sex (%females)	78.28 (56.14, 92.52)	76.92 (46.16, 94.89)	76.92 (46.16, 94.89)	14.29 (3.68, 57.62)	<0.001
Pain duration (weeks)	21.04 (12.91, 29.17)	24.31 (10.23, 38.38)	24.31 (10.23, 38.38)	12.57 (7.16, 17.99)	0.60
VAS	68.04 (61.25, 74.84)	71.15 (62.77, 79.53)	71.15 (62.77, 79.53)	60.00 (52.45, 67.55)	0.34
Jianliao (SJ 14)					
Age (years)	48.09 (38.47, 57.71)	49.59 (43.35, 55.82)	48.89 (38.97, 58.81)	53.27 (46.22, 60.33)	0.78
Sex (%females)	100	82.35 (56.42, 96.62)	100	27.27 (6.01, 60.95)	<0.001
Pain duration (weeks)	22.55 (10.81, 34.28)	23.29 (12.80, 33.79)	22.22 (7.96, 36.49)	11.64 (7.07, 16.21)	0.32
VAS	70.00 (59.17, 80.83)	68.82 (61.14, 76.51)	72.22 (59.60, 84.84)	63.18 (53.41, 72.95)	0.59
Jianyu (LI 15)					
Age (years)	46.92 (38.29, 55.56)	47.47 (41.20, 53.75)	45.42 (36.64, 54.19)	55.10 (48.95, 61.25)	0.32
Sex (%females)	92.31 (63.76, 99.81)	89.47 (66.90, 98.70)	100	20.00 (2.54, 55.41)	<0.001
Pain duration (weeks)	19.69 (9.51, 29.87)	21.89 (12.14, 31.65)	20.67 (9.73, 31.60)	14.80 (9.23, 20.37)	0.76
VAS	68.46 (59.62, 77.30)	66.84 (59.65, 74.04)	66.67 (57.96, 75.38)	62.50 (53.38, 71.62)	0.79
Jianzhen (SI 9)					
Age (years)	48.22 (41.53, 54.91)	48.00 (41.35, 54.65)	46.27 (38.65, 53.88)	53.33 (45.49, 61.17)	0.64
Sex (%females)	83.33 (58.57, 96.44)	83.33 (58.57, 96.44)	93.33 (67.97, 99.83)	33.33 (7.45, 70.06)	<0.001
Pain duration (weeks)	17.11 (9.58, 24.64)	18.22 (10.77, 25.67)	18.67 (9.70, 27.63)	24.00 (6.74, 41.26)	0.78
VAS	67.78 (60.43, 75.13)	66.67 (58.85, 74.48)	68.00 (59.32, 76.68)	65.00 (53.79, 76.21)	0.97

Values presented as % (for sex) or mean with [95% CI]. Abbreviations: VAS – Visual Analogue Acale.

DISCUSSION

The current results were in agreement with a peripherally sensitized state at acupoints which is determined by the side-to-side difference of PPT values in clinical patients with unilateral shoulder pain. Central sensitization at acupoints was conducted by comparing pressure sensitivity in patients with unilateral shoulder pain with healthy age- and sex-matched participants. No obvious PPT values difference was found at the non-acupoint among the painful side, non-painful side and ipsilateral side. To advance this line of research, association between peripheral and central sensitization at acupoints was examined in this paper. We found that individuals behaved significant association between peripheral and central sensitization at acupoints. This finding demonstrated that there existed two pattern of sensitization in acupoints. In addition, three acupoints Jianliao (SJ 14), Jianyu (LI 15) and Jianzhen (SI 9) that normally be used for treating shoulder pain had correlation.

Previous investigations had reported that splanchnic diseases can induce mechanical hyperalgesia on the corresponding acupoints when pressed [17]. Acupoints turned to the “activated mode” or “sensitized mode” from the “silent mode” in pathological condition. This phenomenon is called “acupoint sensitization”. Some acupoints appeared the hypersensitivity of temperature (heat-sensitization) or pain threshold (pain-sensitization) under visceral pain [8, 18]. But unlike those studies, we examined acupoints in patients with musculoskeletal pain in this study. The contribution of this study proves the existence of peripheral and central sensitization at acupoints under musculoskeletal pain, and manifests the pain sensitivity of acupoints are dynamic, by comparing the clinical patients with healthy participants.

Collectively, the findings of the current paper were to support the alteration in both peripheral and central sensitization at acupoints in patients with musculoskeletal pain. To determine whether peripheral and central sensitization were more likely to occur together or alone, we used sensitization indexes to explore the phenomenon. The results demonstrated the PSI and CSI presented association at all the pressure pain assessment acupoints. In PSI or CSI, a strong association was found at three acupoints of Jianliao (SJ 14), Jianyu (LI 15) and Jianzhen (SI 9). No distinct association was found between Tianzong (SI 11) and other acupoints by comparing PSI or CSI. From the clinical perspective, Jianliao (SJ 14), Jianyu (LI 15) and Jianzhen (SI 9) were so frequent to be choose to treat shoulder pain in clinical practice, that they were named

"shoulder three acupoints" by acupuncturists [19, 20]. According to classical acupuncture theory, it is believed that the shoulder pain with limited movement of lifting, abduction and backward extension was mainly caused by the disorder of the Large Intestine Channel and the Small Intestine Channel. Jianyu (LI 15), Jianliao (SJ 14) and Jianzhen (SI 9), are highly-refined acupoints to treat shoulder pain in the clinic, and have been proved to be effective to regulate muscle strength and tension of shoulder joint [21]. The result of the strong association among three acupoints of Jianliao (SJ 14), Jianyu (LI 15) and Jianzhen (SI 9) is consistent with the conception of traditional Chinese medicine and proved evidence for the clinical experience.

In result, we found peripheral and central sensitization had association with sex rather than age, pain duration or VAS. Gender differences in pain has become an increased topic in recent years, and lower PPT value in women than in men was found either in healthy subjects or clinical patients [22]. Our study also provides evidence for gender differences of lower PPT in women at acupoints. Specifically, the result indicates that peripheral and central sensitization at acupoints is not relevant to pain duration, and suggests that peripheral sensitization is not a prerequisite for the presence of central sensitization at acupoints. Moreover, there is no obvious evidence that the pattern of sensitization has relation with the degree of severity of the clinical condition as measured by VAS. These changes are consistent with the idea of traditional Chinese medicine that acupoints are the crucial reflex points of body lesions under pathological circumstance. Hyperalgesia and skin sensitization can occur at corresponding acupoints when under some diseases [23]. Morphological structure studies have reported that the nervous system and blood vessels might have a close relationship with acupoints [24-26]. For example, abundant microvessels existed at the acupoints of Zhongji (RN3) and Zusanli (ST36) in contrast to the surrounding tissues [27]. The acupoints also have a high density of nerve endings including A- and C- afferent fibers [28, 29]. Those characteristics of higher concentration of neural, vascular elements and mast cells could make pain perception more sensitive, and might contribute to peripheral sensitivity. To confirm the specificity of acupoints, we selected a non-acupoint in the infraspinatus muscle. Obviously, no significant difference was found in PPT values among the painful/ non-painful side of clinical patients with unilateral shoulder pain and the ipsilateral side of healthy participants. The finding proved that acupoints become a specific reflex point that under musculoskeletal pain.

There are limitations that should be kept in mind in this research. Pain perception is a multidimensional feature. PPT measurement is just a mechanical and standardized stimulation and inadequate to describe the complexity of pain perception. The multimodal approach should be used to provide the details of the pain system in both normal and pathophysiological situations, such as different stimulus modalities and quantitative assessment of various pain mechanisms. Second, our study is a small sample. It may be appropriate that larger samples provide further information about acupoints sensitivity. Third, the evaluators may not be blinded in the group practicely, although we demand participants not to tell the evaluators which side is the pain side in shoulder. Further studies need to be conducted to confirm the phenomena observed here.

CONCLUSIONS

In conclusion, there exists mixed presence of sensitization patterns at acupoints in patients with unilateral shoulder pain and a strong correlation among Jianliao (SJ 14), Jianyu (LI 15) and Jianzhen (SI 9). Future research of multimodal pain approach should be conducted, such as suprathreshold heat pain response, to determine various sensitivity mechanisms.

Legends

Figure 1. The pressure pain assessment sites in the present study. (A) Locations for the pressure pain threshold (PPT) measurement at Jianliao (SJ 14), Jianyu (LI 15) and Jianzhen (SI 9). (B) Locations for the PPT measurement at Tianzong (SI 11) and Non-acupoint.

Figure 2. The values of pressure pain threshold (PPT) at the non-acupoint. Values are mean ± SD.

Footnotes

Contributors Conceived and designed the experiments: Qian-Qian Li, Cun-Zhi Liu. Performed the experiments: Guang-Xia Shi, Qing-Nan Fu, Chao-Qun Yan, Shuai Zhang. Analyzed the data: Li-Wen Zhang, Xue-Rui Wang. Wrote the paper: Chao-Qun Yan, Qian-Qian Li. All authors approved the final manuscript.

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Patient consent Obtained.

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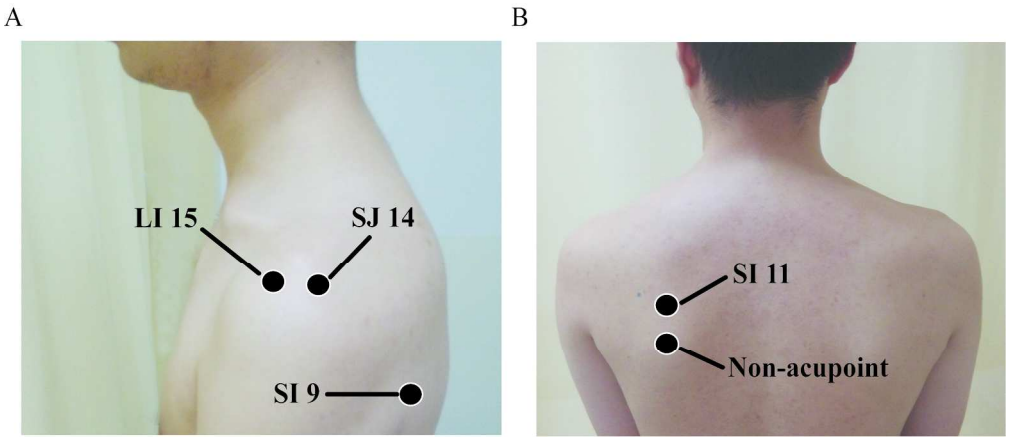

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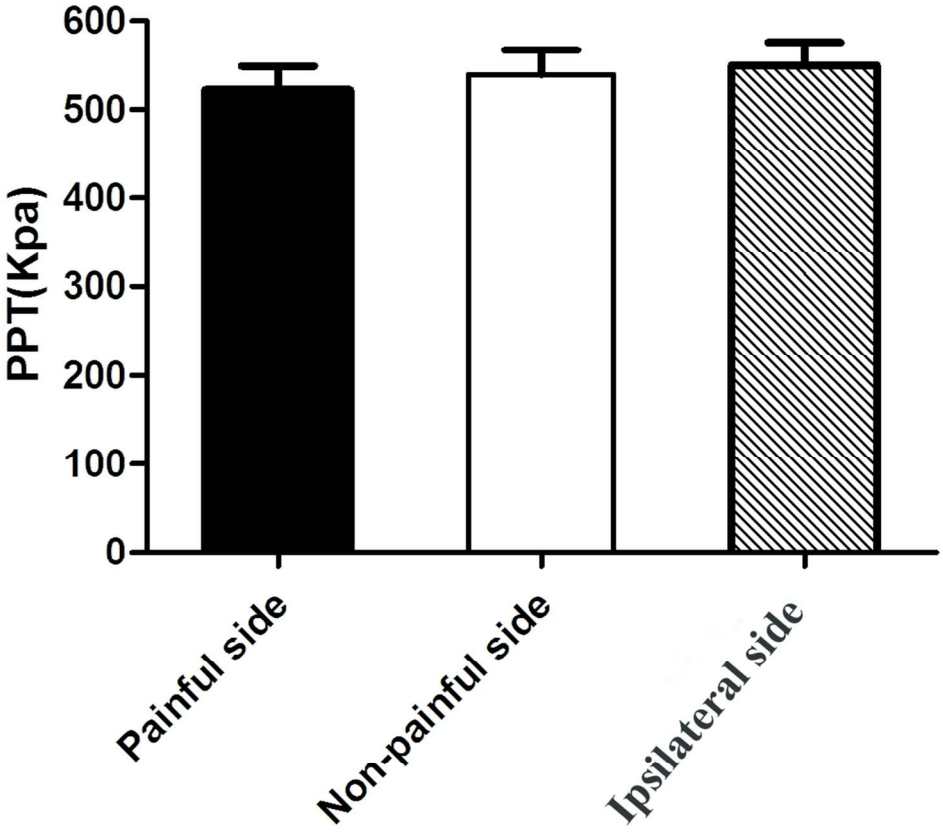

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Appendix Table 1. Locations for the pressure pain assessment sites in the present study.

Points	Location
Jianliao (SJ 14)	In the depression posterior and inferior to the acromion when arm is abducted.
Jianyu (LI 15)	In the depression between the acromial extremity of the clavicle and the great tuberosity of humerus; or when the arm is in full abduction, the acupoint is in the depression at the anterior border of the acromioclavicular joint, and superior to the shoulder joint.
Jianzhen (SI 9)	1 cun directly above the posterior end of the axillary fold when the arm is abducted.
Tianzong (SI 11)	In the depression in the center of the subscapular fossa, at the point 1/3 of the line between the lower border of the mesoscapula and lower angle of capula.
Non-acupoint	2 cm down to Tianzong (SI 11).

Appendix Table 2. Correlation of peripheral sensitization index (PSI) among acupoints.

		PSI			
		Tianzong (SI11)	Jianliao (SJ 14)	Jianyu (LI 15)	Jianzhen (SI 9)
PSI	Tianzong (SI11)	1.00	0.26	0.32	0.35
	Jianliao (SJ 14)	0.26	1.00	0.73*	0.62*
	Jianyu (LI 15)	0.32	0.73*	1.00	0.25
	Jianzhen (SI 9)	0.35	0.62*	0.44*	1.00

Values are Pearson's correlation. *Significant association between variables ($p < .01$).

Appendix Table 3. Correlation between peripheral sensitization indexes (PSI) and relevant baseline characteristics.

	PSI			
	Tianzong (SI 11)	Jianliao (SJ 14)	Jianyu (LI 15)	Jianzhen (SI 9)
Age (years)	-0.28	-0.13	-0.21	-0.22
Sex (%females)	-0.56*	-0.58*	-0.53*	-0.51*
Pain duration (weeks)	0.21	0.20	-0.10	-0.20
VAS	0.24	0.16	0.27	-0.12

Values are Pearson's correlation. *Significant association between variables ($p < 0.01$).

Appendix Table 4. Correlation of central sensitization index (CSI) among acupoints.

		CSI			
		Tianzong (SI11)	Jianliao (SJ 14)	Jianyu (LI 15)	Jianzhen (SI 9)
CSI	Tianzong (SI11)	1.00	0.86	0.11	0.17
	Jianliao (SJ 14)	0.09	1.00	0.59*	0.25
	Jianyu (LI 15)	0.11	0.59*	1.00	0.51*
	Jianzhen (SI 9)	0.17	0.25	0.51*	1.00

Values are Pearson's correlation. *Significant association between variables ($p < 0.01$).

Appendix Table 5. Correlation between central sensitization index (CSI) and relevant baseline characteristics.

	CSI			
	Tianzong (SI 11)	Jianliao (SJ 14)	Jianyu (LI 15)	Jianzhen (SI 9)
Age (years)	-0.10	-0.12	-0.32	-0.24
Sex (%females)	-0.25	-0.45*	-0.71*	-0.51*
Pain duration (weeks)	0.13	0.29	-0.16	-0.05
VAS	0.33	0.21	0.04	0.01

Values are Pearson's correlation. *Significant association between variables ($p < 0.01$).

Appendix Table 6. Correlation between peripheral sensitization index (PSI) and central sensitization index (CSI).

		CSI			
		Tianzong (SI11)	Jianliao (SJ 14)	Jianyu (LI 15)	Jianzhen (SI 9)
PSI	Tianzong (SI11)	0.48*	0.15	0.40*	0.52*
	Jianliao (SJ 14)	0.17	3.9*	0.58*	0.62*
	Jianyu (LI 15)	0.32	0.36	0.53*	0.58*
	Jianzhen (SI 9)	0.17	0.25	0.65*	0.58*

Values are Pearson's correlation. *Significant association between variables ($p < 0.01$).

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The existence of peripheral and central sensitization at acupoints in patients with unilateral shoulder pain: a cross-sectional matched case-control study

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The existence of peripheral and central sensitization at acupoints in patients with unilateral shoulder pain: a cross-sectional matched case-control study

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Abstract

Objective—To investigate the pattern of experimental pain responses at acupoints in patients with unilateral shoulder pain.

Design— A cross-sectional matched case-control study.

Setting—Acupuncture and Moxibustion Department, Beijing Hospital of Traditional Chinese Medicine Affiliated to Capital Medical University.

Participants—Volunteer sample of 60 participants (30 patients with unilateral shoulder pain, 30 healthy controls).

Interventions—Not applicable.

Main Outcome Measures—Pressure pain thresholds (PPTs) were measured at four acupoints, including Tianzong (SI 11), Jianliao (SJ 14), Jianyu (LI 15) and Jianzhen (SI 9) on the painful/non-painful side in patients with unilateral shoulder pain or healthy controls, respectively. The correlations between peripheral sensitization indexes (PSI) and central sensitization indexes (CSI) were compared in this study.

Results—The analysis revealed significantly lower PPT values at acupoints on the painful side compared with non-painful side in patients with shoulder pain ($P < 0.025$). Meanwhile, PPTs on the non-painful side of these patients were lower than the ipsilateral side of healthy controls ($P < 0.025$). No distinct differences of PPT values were found at non-acupoint among the painful/non-painful side in patients with shoulder pain and the ipsilateral side of healthy controls ($P > 0.05$). Additionally, it was observed that the pressure pain assessment acupoints have strong association between the PSI and CSI, and three acupoints of SJ 14, LI 15 and SI 9 showed correlation in PSI or CSI particularly.

Conclusion—The results suggest the presence of peripheral and central sensitization at acupoints in participants with unilateral shoulder pain. There exists an obvious relationship among the three acupoints of SJ 14, LI 15 and SI 9, which are usually chosen to treat shoulder pain. The results provide the evidence for acupoints selection to treat shoulder pain by acupuncture.

Keywords: acupoints; peripheral sensitization; central sensitization; pressure pain threshold; shoulder pain

Strengths and limitations of this study

This is, to the best of our knowledge, the first study to support an alteration in both peripheral and central sensitization at acupoints in patients with shoulder pain.

The peripheral and central sensitization at acupoints seemed to occur correlatively independently from the relevant baseline variable.

A limitation of this study was that only a pressure pain assessment performed at acupoints, which might be inadequate to describe the complexity of pain perception.

This study was conducted in a single institution and consisted of a primarily female sample, so the external validity was not clear.

INTRODUCTION

Acupuncture is one of the most widely-used form of complementary and alternative medicines. There are about 183 countries using acupuncture treatment according to a survey conducted by the World Federation of Acupuncture and Moxibustion Societies in 2013 [1]. The WHO reports that acupuncture treatment can be beneficial for more than 40 disorders [2]. Pain is one of those disorders, which is particularly amenable to acupuncture. The evidence in reviews demonstrates that acupuncture treatment can reduce various types of clinical pain effectively in multiple clinical trials [3-5]. Recently, a meta-analysis reported that shoulder pain, low back pain, neck pain, osteoarthritis and chronic headache can be alleviated significantly after acupuncture treatment [6]. Shoulder pain is the third most common musculoskeletal disorder, of which the prevalence rate varies from 6.9% to 26% for point prevalence and even increases to 66.7% for lifetime prevalence in general population [7, 8]. In our previous study, we identified acupuncture treatment can alleviate shoulder pain, particularly in special acupoints (data unpublished).

Acupoints are special sites at precise locations and lie on ‘meridians’ without a physical structure. According to the theory of meridians in traditional Chinese medicine, it is well known that the acupoints reflect disorders of visceral conditions. Many studies have observed visceral lesions result in changed pain perception of some special acupoints on the body surface [9]. Ben

et al assessed the phenomenon of experimental pain-sensitive points in patients with gastric ulcer or gastritis and found some special acupoints were more sensitive than other areas [10]. Nevertheless, whether acupoints behave more sensitively under musculoskeletal pain has not been described in detail in previous studies.

Sensitization is a nervous system phenomenon that can occur in conjunction with pain [11]. When sensitization is present, there are two types of hypersensitivity including peripheral sensitization and central sensitization, which are important mechanisms for musculoskeletal pain conditions [12]. Peripheral sensitization is defined as the broadening of nociception in pain perception during activities or movements, which typically would be not painful [11]. Central sensitization refers to an amplification of neural signaling within the central nervous system that elicits pain hypersensitivity. It reflects increased activity of pain facilitation pathways and malfunctioning of descending pain inhibitory pathways [13-17].

We performed a multicenter, single blind, factorial randomized controlled clinical trial previously (Number Register: ISRCTN61861069). In the study, we found the phenomenon that the pain threshold decreased at related acupoints in patients with unilateral shoulder pain was widespread. Therefore, we chose some patients from the trial to detect whether acupoints are hypersensitive in musculoskeletal pain disease. We hypothesized that the patients with unilateral shoulder pain would present peripheral or central sensitization at acupoints as evidenced by Pressure pain threshold (PPT) detection, when compared with healthy controls. Specifically, acupoints on the painful side would have lower PPT than on the non-painful side of patients. Also, acupoints on the non-painful side of patients would be hypersensitive as assessed by PPTs, with respect to the ipsilateral side of healthy controls. If the results confirm these hypotheses, then it provides evidence that acupoints exist for peripheral sensitization and central sensitization phenomenon in patients with unilateral shoulder pain.

MEDTHODS AND ANALYSIS

Study design

This cross-sectional matched case-control study was conducted at the Department of Acupuncture and Moxibustion, Beijing Hospital of Traditional Chinese Medicine Affiliated to Capital Medical University.

Participants

Clinical patients—The 30 patients with shoulder pain were chosen from a multicenter, randomized trial. In total, 164 patients with shoulder pain were recruited from three centers in the random trial between January 2014 and September 2014. We enrolled the final 30 patients from 76 patients that screened in the center of Beijing Hospital of Traditional Chinese Medicine before any treatments were dispensed. The Research Ethical Committee of Beijing Hospital of Traditional Chinese Medicine Affiliated to Capital Medical University approved the trial (reference: 201315).

The inclusion criteria included presenting with unilateral shoulder pain for at least six weeks and up to two years, reporting the pain intensity > 50 mm on a visual analogue scale (VAS), and being right hand dominant, who have not received acupuncture or other analgesic therapies in the preceding month.

The exclusion criteria were as follows: pain in both shoulders; referred pain from the cervical spine; previous history of shoulder surgery, pectorial muscle pain, thoracic outlet syndrome, stroke or ipsilateral breast surgery; heart diseases and severe hypertension; osteoarthritis of the glenohumeral joint or systemic bone and joint disorder (rheumatoid arthritis); endocrine diseases such as hyperthyroidism; severe infection; undergoing current therapy involving analgesics and especially major illness depression.

Healthy controls—The healthy controls were matched to the patients with shoulder pain individually. Each healthy control was matched for gender, age (±1 year), ethnicity and dominant hand to one patient. Healthy controls were recruited from the community via posted flyers and general advertisements between May 2014 and September 2014. Healthy controls were eligible if they were not currently performing resistance exercise for the upper extremity. They were excluded based on the following criteria: receiving acupuncture or other analgesic therapies in the preceding month, experiencing neck or shoulder pain, having a history of shoulder surgery or neurological impairments of the upper extremity, a shoulder skin infection, having difficulty in understanding instructions, and taking any pain medication currently.

Protocol

All participants completed a packet of questionnaires including demographic data (age, sex, race, hand dominance) and psychological information before informed consent was obtained. The patients were initially examined by a researcher who assessed compliance using the inclusion and exclusion criteria. Beck Depression Inventory (BDI) is a multiple choice self-reported inventory for measuring the severity of depression [18]. If the BDI outcome is more than 4, the participants were excluded [19]. Clinical pain intensity was assessed by VAS, which consists a line of 0–100 mm: 0 representing 'no pain at all' and 100 mm representing 'the most intense pain imaginable'.

After finishing the questionnaires, both shoulders of participants were exposed and the measurement sites were marked. An expert acupuncturist was responsible for the operation. Five points were marked in this study and shown in Figure 1 and Appendix Table 1. To assess the pain sensitivity of acupoints, four acupoints Jianliao (SJ 14), Jianyu (LI 15), Jianzhen (SI 9), and Tianzong (SI 11) were marked bilaterally with a marker pen drawing a circle (Figure 1. A. B). To assess the pain sensitivity in the non-acupoint, 2 cm down to Tianzong (SI 11) was marked bilaterally in participants (Figure 1. B).

Measurement of pressure pain thresholds (PPT)

Both shoulders were exposed for examination by an operator. The participants were asked to take a prone position on the examination bed with a suitable pillow under the chest when Jianzhen (SI 9), Tianzong (SI 11) and non-acupoint were measured. Then the participants were required to sit on a chair with a researcher to keep the arm and shoulder in parallel when Jianliao (SJ 14) and Jianyu (LI 15) were measured.

PPT is widely used to evaluate the pain sensory threshold for the patients with shoulder pain [20]. The lower value of PPT indicates the decreased nociceptive threshold of pain perception [21]. A handheld electronic pressure algometer (Wagner Instruments, Greenwich, CT) mounted with a 1 cm^2 rubber tipped plunger was used in this study. The probe was held perpendicular to marked points bilaterally, and the pressure was increased at a rate of 30kPa/s, in order to avoid potential skin penetration. All participants were instructed to indicate when the pressure became painful, at that time the pressure was immediately stopped and the digits were recorded. PPT was calculated as the mean of three trials on each point. There was an approximately 2 min interval between the repetitions. The operator was not to be told which side is the painful shoulder of

patients and blinded to patients and healthy controls. Additionally, the operator did not have any basic knowledge of acupoints and did not know whether the measuring sites were acupoints or not during testing.

Data Analysis

The data were double entered with an adequate check in EpiData. SPSS 17.0 software (SPSS Inc., Chicago, IL, USA) was used for analysis. Discrete variables were summarized by frequencies and percentages. Distributed data were summarized using mean ± standard deviation (SD) or median and interquartile range (IRQ). The distributed data were analyzed using a parametric statistical test (Pair t-test) if it agreed with normal distribution. Otherwise, the data were analyzed using a non-parametric (Wilcoxon's signed rank test) statistical test. Shapiro-Wilk test and observation of histograms and normal probability plots were used for all study variables to determine whether they followed a normal distribution. To adjust multiple comparisons, an alpha level of 0.025 was used for all pairwise comparisons.

Peripheral and central sensitization index was used to determine whether pain-sensitivity existed at the pressure pain assessment sites. We examined a patient's ratio response for PPT in painful side fell below the 25th percentile among the non-painful side which would indicate peripheral sensitization. The ratio responses were referred as the peripheral sensitization index (PSI) [22]. Central sensitization indicates a patient's response for PPT in non-painful side fell below the 25th percentile among the ipsilateral side of healthy controls. The ratio responses were termed the central sensitization index (CSI). The 25th percentile was suggested as a lower limit reference value for enhanced sensitivity [23]. Each index was examined by Pearson's correlation. We analyzed association between sensitization subgroups and the relevant baseline characteristics including demographic and clinical variables. Comparisons among the variables were examined using one way analysis of variance or Chi-square and assessment of 95% confidence intervals (CI). A *P* value <0.05 was defined as statistically significant.

RESULTS

Recruitment and baseline characteristics

Between January 2014 and September 2014, thirty patients with shoulder pain (11 males and 19 females) were enrolled in this study. The average age of the thirty patients was 50.60 ± 12.19

years (Table 1). The healthy controls included 11 males and 19 females with a mean age of 50.63 ± 12.20 years. All participants were right hand dominant.

Table. 1 Characteristics of the patients with shoulder pain and healthy controls.

	Patients With Shoulder Pain (N = 30)	Healthy Controls (N = 30)
Age (years)	50.60 ± 12.19	50.63±12.20
Sex (females), n (%)	19 (63.33)	19 (63.33)
Race (number of Han), n (%)	30 (100)	30 (100)
Pain duration (weeks)	19.07 ± 16.99	-
VAS (median, IQR)	70 (50 - 80)	-
BDI (median, IQR)	0 (0 - 0)	0 (0 - 0)
BMI	25.27 ± 4.17	24.91 ± 3.77
Normal (≤ 23.9)	11	12
Overweight (24 - 27.9)	12	14
Obese (≥ 28)	7	4

Values represented as N, mean ± SD or median, IQR.

Abbreviations: N – Number, SD – standard deviation, VAS – Visual Analogue Scale, BDI– Beck Depression Inventory, BMI – Body Mass Index, IQR – interquartile range.

PPT comparison of related acupoints

For the patients with shoulder pain, lower PPT levels were detected at acupoints on the painful side compared with the non-painful side (Table 2). The differences were 94.9 (95% CI, 53.47 to 136.33; $p < 0.001$) at Tianzong (SI 11), 102.91 (95% CI, 79.85 to 125.97; $p < 0.001$) at Jianliao (SJ 14), 91.19 (95% CI, 44.82 to 137.56; $p < 0.001$) at Jianyu (LI 15), and 86.79 (95% CI, 56.30 to 117.28; $p < 0.001$) at Jianzhen (SI 9), respectively.

The PPT value difference between the non-painful side of the patients and the ipsilateral side of healthy controls was also compared. PPTs at the acupoints on non-painful side of the patients were lower than on the ipsilateral side of healthy controls. The differences were 57.71 (95% CI, 24.72 to 90.70; $p = 0.001$) at Tianzong (SI 11), 81.03 (95% CI, 41.40 to 120.67; $p < 0.001$) at Jianliao (SJ 14), 72.20 (95% CI, 10.90 to 133.50; $p = 0.02$) at Jianyu (LI 15), and 88.09 (95% CI, 39.88 to 136.30; $p = 0.001$) at Jianzhen (SI 9), respectively.

Table. 2 Pressure pain threshold values for participants

	Patients With Shoulder Pain		Painful vs Non-painful Side		Health Controls	Non-painful Side vs Ipsilateral Side	
	Painful Side	**Non-painful Side**	**Mean (95% CI)**	**P value**	**Ipsilateral Side**	**Mean (95% CI)**	**P value**
Tianzong (SI 11)	414.83 ± 135.61	509.73 ± 168.05	94.90 (53.47, 136.33)	<0.001	567.44 ± 153.84	57.71 (24.72, 90.70)	0.001
Jianliao (SJ 14)	469.70 ± 181.07	572.61 ± 196.71	102.91 (79.85, 125.97)	<0.001	653.64 ± 211.50	81.03 (41.40, 120.67)	<0.001
Jianyu (LI 15)	434.79 ± 140.39	525.98 ± 160.16	91.19 (44.82, 137.56)	<0.001	598.18 ± 181.76	72.20 (10.90, 133.50)	0.02
Jianzhen (SI 9)	453.08 ± 154.54	539.87 ± 167.59	86.79 (56.30, 117.28)	<0.001	627.96 ± 209.67	88.09 (39.88, 136.30)	0.001
Non-acupoints	521.34 ± 147.02	538.67 ± 153.89	17.33 (-36.99, 2.34)	0.08	549.18 ± 143.66	10.51 (-18.87, 39.89)	0.47

Values represented as mean ± SD. The unit of values was Kpa. Abbreviations: SD – Standard Deviation, CI –Confidence Intervals.

PPT comparison of non-acupoint

Figure 2 showed PPT at the non-acupoint. For the patients, the analysis revealed no obvious difference of 17.33 (95% CI, -36.99 to 2.34; p = 0.08) in PPT value on the painful side compared to the non-painful side. Meanwhile, no significant difference of PPT level, which behaved 10.51 (95% CI, -18.87 to 39.89; p = 0.47), was found between the non-painful side of the patients and the ipsilateral side of healthy controls.

Peripheral Sensitization Index

All measured acupoints demonstrated side-to-side difference in the patients. They were used to compute a peripheral sensitization index (PSI). PPT values at acupoints on the painful side below the 25th percentile of the non-painful side indicated peripheral sensitization. The proportion of the patients with peripheral sensitization were 77% at Tianzong (SI 11), 37% at Jianliao (SJ 14), 43% at Jianyu (LI 15) and 60% at Jianzhen (SI 9) (Table 3). Significant correlation (Appendix Table 2) was observed in PSI among Jianliao (SJ 14), Jianyu (LI 15) and Jianzhen (SI 9) ($p < 0.01$). There was no significant association between PSI and other baseline variables (Appendix Table 3).

Table 3. Frequencies meeting the peripheral sensitization index (PSI) and central sensitization index (CSI) based on PPT response.

Sites	PSI	CSI	PSI×CSI
Tianzong (SI11)	23 (77)	13 (43)	13 (43)
Jianliao (SJ 14)	11 (37)	17 (57)	9 (30)
Jianyu (LI 15)	13 (43)	19 (63)	12 (40)
Jianzhen (SI 9)	18 (60)	18 (60)	15 (50)

Values are individual counts (percentages). Abbreviations: PPT – Pressure-pain threshold.

Central Sensitization Index

Four measured acupoints demonstrated difference between the painful side of patients and the ipsilateral side of healthy controls. Therefore, they were used to compute central sensitization index (CSI). The proportion of the patients were 43% at Tianzong (SI 11), 57% at Jianliao (SJ

14), 63% at Jianyu (LI 15) and 60% at Jianzhen (SI 9) (Table 3). Meanwhile, distinct significant association (Appendix Table 4) was observed among Jianliao (SJ 14), Jianyu (LI 15) and Jianzhen (SI 9) in CSI ($p < 0.01$). Appendix Table 5 showed no statistical significance correlations between CSI and relevant baseline characteristics ($p < 0.01$).

Comparison of Peripheral and Central Sensitization Index

The frequencies of the patients who had both PSI and CSI were 43% at Tianzong (SI 11), 30% at Jianliao (SJ 14), 40% at Jianyu (LI 15) and 50% at Jianzhen (SI 9), respectively (Table 3). Significant correlation was observed between PSI and CSI in measured acupoints (Appendix Table 6). Table 4 showed that association between subgroups and sex approached statistical significance at the assessment acupoints ($p < 0.001$). No significant association was observed between subgroups and other baseline variable ($p > 0.05$).

Table. 4 Demographic, clinical, and psychological characteristics of sensitization groups.

	Peripheral Sensitization	Central Sensitization	Peripheral and Central Sensitization	No Sensitization	P value
Tianzong (SI11)					
Age (years)	48.78 (43.45, 54.21)	50.38 (44.02, 56.75)	50.38 (44.02, 56.75)	56.57 (48.00, 65.15)	0.47
Sex (%females)	78.28 (56.14, 92.52)	76.92 (46.16, 94.89)	76.92 (46.16, 94.89)	14.29 (3.68, 57.62)	<0.001
Pain duration (weeks)	21.04 (12.91, 29.17)	24.31 (10.23, 38.38)	24.31 (10.23, 38.38)	12.57 (7.16, 17.99)	0.60
VAS	68.04 (61.25, 74.84)	71.15 (62.77, 79.53)	71.15 (62.77, 79.53)	60.00 (52.45, 67.55)	0.34
Jianliao (SJ 14)					
Age (years)	48.09 (38.47, 57.71)	49.59 (43.35, 55.82)	48.89 (38.97, 58.81)	53.27 (46.22, 60.33)	0.78
Sex (%females)	100	82.35 (56.42, 96.62)	100	27.27 (6.01, 60.95)	<0.001
Pain duration (weeks)	22.55 (10.81, 34.28)	23.29 (12.80, 33.79)	22.22 (7.96, 36.49)	11.64 (7.07, 16.21)	0.32
VAS	70.00 (59.17, 80.83)	68.82 (61.14, 76.51)	72.22 (59.60, 84.84)	63.18 (53.41, 72.95)	0.59
Jianyu (LI 15)					
Age (years)	46.92 (38.29, 55.56)	47.47 (41.20, 53.75)	45.42 (36.64, 54.19)	55.10 (48.95, 61.25)	0.32
Sex (%females)	92.31 (63.76, 99.81)	89.47 (66.90, 98.70)	100	20.00 (2.54, 55.41)	<0.001
Pain duration (weeks)	19.69 (9.51, 29.87)	21.89 (12.14, 31.65)	20.67 (9.73, 31.60)	14.80 (9.23, 20.37)	0.76
VAS	68.46 (59.62, 77.30)	66.84 (59.65, 74.04)	66.67 (57.96, 75.38)	62.50 (53.38, 71.62)	0.79
Jianzhen (SI 9)					
Age (years)	48.22 (41.53, 54.91)	48.00 (41.35, 54.65)	46.27 (38.65, 53.88)	53.33 (45.49, 61.17)	0.64
Sex (%females)	83.33 (58.57, 96.44)	83.33 (58.57, 96.44)	93.33 (67.97, 99.83)	33.33 (7.45, 70.06)	<0.001
Pain duration (weeks)	17.11 (9.58, 24.64)	18.22 (10.77, 25.67)	18.67 (9.70, 27.63)	24.00 (6.74, 41.26)	0.78
VAS	67.78 (60.43, 75.13)	66.67 (58.85, 74.48)	68.00 (59.32, 76.68)	65.00 (53.79, 76.21)	0.97

Values presented as % (for sex) or mean with [95% CI]. Abbreviations: VAS – Visual Analogue Acale.

DISCUSSION

The current results were in agreement with a peripherally sensitized state at acupoints which is determined by the side-to-side difference of PPT values in patients with unilateral shoulder pain. Central sensitization at acupoints was conducted by comparing pressure sensitivity in patients with healthy age- and sex-matched healthy controls. No obvious PPT values difference was found at the non-acupoint among the painful side, non-painful side and ipsilateral side. To advance this line of research, association between peripheral and central sensitization at acupoints was examined. We found that the patients displayed a significant association between peripheral and central sensitization at measured acupoints. This finding demonstrated that there existed two patterns of sensitization in acupoints. In addition, three acupoints Jianliao (SJ 14), Jianyu (LI 15) and Jianzhen (SI 9) that are normally used for treating shoulder pain had correlation.

Previous investigations had reported that splanchnic diseases can induce mechanical hyperalgesia on the corresponding acupoints when pressed [24]. Acupoints turned to the "activated mode" or "sensitized mode" from the "silent mode" in pathological conditions. This phenomenon is called "acupoint sensitization". At some acupoints there appeared to be a hypersensitivity of temperature (heat-sensitization) or pain threshold (pain-sensitization) under visceral pain [9, 25]. But unlike those studies, we examined acupoints in patients with musculoskeletal pain in this study.

Central sensitization is challenging clinically, since no standard assessment exists. Some studies recommended the use of various modalities for pain sensitivity at local and distal locations [21, 26]. However, other researches showed that decreased PPTs at the painful and non-painful shoulder, but not at the muscle tibialis anterior [27-29]. According to "Criteria for the Classification of Central Sensitization Pain", patients with diffuse pain distribution, allodynia, and hyperalgesia are more likely to present with central sensitization. One of the patterns of pain distribution is that patients have bilateral pain/mirror pain [17]. In the patients with shoulder pain, the increased sensitivity to mechanical input in the contralateral shoulder would be interpreted as central sensitization [11]. A large number of studies define central sensitization as pain sensitivity at local and distal locations. We chose bilateral pain to define central sensitization,

unlike earlier studies, and to determine whether there existed patterns of experimental pain responses at shoulder acupoints.

Collectively, the findings of the study support the alteration in both peripheral and central sensitization at acupoints in patients with musculoskeletal pain. We determined whether peripheral and central sensitization were more likely to occur together or alone, and found peripheral sensitization was not a prerequisite for the presence of central sensitization at acupoints. The results demonstrated that PSI and CSI presented in association at all the pressure pain assessment acupoints. In PSI or CSI, the strong association was found at three acupoints of Jianliao (SJ 14), Jianyu (LI 15) and Jianzhen (SI 9). No distinct association was found between Tianzong (SI 11) and other acupoints by comparing PSI or CSI. From the clinical perspective, Jianliao (SJ 14), Jianyu (LI 15) and Jianzhen (SI 9) are frequently chosen to treat shoulder pain in clinical practice, and have been called "shoulder three acupoints" by acupuncturists [30, 31]. Jianyu (LI 15), Jianliao (SJ 14) and Jianzhen (SI 9), are highly-refined acupoints used to treat shoulder pain in the clinic, and have been proven to be effective at regulating muscle strength and tension of shoulder joint [32]. The strong association among Jianliao (SJ 14), Jianyu (LI 15) and Jianzhen (SI 9) is consistent with the conception of traditional Chinese medicine.

To our knowledge, this is the first study to research peripheral and central sensitization at acupoints in patients with shoulder pain. One of the advantages of the study is that the measured acupoints and non-acupoint were marked by an acupuncturist with 24 years of experience in clinical acupuncture treatment. The evaluator who measured PPTs also has extensive experience with using the algometer and without basic knowledge of acupoints. The internal validity is increased by blinding the evaluator who did not know whether the measured sites were acupoints or not during testing. In addition, the evaluator was blinded as to whether the test participant was a patient or a healthy control. The participants were asked to take different positions when different acupoints were measured. For example, the participants were required take a prone position on the examination bed with a suitable pillow under the chest and the arms close to the body when Jianzhen (SI 9) was measured. It increases the reliability of testing PPT over a soft area.

Specifically, the result indicates that peripheral and central sensitization at acupoints is not relevant to pain duration. Moreover, there is no obvious evidence that the pattern of sensitization

has relation with the degree of severity of the clinical condition as measured by VAS. These changes are consistent with the idea of traditional Chinese medicine that acupoints are the crucial reflex points of body lesions under pathological circumstance. Hyperalgesia and skin sensitization can occur at corresponding acupoints in the presence of some diseases [33]. Morphological structure studies have reported that the nervous system and blood vessels might have a close relationship with acupoints [34-36]. For example, abundant microvessels existed at the acupoints of Zhongji (RN3) and Zusanli (ST36) in contrast to the surrounding tissues [37]. The acupoints also have a high density of nerve endings including A- and C- afferent fibers [38, 39]. Those characteristics of higher concentration of neural, vascular elements and mast cells could make pain perception more sensitive, and might contribute to peripheral sensitivity. To confirm the specificity of acupoints, we selected a non-acupoint in the infraspinatus muscle. Obviously, no significant difference was found in PPT values among the painful/ non-painful side of patients and the ipsilateral side of healthy controls. The finding proved that acupoints become a specific reflex point that respond to the presence of musculoskeletal pain. Our study provides evidence that there is an association with acupoints sensitization and gender. Pain difference in gender has become an increased topic in recent years, and lower PPT values in women than that in men were found either in healthy subjects or in clinical patients [40]. But, the significant difference may be accounted for by the fact that the majority of the patients were female (63.33%) in the study.

There are limitations that should be kept in mind regarding this study. Pain perception is multidimensional. PPT measurements are just a mechanical and standardized stimulation and are inadequate to describe the complexity of pain perception. The multimodal approach should be used to provide the details of the pain system in both normal and pathophysiological situations, such as different stimulus modalities and quantitative assessment of various pain mechanisms. Second, the non-acupoint was chosen as 2 cm down from Tianzong (SI 11) because the shoulder blade is relatively flat and may reduce the measurement errors between acupoints and non-acupoints. In the clinical trial published recently, the distance was 1 cun (2-3 cm) between the non-acupoints and acupoints, and clinical outcome showed that acupoints treatment alleviated symptoms superior to non-acupoints treatment [41]. The measured sites are adjacent in some studies including PPT measured [42, 43]. The distance is 2 cm between the non-acupoint and Tianzong (SI 11), which is acceptable in our study. For reducing the stimulation effect, there is

an approximately 2 min interval between the repetitions. The probability of a possible stimulation by too close to acupoint is low but it could not be ruled out completely. In addition, Ashi points also named reflexing points or tender spots. They are the phenomenon acupoints or temporary acupoints, which are dissimilar from acupoints of the fourteen meridians or extraordinary points. Generally, Ashi points have no specific names and definite locations, and will vanish after disease recovered. The aim of our study is to investigate the pattern of experimental pain responses at acupoints, which have specific names and definite locations. For that reason, the Ashi points do not take into account in this study. Further studies need to be conducted to confirm the phenomena observed here.

CONCLUSIONS

In conclusion, there exists a mixed presence of sensitization patterns at acupoints in patients with unilateral shoulder pain and a strong correlation among Jianliao (SJ 14), Jianyu (LI 15) and Jianzhen (SI 9). Future research utilizing the multimodal pain approach should be conducted, such as suprathreshold heat pain response, to determine various sensitivity mechanisms.

Legends

Figure 1. The pressure pain assessment sites in the present study. (A) Locations for the pressure pain threshold (PPT) measurement at Jianliao (SJ 14), Jianyu (LI 15) and Jianzhen (SI 9). (B) Locations for the PPT measurement at Tianzong (SI 11) and Non-acupoint.

Figure 2. The values of pressure pain threshold (PPT) at the non-acupoint. Values are mean ± SD.

Footnotes

Contributors Conceived and designed the experiments: Qian-Qian Li, Cun-Zhi Liu. Performed the experiments: Guang-Xia Shi, Qing-Nan Fu, Chao-Qun Yan, Shuai Zhang. Analyzed the data:

Li-Wen Zhang, Xue-Rui Wang. Wrote the paper: Chao-Qun Yan, Qian-Qian Li. All authors approved the final manuscript.

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Competing interests We have read and understood BMJ policy on declaration of interests and declare that we have no competing interests.

Ethics approval The Research Ethical Committee of Beijing Hospital of Traditional Chinese Medicine Affiliated to Capital Medical University approved the trial.

Patient consent Obtained.

Provenance and peer review Not commissioned; externally peer reviewed.

Data Sharing Statement No additional data are available.

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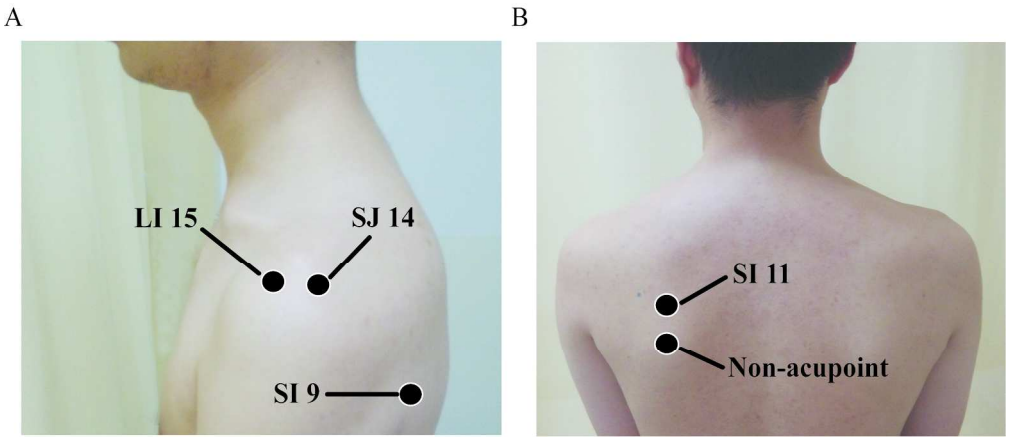

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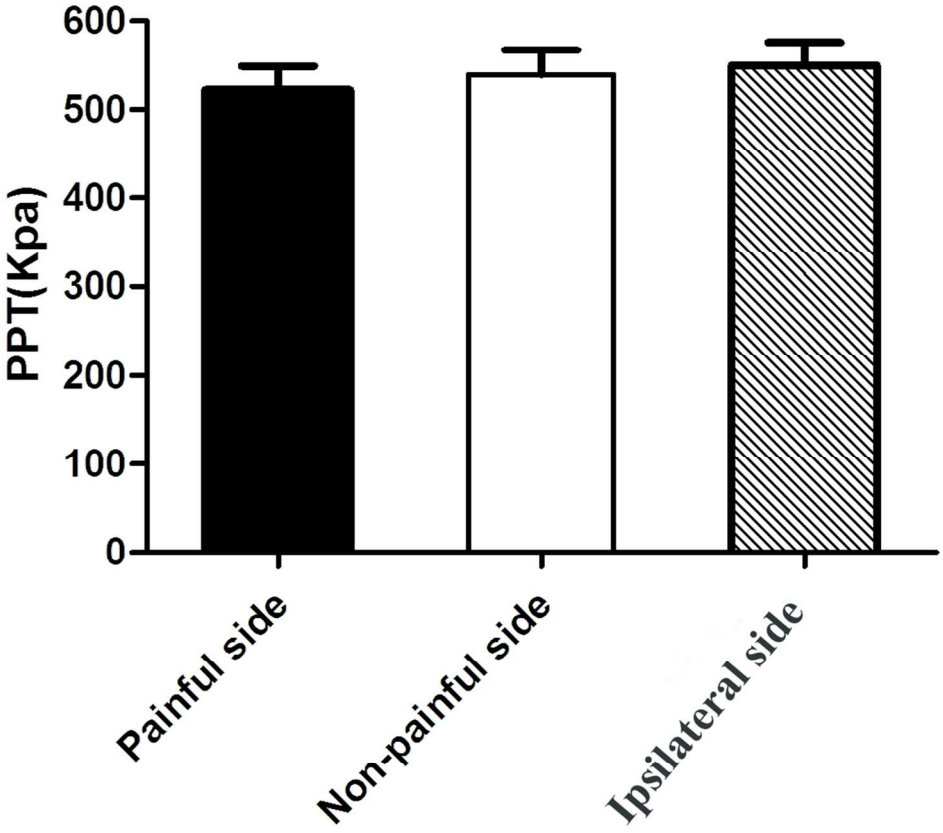

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Appendix Table 1. Locations for the pressure pain assessment sites in the present study.

Points	Location
Jianliao (SJ 14)	In the depression posterior and inferior to the acromion when arm is abducted.
Jianyu (LI 15)	In the depression between the acromial extremity of the clavicle and the great tuberosity of humerus; or when the arm is in full abduction, the acupoint is in the depression at the anterior border of the acromioclavicular joint, and superior to the shoulder joint.
Jianzhen (SI 9)	1 cun directly above the posterior end of the axillary fold when the arm is abducted.
Tianzong (SI 11)	In the depression in the center of the subscapular fossa, at the point 1/3 of the line between the lower border of the mesoscapula and lower angle of capula.
Non-acupoint	2 cm down to Tianzong (SI 11).

Appendix Table 2. Correlation of peripheral sensitization index (PSI) among acupoints.

		PSI			
		Tianzong (SI11)	Jianliao (SJ 14)	Jianyu (LI 15)	Jianzhen (SI 9)
PSI	Tianzong (SI11)	1.00	0.26	0.32	0.35
	Jianliao (SJ 14)	0.26	1.00	0.73*	0.62*
	Jianyu (LI 15)	0.32	0.73*	1.00	0.25
	Jianzhen (SI 9)	0.35	0.62*	0.44*	1.00

Values are Pearson's correlation. *Significant association between variables ($p < .01$).

Appendix Table 3. Correlation between peripheral sensitization indexes (PSI) and relevant baseline characteristics.

	PSI			
	Tianzong (SI 11)	Jianliao (SJ 14)	Jianyu (LI 15)	Jianzhen (SI 9)
Age (years)	-0.28	-0.13	-0.21	-0.22
Pain duration (weeks)	0.21	0.20	-0.10	-0.20
VAS	0.24	0.16	0.27	-0.12

Values are Pearson's correlation.

Appendix Table 4. Correlation of central sensitization index (CSI) among acupoints.

		CSI			
		Tianzong (SI11)	Jianliao (SJ 14)	Jianyu (LI 15)	Jianzhen (SI 9)
CSI	Tianzong (SI11)	1.00	0.86	0.11	0.17
	Jianliao (SJ 14)	0.09	1.00	0.59*	0.25
	Jianyu (LI 15)	0.11	0.59*	1.00	0.51*
	Jianzhen (SI 9)	0.17	0.25	0.51*	1.00

Values are Pearson's correlation. *Significant association between variables ($p < 0.01$).

Appendix Table 5. Correlation between central sensitization index (CSI) and relevant baseline characteristics.

	CSI			
	Tianzong (SI 11)	Jianliao (SJ 14)	Jianyu (LI 15)	Jianzhen (SI 9)
Age (years)	-0.10	-0.12	-0.32	-0.24
Pain duration (weeks)	0.13	0.29	-0.16	-0.05
VAS	0.33	0.21	0.04	0.01

Values are Pearson's correlation.

Appendix Table 6. Correlation between peripheral sensitization index (PSI) and central sensitization index (CSI).

		CSI			
		Tianzong (SI11)	Jianliao (SJ 14)	Jianyu (LI 15)	Jianzhen (SI 9)
PSI	Tianzong (SI11)	0.48*	0.15	0.40*	0.52*
	Jianliao (SJ 14)	0.17	0.39*	0.58*	0.62*
	Jianyu (LI 15)	0.32	0.36	0.53*	0.58*
	Jianzhen (SI 9)	0.17	0.25	0.65*	0.58*

Values are Pearson's correlation. *Significant association between variables ($p < 0.01$).

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Detection for peripheral and central sensitization at acupoints in patients with unilateral shoulder pain: a cross-sectional matched case-control study

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Detection for peripheral and central sensitization at acupoints in patients with unilateral shoulder pain: a cross-sectional matched case-control study

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Abstract

Objective—To investigate the pattern of experimental pain responses at acupoints in patients with unilateral shoulder pain.

Design— A cross-sectional matched case-control study.

Setting—Acupuncture and Moxibustion Department, Beijing Hospital of Traditional Chinese Medicine Affiliated to Capital Medical University.

Participants—Volunteer sample of 60 participants (30 patients with unilateral shoulder pain, 30 healthy controls).

Interventions—Not applicable.

Main Outcome Measures—Pressure pain thresholds (PPTs) were measured at four acupoints, including Tianzong (SI 11), Jianliao (SJ 14), Jianyu (LI 15) and Jianzhen (SI 9) on the painful/non-painful side in patients with unilateral shoulder pain or healthy controls, respectively. The correlations between peripheral sensitization index (PSI) and central sensitization index (CSI) were compared in this study.

Results—The analysis revealed significantly lower PPT values at acupoints on the painful side compared with non-painful side in patients with shoulder pain ($P < 0.025$). Meanwhile, PPTs on the non-painful side of these patients were lower than the ipsilateral side of healthy controls ($P < 0.025$). No distinct differences of PPT values were found at non-acupoint among the painful/non-painful side in patients with shoulder pain and the ipsilateral side of healthy controls ($P > 0.05$). Additionally, it was observed that the pressure pain assessment acupoints have strong association between the PSI and CSI, and three acupoints of SJ 14, LI 15 and SI 9 showed correlation in PSI or CSI particularly.

Conclusion—The results suggest the presence of peripheral and central sensitization at acupoints in participants with unilateral shoulder pain. There exists an obvious relationship among the three acupoints of SJ 14, LI 15 and SI 9, which are usually chosen to treat shoulder pain. The results provide the evidence for acupoints selection to treat shoulder pain by acupuncture.

Keywords: acupoints; peripheral sensitization; central sensitization; pressure pain threshold; shoulder pain

Strengths and limitations of this study

This is, to the best of our knowledge, the first study to support an alteration in both peripheral and central sensitization at acupoints in patients with shoulder pain.

The peripheral and central sensitization at acupoints seemed to occur correlatively independently from the relevant baseline variable.

A limitation of this study was that only a pressure pain assessment performed at acupoints, which might be inadequate to describe the complexity of pain perception.

This study was conducted in a single institution and consisted of a primarily female sample, so the external validity was not clear.

INTRODUCTION

Acupuncture is one of the most widely-used forms of complementary and alternative medicines. There are approximately 183 countries using acupuncture treatment according to a survey conducted by the World Federation of Acupuncture and Moxibustion Societies in 2013 [1]. The WHO reports that acupuncture treatment can be beneficial for more than 40 disorders [2]. Pain is one of those disorders, which is particularly amenable to acupuncture. The evidence in reviews demonstrates that acupuncture treatment can reduce various types of clinical pain effectively in multiple clinical trials [3-5]. Recently, a meta-analysis reported that shoulder pain, low back pain, neck pain, osteoarthritis and chronic headache can be alleviated significantly after acupuncture treatment [6]. Shoulder pain is the third most common musculoskeletal disorder, of which the prevalence rate varies from 6.9% to 26% for point prevalence and even increases to 66.7% for lifetime prevalence in a general population [7, 8]. In our previous study, we identified acupuncture treatment can alleviate shoulder pain, particularly in special acupoints (data unpublished).

Acupoints are special sites at precise locations and lie on ‘meridians’ without a physical structure. According to the theory of meridians in traditional Chinese medicine, it is well known that the acupoints reflect disorders of visceral conditions. Many studies have observed visceral

lesions result in changed pain perception of some special acupoints on the body surface [9]. Ben et al assessed the phenomenon of experimental pain-sensitive points in patients with gastric ulcer or gastritis and found some special acupoints were more sensitive than other areas [10]. Nevertheless, whether acupoints behave more sensitively under musculoskeletal pain has not been described in detail in previous studies.

Sensitization is a nervous system phenomenon that can occur in conjunction with pain [11]. When sensitization is present, there are two types of hypersensitivity including peripheral sensitization and central sensitization, which are important mechanisms in musculoskeletal pain conditions [12]. Peripheral sensitization is defined as the broadening of nociception in pain perception during activities or movements, which typically would be not painful [11]. Central sensitization refers to an amplification of neural signaling within the central nervous system that elicits pain hypersensitivity. It reflects increased activity of pain facilitation pathways and malfunctioning of descending pain inhibitory pathways [13-17].

We performed a multicenter, single blind, factorial randomized controlled clinical trial previously (Number Register: ISRCTN61861069). In the study, we found the phenomenon that the pain threshold decreased at related acupoints in patients with unilateral shoulder pain was widespread. Therefore, we chose some patients from the trial to detect whether acupoints are hypersensitive in musculoskeletal pain disease. We hypothesized that the patients with unilateral shoulder pain would present peripheral or central sensitization at acupoints as evidenced by Pressure pain threshold (PPT) detection, when compared with healthy controls. Specifically, acupoints on the painful side would have lower PPT than on the non-painful side of patients. Also, acupoints on the non-painful side of patients would be hypersensitive as assessed by PPTs, with respect to the ipsilateral side of healthy controls. If the results confirm these hypotheses, then it provides evidence that acupoints exist for peripheral sensitization and central sensitization phenomenon in patients with unilateral shoulder pain.

MEDTHODS AND ANALYSIS

Study design

This cross-sectional matched case-control study was conducted at the Department of Acupuncture and Moxibustion, Beijing Hospital of Traditional Chinese Medicine Affiliated to Capital Medical University.

Participants

Clinical patients—The 30 patients with shoulder pain were chosen from a multicenter, randomized trial. In total, 164 patients with shoulder pain were recruited from three centers in the random trial between January 2014 and September 2014. We enrolled the final 30 patients from 76 patients that screened in the center of Beijing Hospital of Traditional Chinese Medicine before any treatments were dispensed. The Research Ethical Committee of Beijing Hospital of Traditional Chinese Medicine Affiliated to Capital Medical University approved the trial (reference: 201315).

The inclusion criteria included presenting with unilateral shoulder pain for at least six weeks and up to two years, reporting the pain intensity > 50 mm on a visual analogue scale (VAS), and being right hand dominant, who have not received acupuncture or other analgesic therapies in the preceding month.

The exclusion criteria were as follows: pain in both shoulders; referred pain from the cervical spine; previous history of shoulder surgery, pectorial muscle pain, thoracic outlet syndrome, stroke or ipsilateral breast surgery; heart diseases and severe hypertension; osteoarthritis of the glenohumeral joint or systemic bone and joint disorder (rheumatoid arthritis); endocrine diseases such as hyperthyroidism; severe infection; undergoing current therapy involving analgesics and especially major illness depression.

Healthy controls—The healthy controls were matched to the patients with shoulder pain individually. Each healthy control was matched for gender, age (±1 year), ethnicity and dominant hand to one patient. Healthy controls were recruited from the community via posted flyers and general advertisements between May 2014 and September 2014. Healthy controls were eligible if they were not currently performing resistance exercise for the upper extremity. They were excluded based on the following criteria: receiving acupuncture or other analgesic therapies in the preceding month, experiencing neck or shoulder pain, having a history of shoulder surgery or

neurological impairments of the upper extremity, a shoulder skin infection, having difficulty in understanding instructions, and taking any pain medication currently.

Protocol

All participants completed a packet of questionnaires including demographic data (age, sex, race, dominant hand) and psychological information before informed consent was obtained. The patients were initially examined by a researcher who assessed compliance using the inclusion and exclusion criteria. Beck Depression Inventory (BDI) is a multiple choice self-reported inventory for measuring the severity of depression [18]. If the BDI outcome is more than 4, the participants were excluded [19]. Clinical pain intensity was assessed by VAS, which consists a line of 0–100 mm: 0 representing 'no pain at all' and 100 mm representing 'the most intense pain imaginable'.

After finishing the questionnaires, both shoulders of participants were exposed and the measurement sites were marked. An expert acupuncturist was responsible for the operation. Five points were marked in this study and shown in Figure 1 and Appendix Table 1. To assess the pain sensitivity of acupoints, four acupoints Jianliao (SJ 14), Jianyu (LI 15), Jianzhen (SI 9), and Tianzong (SI 11) were marked bilaterally with a marker pen drawing a circle (Figure 1. A. B). To assess the pain sensitivity in the non-acupoint, 2 cm down to Tianzong (SI 11) was marked bilaterally in participants (Figure 1. B).

Measurement of pressure pain thresholds (PPT)

Both shoulders were exposed for examination by an operator. The participants were asked to take a prone position on the examination bed with a suitable pillow under the chest when Jianzhen (SI 9), Tianzong (SI 11) and non-acupoint were measured. Then the participants were required to sit on a chair with a researcher to keep the arm and shoulder in parallel when Jianliao (SJ 14) and Jianyu (LI 15) were measured.

PPT is widely used to evaluate the pain sensory threshold for the patients with shoulder pain [20]. The lower value of PPT indicates the decreased nociceptive threshold of pain perception [21]. A handheld electronic pressure algometer (Wagner Instruments, Greenwich, CT) mounted with a 1 cm^2 rubber tipped plunger was used in this study. The probe was held perpendicular to marked points bilaterally, and the pressure was increased at a rate of 30kPa/s, in order to avoid

potential skin penetration. All participants were instructed to indicate when the pressure became painful, at that time the pressure was immediately stopped and the digits were recorded. PPT was calculated as the mean of three trials on each point. There was an approximately 2 min interval between the repetitions. The operator was not to be told which side is the painful shoulder of patients and blinded to patients and healthy controls. Additionally, the operator did not have any basic knowledge of acupoints and did not know whether the measuring sites were acupoints or not during testing.

Data Analysis

The data were double entered with an adequate check in EpiData. SPSS 17.0 software (SPSS Inc., Chicago, IL, USA) was used for analysis. Discrete variables were summarized by frequencies and percentages. Distributed data were summarized using mean ± standard deviation (SD) or median and interquartile range (IQR). The distributed data were analyzed using a parametric statistical test (Pair t-test) if it agreed with normal distribution. Otherwise, the data were analyzed using a non-parametric (Wilcoxon's signed rank test) statistical test. Shapiro-Wilk test and observation of histograms and normal probability plots were used for all study variables to determine whether they followed a normal distribution. To adjust multiple comparisons, an alpha level of 0.025 was used for all pairwise comparisons.

Peripheral and central sensitization index was used to determine whether pain-sensitivity existed at the pressure pain assessment sites. Peripheral sensitization is referred to a patient's response for PPT on the painful side that fell below the 25^{th} percentile among the non-painful side [22]. The PPT value of the 25^{th} percentile is determined by the average value of 7^{th} and 8^{th} lowest observations on the non-painful side. We examined a patient's proportional response for PPT on the painful side fell below the 25^{th} percentile. Each response PPT is considered for peripheral sensitization index (PSI). Central sensitization indicates that a patient's response for PPT on the non-painful side fell below the 25^{th} percentile (the average value of 7^{th} and 8^{th} lowest observations) among the ipsilateral side of healthy controls. We computed the proportional responses for PPT on the non-painful side fell below the 25^{th} percentile among the ipsilateral side of healthy controls. Each response PPT is considered for central sensitization index (CSI). The 25^{th} percentile was suggested as a lower limit reference value for enhanced sensitivity [23]. Each index was examined by Pearson's correlation. We determine whether patients with shoulder pain

demonstrated peripheral, central, a mixed-pattern or no sensitization, and analyzed the association between sensitization subgroups and the relevant baseline characteristics including demographic and clinical variables. We analyzed association between sensitization subgroups and the relevant baseline characteristics including demographic and clinical variables. Comparisons among the variables were examined using one way analysis of variance or Chi-square and assessment of 95% confidence intervals (CI). A *P* value <0.05 was defined as statistically significant.

RESULTS

Recruitment and baseline characteristics

Between January 2014 and September 2014, thirty patients with shoulder pain (11 males and 19 females) were enrolled in this study. The average age of the thirty patients was 50.60 ± 12.19 years (Table 1). The healthy controls included 11 males and 19 females with a mean age of 50.63 ± 12.20 years. All participants were right hand dominant.

Table. 1 Characteristics of the patients with shoulder pain and healthy controls.

	Patients With Shoulder Pain (N = 30)	Healthy Controls (N = 30)
Age (years)	50.60 ± 12.19	50.63±12.20
Sex (females), n (%)	19 (63.33)	19 (63.33)
Race (number of Han), n (%)	30 (100)	30 (100)
Pain duration (weeks)	19.07 ± 16.99	-
VAS (median, IQR)	70 (50 - 80)	-
BDI (median, IQR)	0 (0 - 0)	0 (0 - 0)
BMI	25.27 ± 4.17	24.91 ± 3.77
Normal (≤ 23.9)	11	12
Overweight (24 - 27.9)	12	14
Obese (≥ 28)	7	4

Values represented as N, mean ± SD or median, IQR.

Abbreviations: N – Number, SD – standard deviation, VAS – Visual Analogue Scale, BDI– Beck Depression Inventory, BMI – Body Mass Index, IQR – interquartile range.

PPT comparison of related acupoints

For the patients with shoulder pain, lower PPT levels were detected at acupoints on the painful side compared with the non-painful side (Table 2). The differences were 94.9 (95% CI, 53.47 to 136.33; $p < 0.001$) at Tianzong (SI 11), 102.91 (95% CI, 79.85 to 125.97; $p < 0.001$) at Jianliao (SJ 14), 91.19 (95% CI, 44.82 to 137.56; $p < 0.001$) at Jianyu (LI 15), and 86.79 (95% CI, 56.30 to 117.28; $p < 0.001$) at Jianzhen (SI 9), respectively.

The PPT value difference between the non-painful side of the patients and the ipsilateral side of healthy controls was also compared. PPTs at the acupoints on non-painful side of the patients were lower than on the ipsilateral side of healthy controls. The differences were 57.71 (95% CI, 24.72 to 90.70; $p = 0.001$) at Tianzong (SI 11), 81.03 (95% CI, 41.40 to 120.67; $p < 0.001$) at Jianliao (SJ 14), 72.20 (95% CI, 10.90 to 133.50; $p = 0.02$) at Jianyu (LI 15), and 88.09 (95% CI, 39.88 to 136.30; $p = 0.001$) at Jianzhen (SI 9), respectively.

Table. 2 Pressure pain threshold values for participants

	Patients With Shoulder Pain		Painful vs Non-painful Side		Health Controls	Non-painful Side vs Ipsilateral Side	
	Painful Side	**Non-painful Side**	**Mean (95% CI)**	**P value**	**Ipsilateral Side**	**Mean (95% CI)**	**P value**
Tianzong (SI 11)	414.83 ± 135.61	509.73 ± 168.05	94.90 (53.47, 136.33)	<0.001	567.44 ± 153.84	57.71 (24.72, 90.70)	0.001
Jianliao (SJ 14)	469.70 ± 181.07	572.61 ± 196.71	102.91 (79.85, 125.97)	<0.001	653.64 ± 211.50	81.03 (41.40, 120.67)	<0.001
Jianyu (LI 15)	434.79 ± 140.39	525.98 ± 160.16	91.19 (44.82, 137.56)	<0.001	598.18 ± 181.76	72.20 (10.90, 133.50)	0.02
Jianzhen (SI 9)	453.08 ± 154.54	539.87 ± 167.59	86.79 (56.30, 117.28)	<0.001	627.96 ± 209.67	88.09 (39.88, 136.30)	0.001
Non-acupoints	521.34 ± 147.02	538.67 ± 153.89	17.33 (-36.99, 2.34)	0.08	549.18 ± 143.66	10.51 (-18.87, 39.89)	0.47

Values represented as mean ± SD. The unit of values was Kpa. Abbreviations: SD – Standard Deviation, CI –Confidence Intervals.

PPT comparison of non-acupoint

Figure 2 showed PPT at the non-acupoint. For the patients, the analysis revealed no obvious difference of 17.33 (95% CI, -36.99 to 2.34; p = 0.08) in PPT value on the painful side compared to the non-painful side. Meanwhile, no significant difference of PPT level, which behaved 10.51 (95% CI, -18.87 to 39.89; p = 0.47), was found between the non-painful side of the patients and the ipsilateral side of healthy controls.

Peripheral Sensitization Index

All measured acupoints demonstrated side-to-side difference in the patients. They were used to compute a peripheral sensitization index (PSI). PPT values at acupoints on the painful side below the 25th percentile of the non-painful side indicated peripheral sensitization. The proportion of the patients with peripheral sensitization were 77% at Tianzong (SI 11), 37% at Jianliao (SJ 14), 43% at Jianyu (LI 15) and 60% at Jianzhen (SI 9) (Table 3). Significant correlation (Appendix Table 2) was observed in PSI among Jianliao (SJ 14), Jianyu (LI 15) and Jianzhen (SI 9) ($p < 0.01$). There was no significant association between PSI and other baseline variables ($p > 0.05$) (Appendix Table 3).

Table 3. Frequencies meeting the peripheral sensitization index (PSI) and central sensitization index (CSI) based on PPT response.

Sites	PSI	CSI	PSI×CSI
Tianzong (SI11)	23 (77)	13 (43)	13 (43)
Jianliao (SJ 14)	11 (37)	17 (57)	9 (30)
Jianyu (LI 15)	13 (43)	19 (63)	12 (40)
Jianzhen (SI 9)	18 (60)	18 (60)	15 (50)

Values are individual counts (percentages). Abbreviations: PPT – Pressure-pain threshold.

Central Sensitization Index

The four measured acupoints demonstrated difference between the non-painful side of patients and the ipsilateral side of healthy controls. Therefore, they were used to compute central sensitization index (CSI). The proportion of the patients were 43% at Tianzong (SI 11), 57% at

Jianliao (SJ 14), 63% at Jianyu (LI 15) and 60% at Jianzhen (SI 9) (Table 3). A distinct and significant association (Appendix Table 4) was observed between Jianliao (SJ 14) and Jianyu (LI 15) ($p < 0.01$), Jianyu (LI 15) and Jianzhen (SI 9) ($p < 0.01$) in CSI. Appendix Table 5 showed no statistical significance correlations between CSI and relevant baseline characteristics ($p > 0.05$).

Comparison of Peripheral and Central Sensitization Index

The frequencies of the patients who had both PSI and CSI were 43% at Tianzong (SI 11), 30% at Jianliao (SJ 14), 40% at Jianyu (LI 15) and 50% at Jianzhen (SI 9), respectively (Table 3). Significant correlation was observed between PSI and CSI in measured acupoints ($p < 0.05$) (Appendix Table 6). Table 4 showed that association between subgroups and sex approached statistical significance at the assessment acupoints ($p < 0.001$). No significant association was observed between subgroups and other baseline variable ($p > 0.05$).

Table. 4 Demographic, clinical, and psychological characteristics of sensitization groups.

	Peripheral Sensitization	Central Sensitization	Peripheral and Central Sensitization	No Sensitization	P value
Tianzong (SI11)					
Age (years)	48.78 (43.45, 54.21)	50.38 (44.02, 56.75)	50.38 (44.02, 56.75)	56.57 (48.00, 65.15)	0.47
Sex (%females)	78.28 (56.14, 92.52)	76.92 (46.16, 94.89)	76.92 (46.16, 94.89)	14.29 (3.68, 57.62)	<0.001
Pain duration (weeks)	21.04 (12.91, 29.17)	24.31 (10.23, 38.38)	24.31 (10.23, 38.38)	12.57 (7.16, 17.99)	0.60
VAS	68.04 (61.25, 74.84)	71.15 (62.77, 79.53)	71.15 (62.77, 79.53)	60.00 (52.45, 67.55)	0.34
Jianliao (SJ 14)					
Age (years)	48.09 (38.47, 57.71)	49.59 (43.35, 55.82)	48.89 (38.97, 58.81)	53.27 (46.22, 60.33)	0.78
Sex (%females)	100	82.35 (56.42, 96.62)	100	27.27 (6.01, 60.95)	<0.001
Pain duration (weeks)	22.55 (10.81, 34.28)	23.29 (12.80, 33.79)	22.22 (7.96, 36.49)	11.64 (7.07, 16.21)	0.32
VAS	70.00 (59.17, 80.83)	68.82 (61.14, 76.51)	72.22 (59.60, 84.84)	63.18 (53.41, 72.95)	0.59
Jianyu (LI 15)					
Age (years)	46.92 (38.29, 55.56)	47.47 (41.20, 53.75)	45.42 (36.64, 54.19)	55.10 (48.95, 61.25)	0.32
Sex (%females)	92.31 (63.76, 99.81)	89.47 (66.90, 98.70)	100	20.00 (2.54, 55.41)	<0.001
Pain duration (weeks)	19.69 (9.51, 29.87)	21.89 (12.14, 31.65)	20.67 (9.73, 31.60)	14.80 (9.23, 20.37)	0.76
VAS	68.46 (59.62, 77.30)	66.84 (59.65, 74.04)	66.67 (57.96, 75.38)	62.50 (53.38, 71.62)	0.79
Jianzhen (SI 9)					
Age (years)	48.22 (41.53, 54.91)	48.00 (41.35, 54.65)	46.27 (38.65, 53.88)	53.33 (45.49, 61.17)	0.64
Sex (%females)	83.33 (58.57, 96.44)	83.33 (58.57, 96.44)	93.33 (67.97, 99.83)	33.33 (7.45, 70.06)	<0.001
Pain duration (weeks)	17.11 (9.58, 24.64)	18.22 (10.77, 25.67)	18.67 (9.70, 27.63)	24.00 (6.74, 41.26)	0.78
VAS	67.78 (60.43, 75.13)	66.67 (58.85, 74.48)	68.00 (59.32, 76.68)	65.00 (53.79, 76.21)	0.97

Values presented as % (for sex) or mean with [95% CI]. Abbreviations: VAS – Visual Analogue Acale.

DISCUSSION

The current results were in agreement with a peripherally sensitized state at acupoints which is determined by the side-to-side difference of PPT values in patients with unilateral shoulder pain. Central sensitization at acupoints was conducted by comparing pressure sensitivity in patients with age- and sex-matched healthy controls. No obvious PPT values difference was found at the non-acupoint among the painful side, non-painful side and ipsilateral side. To advance this line of research, association between peripheral and central sensitization at acupoints was examined. We found that the patients displayed a significant association between peripheral and central sensitization at measured acupoints. This finding demonstrated that there existed two patterns of sensitization in acupoints. In addition, three acupoints Jianliao (SJ 14), Jianyu (LI 15) and Jianzhen (SI 9) that are normally used for treating shoulder pain had correlation.

Previous investigations had reported that splanchnic diseases can induce mechanical hyperalgesia on the corresponding acupoints when pressed [24]. Acupoints turned to the "activated mode" or "sensitized mode" from the "silent mode" in pathological conditions. This phenomenon is called "acupoint sensitization". At some acupoints there appeared to be a hypersensitivity of temperature (heat-sensitization) or pain threshold (pain-sensitization) under visceral pain [9, 25]. But unlike those studies, we examined acupoints in patients with musculoskeletal pain in this study.

Central sensitization is challenging clinically, since no standard assessment exists. Some studies recommended the use of various modalities for pain sensitivity at local and distal locations [21, 26]. However, other researches showed that decreased PPTs at the painful and non-painful shoulder, but not at the muscle tibialis anterior [27-29]. According to "Criteria for the Classification of Central Sensitization Pain", patients with diffuse pain distribution, allodynia, and hyperalgesia are more likely to present with central sensitization. One of the patterns of pain distribution is that patients have bilateral pain/mirror pain [17]. In the patients with shoulder pain, the increased sensitivity to mechanical input in the contralateral shoulder would be interpreted as central sensitization [11]. A large number of studies define central sensitization as pain sensitivity at local and distal locations. We chose bilateral pain to define central sensitization, unlike earlier studies, and to determine whether there existed patterns of experimental pain responses at shoulder acupoints.

Collectively, the findings of the study support the alteration in both peripheral and central sensitization at acupoints in patients with musculoskeletal pain. We determined whether peripheral and central sensitization were more likely to occur together or alone. In Table 3, we describe that 19 patients had central sensitization and 13 patients had peripheral sensitization in Jianyu (LI 15). Previous study showed that long-term peripheral sensitization can lead central nervous system changes occurring and resulting in central sensitization [11]. However, there were 6 patients having only central sensitization without peripheral sensitization. This result indicates that peripheral sensitization is not a prerequisite for the presence of central sensitization at acupoints. In PSI or CSI, the strong association was found at three acupoints of Jianliao (SJ 14), Jianyu (LI 15) and Jianzhen (SI 9). From the clinical perspective, Jianliao (SJ 14), Jianyu (LI 15) and Jianzhen (SI 9) are frequently chosen to treat shoulder pain in clinical practice, and have been called "shoulder three acupoints" by acupuncturists [30, 31]. Jianyu (LI 15), Jianliao (SJ 14) and Jianzhen (SI 9), are highly-refined acupoints used to treat shoulder pain in the clinic, and have been proven to be effective at regulating muscle strength and tension of shoulder joint [32]. The strong association among Jianliao (SJ 14), Jianyu (LI 15) and Jianzhen (SI 9) is consistent with the conception of traditional Chinese medicine.

To our knowledge, this is the first study to research peripheral and central sensitization at acupoints in patients with shoulder pain. One of the advantages of the study is that the measured acupoints and non-acupoint were marked by an acupuncturist with 24 years of experience in clinical acupuncture treatment. The evaluator who measured PPTs also has extensive experience with using the algometer and without basic knowledge of acupoints. The internal validity is increased by blinding the evaluator who did not know whether the measured sites were acupoints or not during testing. In addition, the evaluator was blinded as to whether the test participant was a patient or a healthy control. The participants were asked to take different positions when different acupoints were measured. For example, the participants were required take a prone position on the examination bed with a suitable pillow under the chest and the arms close to the body when Jianzhen (SI 9) was measured. It increases the reliability of testing PPT over a soft area.

Specifically, the result indicates that peripheral and central sensitization at acupoints is not relevant to pain duration. Moreover, there is no obvious evidence that the pattern of sensitization

has relation with the degree of severity of the clinical condition as measured by VAS. These changes are consistent with the idea of traditional Chinese medicine that acupoints are the crucial reflex points of body lesions under pathological circumstance. Hyperalgesia and skin sensitization can occur at corresponding acupoints in the presence of some diseases [33]. Morphological structure studies have reported that the nervous system and blood vessels might have a close relationship with acupoints [34-36]. For example, abundant microvessels existed at the acupoints of Zhongji (RN3) and Zusanli (ST36) in contrast to the surrounding tissues [37]. The acupoints also have a high density of nerve endings including A- and C- afferent fibers [38, 39]. Those characteristics of higher concentration of neural, vascular elements and mast cells could make pain perception more sensitive, and might contribute to peripheral sensitivity. To confirm the specificity of acupoints, we selected a non-acupoint in the infraspinatus muscle. Obviously, no significant difference was found in PPT values among the painful/ non-painful side of patients and the ipsilateral side of healthy controls. The finding proved that acupoints become a specific reflex point that respond to the presence of musculoskeletal pain. Our study provides evidence that there is an association with acupoints sensitization and gender. Pain difference in gender has become an increased topic in recent years, and lower PPT values in women than that in men were found either in healthy subjects or in clinical patients [40]. But, the significant difference may be accounted for by the fact that the majority of the patients were female (63.33%) in the study.

There are limitations that should be kept in mind regarding this study. Pain perception is multidimensional. PPT measurements are just a mechanical and standardized stimulation and are inadequate to describe the complexity of pain perception. The multimodal approach should be used to provide the details of the pain system in both normal and pathophysiological situations, such as different stimulus modalities and quantitative assessment of various pain mechanisms. Second, the non-acupoint was chosen as 2 cm down from Tianzong (SI 11) because the shoulder blade is relatively flat and may reduce the measurement errors between acupoints and non-acupoints. In the clinical trial published recently, the distance was 1 cun (2-3 cm) between the non-acupoints and acupoints, and clinical outcome showed that acupoints treatment alleviated symptoms superior to non-acupoints treatment [41]. The measured sites are adjacent in some studies including PPT measured [20, 42]. The distance is 2 cm between the non-acupoint and Tianzong (SI 11), which is acceptable in our study. For reducing the stimulation effect, there is

an approximately 2 min interval between the repetitions. The probability of a possible stimulation by too close to an acupoint is low but it could not be ruled out completely. In addition, Ashi points also named reflexing points or tender spots. They are the phenomenon acupoints or temporary acupoints, which are dissimilar from acupoints of the fourteen meridians or extraordinary points. Generally, Ashi points have no specific names and definite locations, and will vanish after disease recovered. The aim of our study is to investigate the pattern of experimental pain responses at acupoints, which have specific names and definite locations. For that reason, the Ashi points do not take into account in this study. Further studies need to be conducted to confirm the phenomena observed here.

CONCLUSIONS

In conclusion, there exists a mixed presence of sensitization patterns at acupoints in patients with unilateral shoulder pain and a strong correlation among Jianliao (SJ 14), Jianyu (LI 15) and Jianzhen (SI 9). Future research utilizing the multimodal pain approach should be conducted, such as suprathreshold heat pain response, to determine various sensitivity mechanisms.

Legends

Figure 1. The pressure pain assessment sites in the present study. (A) Locations for the pressure pain threshold (PPT) measurement at Jianliao (SJ 14), Jianyu (LI 15) and Jianzhen (SI 9). (B) Locations for the PPT measurement at Tianzong (SI 11) and Non-acupoint.

Figure 2. The values of pressure pain threshold (PPT) at the non-acupoint. Values are mean ± SD.

Footnotes

Contributors Conceived and designed the experiments: Qian-Qian Li, Cun-Zhi Liu. Performed the experiments: Guang-Xia Shi, Qing-Nan Fu, Chao-Qun Yan, Shuai Zhang. Analyzed the data:

Li-Wen Zhang, Xue-Rui Wang. Wrote the paper: Chao-Qun Yan, Qian-Qian Li. All authors approved the final manuscript.

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Patient consent Obtained.

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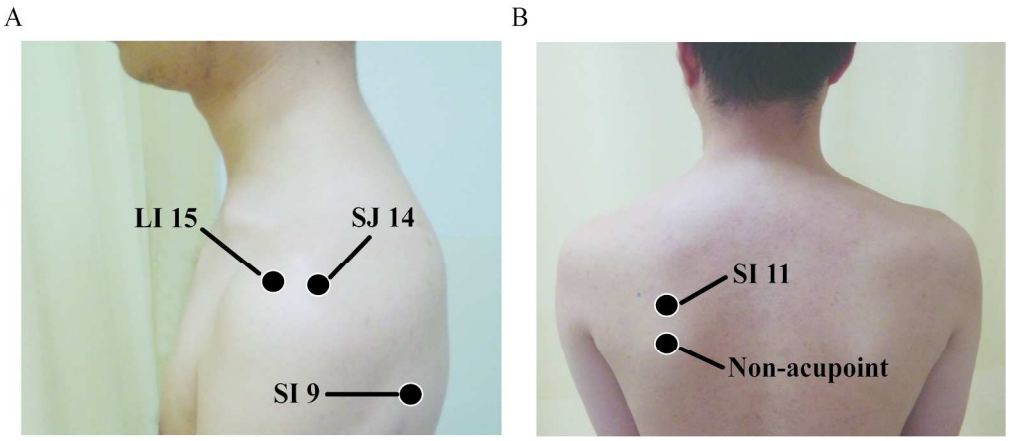

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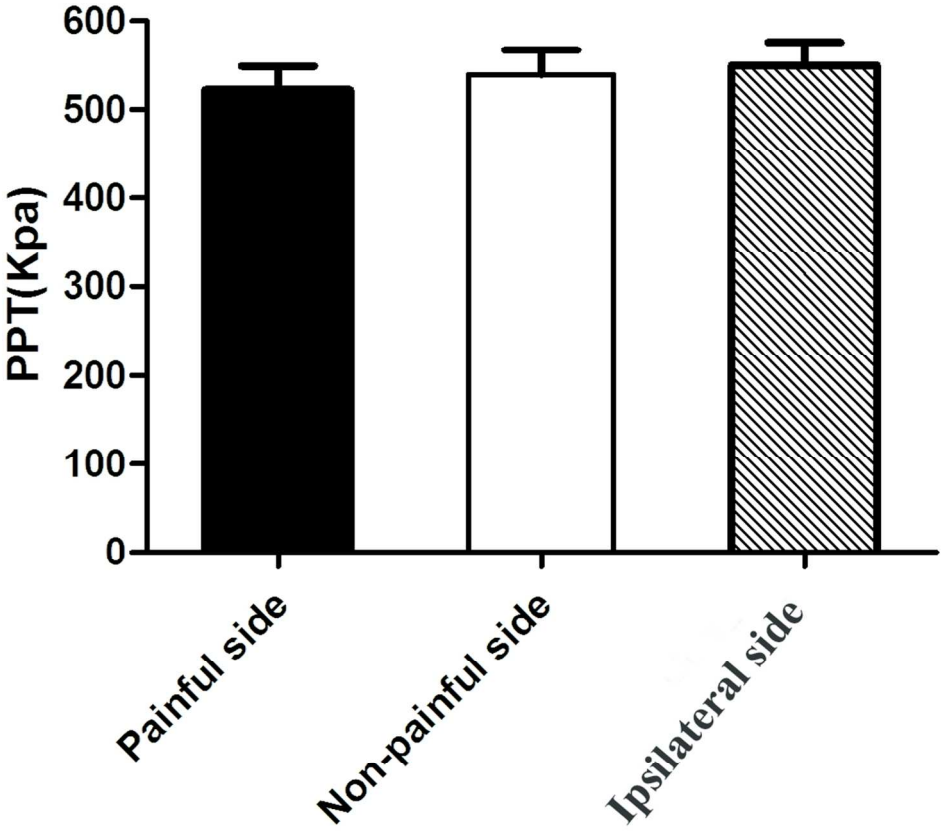

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Appendix Table 1. Locations for the pressure pain assessment sites in the present study.

Points	Location
Jianliao (SJ 14)	In the depression posterior and inferior to the acromion when arm is abducted.
Jianyu (LI 15)	In the depression between the acromial extremity of the clavicle and the great tuberosity of humerus; or when the arm is in full abduction, the acupoint is in the depression at the anterior border of the acromioclavicular joint, and superior to the shoulder joint.
Jianzhen (SI 9)	1 cun directly above the posterior end of the axillary fold when the arm is abducted.
Tianzong (SI 11)	In the depression in the center of the subscapular fossa, at the point 1/3 of the line between the lower border of the mesoscapula and lower angle of capula.
Non-acupoint	2 cm down to Tianzong (SI 11).

Appendix Table 2. Correlation of peripheral sensitization index (PSI) among acupoints.

		PSI			
		Tianzong (SI11)	Jianliao (SJ 14)	Jianyu (LI 15)	Jianzhen (SI 9)
PSI	Tianzong (SI11)	1.00	0.26	0.32	0.35
	Jianliao (SJ 14)	0.26	1.00	0.73*	0.62*
	Jianyu (LI 15)	0.32	0.73*	1.00	0.44*
	Jianzhen (SI 9)	0.35	0.62*	0.44*	1.00

Values are Pearson's correlation. *Significant association between variables ($p < 0.01$).

Appendix Table 3. Correlation between peripheral sensitization indexes (PSI) and relevant baseline characteristics.

	PSI			
	Tianzong (SI 11)	Jianliao (SJ 14)	Jianyu (LI 15)	Jianzhen (SI 9)
Age (years)	-0.28	-0.13	-0.21	-0.22
Pain duration (weeks)	0.21	0.20	-0.10	-0.20
VAS	0.24	0.16	0.27	-0.12

Values are Pearson's correlation.

Appendix Table 4. Correlation of central sensitization index (CSI) among acupoints.

		CSI			
		Tianzong (SI11)	Jianliao (SJ 14)	Jianyu (LI 15)	Jianzhen (SI 9)
CSI	Tianzong (SI11)	1.00	0.09	0.11	0.17
	Jianliao (SJ 14)	0.09	1.00	0.59*	0.25
	Jianyu (LI 15)	0.11	0.59*	1.00	0.51*
	Jianzhen (SI 9)	0.17	0.25	0.51*	1.00

Values are Pearson's correlation. *Significant association between variables ($p < 0.01$).

Appendix Table 5. Correlation between central sensitization index (CSI) and relevant baseline characteristics.

	CSI			
	Tianzong (SI 11)	Jianliao (SJ 14)	Jianyu (LI 15)	Jianzhen (SI 9)
Age (years)	-0.10	-0.12	-0.32	-0.24
Pain duration (weeks)	0.13	0.29	-0.16	-0.05
VAS	0.33	0.21	0.04	0.01

Values are Pearson's correlation.

Appendix Table 6. Correlation between peripheral sensitization index (PSI) and central sensitization index (CSI).

		CSI			
		Tianzong (SI11)	Jianliao (SJ 14)	Jianyu (LI 15)	Jianzhen (SI 9)
PSI	Tianzong (SI11)	0.48*	0.15	0.40*	0.52*
	Jianliao (SJ 14)	0.17	0.39*	0.58*	0.62*
	Jianyu (LI 15)	0.32	0.36	0.53*	0.58*
	Jianzhen (SI 9)	0.17	0.25	0.65*	0.58*

Values are Pearson's correlation. *Significant association between variables ($p < 0.05$).

STROBE Statement—Checklist of items that should be included in reports of ***case-control studies***

	Item No	Recommendation
Title and abstract	1 **(P1-2)**	(*a*) Indicate the study's design with a commonly used term in the title or the abstract
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found
Introduction		
Background/rationale	2 **(P4)**	Explain the scientific background and rationale for the investigation being reported
Objectives	3 **(P4)**	State specific objectives, including any prespecified hypotheses
Methods		
Study design	4 **(P4)**	Present key elements of study design early in the paper
Setting	5 **(P5-7)**	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls **(P5)**
		(*b*) For matched studies, give matching criteria and the number of controls per case **(P5-6)**
Variables	7 **(P6-7)**	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
Data sources/ measurement	8* **(P6-7)**	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias	9 **(P7)**	Describe any efforts to address potential sources of bias
Study size	10 **(P5)**	Explain how the study size was arrived at
Quantitative variables	11 **(P7)**	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding **(P7)**
		(*b*) Describe any methods used to examine subgroups and interactions **(P8)**
		(*c*) Explain how missing data were addressed **(NONE)**
		(*d*) If applicable, explain how matching of cases and controls was addressed **(NONE)**
		(*e*) Describe any sensitivity analyses **(NONE)**
Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed **(P8)**
		(b) Give reasons for non-participation at each stage **(NONE)**
		(c) Consider use of a flow diagram **(NONE)**
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders **(P8)**
		(b) Indicate number of participants with missing data for each variable of interest **(NONE)**
Outcome data	15* **(NONE)**	Report numbers in each exposure category, or summary measures of exposure

Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included **(P9-11)**
		(*b*) Report category boundaries when continuous variables were categorized **(NONE)**
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period **(NONE)**
Other analyses	17 **(P8)**	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion		
Key results	18 **(P14)**	Summarise key results with reference to study objectives
Limitations	19 **(P16−17)**	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	20 **(P14−16)**	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	21 **(P3)**	Discuss the generalisability (external validity) of the study results
Other information		
Funding	22 **(P18)**	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for cases and controls.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.

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Detection for peripheral and central sensitization at acupoints in patients with unilateral shoulder pain in Beijing: a cross-sectional matched case-control study

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Primary Subject Heading:	Complementary medicine
Secondary Subject Heading:	Rehabilitation medicine
Keywords:	acupoints, peripheral sensitization, central sensitization, pressure pain threshold, shoulder pain

Detection for peripheral and central sensitization at acupoints in patients with unilateral shoulder pain in Beijing: a cross-sectional matched case-control study

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Abstract

Objective—To investigate the pattern of experimental pain responses at acupoints in patients with unilateral shoulder pain.

Design— A cross-sectional matched case-control study.

Setting—Acupuncture and Moxibustion Department, Beijing Hospital of Traditional Chinese Medicine Affiliated to Capital Medical University.

Participants—Volunteer sample of 60 participants (30 patients with unilateral shoulder pain, 30 healthy controls).

Interventions—Not applicable.

Main Outcome Measures—Pressure pain thresholds (PPTs) were measured at four acupoints, including Tianzong (SI 11), Jianliao (SJ 14), Jianyu (LI 15) and Jianzhen (SI 9) on the painful/non-painful side in patients with unilateral shoulder pain or healthy controls, respectively. The correlations between peripheral sensitization index (PSI) and central sensitization index (CSI) were compared in this study.

Results—The analysis revealed significantly lower PPT values at acupoints on the painful side compared with non-painful side in patients with shoulder pain ($P < 0.025$). Meanwhile, PPTs on the non-painful side of these patients were lower than the ipsilateral side of healthy controls ($P < 0.025$). No distinct differences of PPT values were found at non-acupoint among the painful/non-painful side in patients with shoulder pain and the ipsilateral side of healthy controls ($P > 0.05$). Additionally, it was observed that the pressure pain assessment acupoints have strong association between the PSI and CSI, and three acupoints of SJ 14, LI 15 and SI 9 showed correlation in PSI or CSI particularly.

Conclusion—The results suggest the presence of peripheral and central sensitization at acupoints in participants with unilateral shoulder pain. There exists an obvious relationship among the three acupoints of SJ 14, LI 15 and SI 9, which are usually chosen to treat shoulder pain. The results provide the evidence for acupoints selection to treat shoulder pain by acupuncture.

Keywords: acupoints; peripheral sensitization; central sensitization; pressure pain threshold; shoulder pain

Strengths and limitations of this study

This is, to our knowledge, the first study to support an alteration in both peripheral and central sensitization at acupoints in patients with shoulder pain.

The internal validity is increased by blinding the evaluator and participants.

A limitation of this study is that only pressure pain assessment was performed at acupoints, which might be inadequate to describe the complexity of pain perception.

This study was conducted in a single institution and consisted of a primarily female sample, so the external validity is not clear.

INTRODUCTION

Acupuncture is one of the most widely-used forms of complementary and alternative medicines. There are approximately 183 countries using acupuncture treatment according to a survey conducted by the World Federation of Acupuncture and Moxibustion Societies in 2013 [1]. The WHO reports that acupuncture treatment can be beneficial for more than 40 disorders [2]. Pain is one of those disorders, which is particularly amenable to acupuncture. The evidence in reviews demonstrates that acupuncture treatment can reduce various types of clinical pain effectively in multiple clinical trials [3-5]. Recently, a meta-analysis reported that shoulder pain, low back pain, neck pain, osteoarthritis and chronic headache can be alleviated significantly after acupuncture treatment [6]. Shoulder pain is the third most common musculoskeletal disorder, of which the prevalence rate varies from 6.9% to 26% for point prevalence and even increases to 66.7% for lifetime prevalence in a general population [7, 8]. In our previous study, we identified acupuncture treatment can alleviate shoulder pain, particularly in special acupoints (data unpublished).

Acupoints are special sites at precise locations and lie on ‘meridians’ without a physical structure. According to the theory of meridians in Traditional Chinese Medicine, it is well known that the acupoints reflect disorders of visceral conditions. Many studies have observed visceral lesions result in changed pain perception of some special acupoints on the body surface [9]. Ben

et al assessed the phenomenon of experimental pain-sensitive points in patients with gastric ulcer or gastritis and found some special acupoints were more sensitive than other areas [10]. Nevertheless, whether acupoints behave more sensitively under musculoskeletal pain has not been described in detail in previous studies.

Sensitization is a nervous system phenomenon that can occur in conjunction with pain [11]. When sensitization is present, there are two types of hypersensitivity including peripheral sensitization and central sensitization, which are important mechanisms in musculoskeletal pain conditions [12]. Peripheral sensitization is defined as the broadening of nociception in pain perception during activities or movements, which typically would be not painful [11]. Central sensitization refers to an amplification of neural signaling within the central nervous system that elicits pain hypersensitivity. It reflects increased activity of pain facilitation pathways and malfunctioning of descending pain inhibitory pathways [13-17].

We performed a multicenter, single blind, factorial randomized controlled clinical trial previously (Number Register: ISRCTN61861069). In the study, we found the phenomenon that the pain threshold decreased at related acupoints in patients with unilateral shoulder pain was widespread. Therefore, we chose some patients from the trial to detect whether acupoints are hypersensitive in musculoskeletal pain disease. We hypothesized that the patients with unilateral shoulder pain would present peripheral or central sensitization at acupoints as evidenced by Pressure pain threshold (PPT) detection, when compared with healthy controls. Specifically, acupoints on the painful side would have lower PPT than on the non-painful side of patients. Also, acupoints on the non-painful side of patients would be hypersensitive as assessed by PPTs, with respect to the ipsilateral side of healthy controls. If the results confirm these hypotheses, then it provides evidence that acupoints exist for peripheral sensitization and central sensitization phenomenon in patients with unilateral shoulder pain.

MEDTHODS AND ANALYSIS

Study design

This cross-sectional matched case-control study was conducted at the Department of Acupuncture and Moxibustion, Beijing Hospital of Traditional Chinese Medicine Affiliated to Capital Medical University.

Participants

Clinical patients—The 30 patients with shoulder pain were chosen from a multicenter, randomized trial. In total, 164 patients with shoulder pain were recruited from three centers in the random trial between January 2014 and September 2014. We enrolled the final 30 patients from 76 patients that screened in the center of Beijing Hospital of Traditional Chinese Medicine before any treatments were dispensed. The Research Ethical Committee of Beijing Hospital of Traditional Chinese Medicine Affiliated to Capital Medical University approved the trial (reference: 201315).

The inclusion criteria included presenting with unilateral shoulder pain for at least six weeks and up to two years, reporting the pain intensity > 50 mm on a visual analogue scale (VAS), and being right hand dominant, who have not received acupuncture or other analgesic therapies in the preceding month.

The exclusion criteria were as follows: pain in both shoulders; referred pain from the cervical spine; previous history of shoulder surgery, pectorial muscle pain, thoracic outlet syndrome, stroke or ipsilateral breast surgery; heart diseases and severe hypertension; osteoarthritis of the glenohumeral joint or systemic bone and joint disorder (rheumatoid arthritis); endocrine diseases such as hyperthyroidism; severe infection; undergoing current therapy involving analgesics and especially major illness depression.

Healthy controls—The healthy controls were matched to the patients with shoulder pain individually. Each healthy control was matched for gender, age (±1 year), ethnicity and dominant hand to one patient. Healthy controls were recruited from the community via posted flyers and general advertisements between May 2014 and September 2014. Healthy controls were eligible if they were not currently performing resistance exercise for the upper extremity. They were excluded based on the following criteria: receiving acupuncture or other analgesic therapies in the preceding month, experiencing neck or shoulder pain, having a history of shoulder surgery or neurological impairments of the upper extremity, a shoulder skin infection, having difficulty in understanding instructions, and taking any pain medication currently.

Protocol

All participants completed a packet of questionnaires including demographic data (age, sex, race, dominant hand) and psychological information before informed consent was obtained. The patients were initially examined by a researcher who assessed compliance using the inclusion and exclusion criteria. Beck Depression Inventory (BDI) is a multiple choice self-reported inventory for measuring the severity of depression [18]. If the BDI outcome is more than 4, the participants were excluded [19]. Clinical pain intensity was assessed by VAS, which consists a line of 0–100 mm: 0 representing 'no pain at all' and 100 mm representing 'the most intense pain imaginable'.

After finishing the questionnaires, both shoulders of participants were exposed and the measurement sites were marked. The participants were not been told which site was non-acupoint. An expert acupuncturist was responsible for the operation. Five points were marked in this study and shown in Figure 1 and Appendix Table 1. To assess the pain sensitivity of acupoints, four acupoints Jianliao (SJ 14), Jianyu (LI 15), Jianzhen (SI 9), and Tianzong (SI 11) were marked bilaterally with a marker pen drawing a circle (Figure 1. A. B). To assess the pain sensitivity in the non-acupoint, 2 cm down to Tianzong (SI 11) was marked bilaterally in participants (Figure 1. B).

Measurement of pressure pain thresholds (PPT)

Both shoulders were exposed for examination by an operator. The participants were asked to take a prone position on the examination bed with a suitable pillow under the chest when Jianzhen (SI 9), Tianzong (SI 11) and non-acupoint were measured. Then the participants were required to sit on a chair with a researcher to keep the arm and shoulder in parallel when Jianliao (SJ 14) and Jianyu (LI 15) were measured.

PPT is widely used to evaluate the pain sensory threshold for the patients with shoulder pain [20]. The lower value of PPT indicates the decreased nociceptive threshold of pain perception [21]. A handheld electronic pressure algometer (Wagner Instruments, Greenwich, CT) mounted with a 1 cm^2 rubber tipped plunger was used in this study. The probe was held perpendicular to marked points bilaterally, and the pressure was increased at a rate of 30kPa/s, in order to avoid potential skin penetration. All participants were instructed to indicate when the pressure became painful, at that time the pressure was immediately stopped and the digits were recorded. PPT was calculated as the mean of three trials on each point. There was an approximately 2 min interval

between the repetitions. The operator was not to be told which side is the painful shoulder of patients and blinded to patients and healthy controls. Additionally, the operator did not have any basic knowledge of acupoints and did not know whether the measuring sites were acupoints or not during testing.

Data Analysis

The data were double entered with an adequate check in EpiData. SPSS 17.0 software (SPSS Inc., Chicago, IL, USA) was used for analysis. Discrete variables were summarized by frequencies and percentages. Distributed data were summarized using mean ± standard deviation (SD) or median and interquartile range (IQR). The distributed data were analyzed using a parametric statistical test (Pair t-test) if it agreed with normal distribution. Otherwise, the data were analyzed using a non-parametric (Wilcoxon's signed rank test) statistical test. Shapiro-Wilk test and observation of histograms and normal probability plots were used for all study variables to determine whether they followed a normal distribution. To adjust multiple comparisons, an alpha level of 0.025 was used for all pairwise comparisons.

Peripheral and central sensitization index was used to determine whether pain-sensitivity existed at the pressure pain assessment sites. Peripheral sensitization is referred to a patient's response for PPT on the painful side that fell below the 25^{th} percentile among the non-painful side [22]. The PPT value of the 25^{th} percentile is determined by the average value of 7^{th} and 8^{th} lowest observations on the non-painful side. We examined a patient's proportional response for PPT on the painful side fell below the 25^{th} percentile. Each response PPT is considered for peripheral sensitization index (PSI). Central sensitization indicates that a patient's response for PPT on the non-painful side fell below the 25^{th} percentile (the average value of 7^{th} and 8^{th} lowest observations) among the ipsilateral side of healthy controls. We computed the proportional responses for PPT on the non-painful side fell below the 25^{th} percentile among the ipsilateral side of healthy controls. Each response PPT is considered for central sensitization index (CSI). The 25^{th} percentile was suggested as a lower limit reference value for enhanced sensitivity [23]. Each index was examined by Pearson's correlation. We determine whether patients with shoulder pain demonstrated peripheral, central, a mixed-pattern or no sensitization, and analyzed the association between sensitization subgroups and the relevant baseline characteristics including demographic and clinical variables. We analyzed association between sensitization subgroups

and the relevant baseline characteristics including demographic and clinical variables. Comparisons among the variables were examined using one way analysis of variance or Chi-square and assessment of 95% confidence intervals (CI). A *P* value <0.05 was defined as statistically significant.

RESULTS

Recruitment and baseline characteristics

Between January 2014 and September 2014, thirty patients with shoulder pain (11 males and 19 females) were enrolled in this study. The average age of the thirty patients was 50.60 ± 12.19 years (Table 1). The healthy controls included 11 males and 19 females with a mean age of 50.63 ± 12.20 years. All participants were right hand dominant.

Table. 1 Characteristics of the patients with shoulder pain and healthy controls.

	Patients With Shoulder Pain (N = 30)	Healthy Controls (N = 30)
Age (years)	50.60 ± 12.19	50.63±12.20
Sex (females), n (%)	19 (63.33)	19 (63.33)
Race (number of Han), n (%)	30 (100)	30 (100)
Pain duration (weeks)	19.07 ± 16.99	-
VAS (median, IQR)	70 (50 - 80)	-
BDI (median, IQR)	0 (0 - 0)	0 (0 - 0)
BMI	25.27 ± 4.17	24.91 ± 3.77
Normal (≤ 23.9)	11	12
Overweight (24 - 27.9)	12	14
Obese (≥ 28)	7	4

Values represented as N, mean ± SD or median, IQR.

Abbreviations: N – Number, SD – standard deviation, VAS – Visual Analogue Scale, BDI– Beck Depression Inventory, BMI – Body Mass Index, IQR – interquartile range.

PPT comparison of related acupoints

For the patients with shoulder pain, lower PPT levels were detected at acupoints on the painful side compared with the non-painful side (Table 2). The differences were 94.9 (95% CI, 53.47 to 136.33; p < 0.001) at Tianzong (SI 11), 102.91 (95% CI, 79.85 to 125.97; p < 0.001) at Jianliao

(SJ 14), 91.19 (95% CI, 44.82 to 137.56; $p < 0.001$) at Jianyu (LI 15), and 86.79 (95% CI, 56.30 to 117.28; $p < 0.001$) at Jianzhen (SI 9), respectively.

The PPT value difference between the non-painful side of the patients and the ipsilateral side of healthy controls was also compared. PPTs at the acupoints on non-painful side of the patients were lower than on the ipsilateral side of healthy controls. The differences were 57.71 (95% CI, 24.72 to 90.70; $p = 0.001$) at Tianzong (SI 11), 81.03 (95% CI, 41.40 to 120.67; $p < 0.001$) at Jianliao (SJ 14), 72.20 (95% CI, 10.90 to 133.50; $p = 0.02$) at Jianyu (LI 15), and 88.09 (95% CI, 39.88 to 136.30; $p = 0.001$) at Jianzhen (SI 9), respectively.

Table. 2 Pressure pain threshold values for participants

	Patients With Shoulder Pain		Painful vs Non-painful Side		Health Controls	Non-painful Side vs Ipsilateral Side	
	Painful Side	**Non-painful Side**	**Mean (95% CI)**	**P value**	**Ipsilateral Side**	**Mean (95% CI)**	**P value**
Tianzong (SI 11)	414.83 ± 135.61	509.73 ± 168.05	94.90 (53.47, 136.33)	<0.001	567.44 ± 153.84	57.71 (24.72, 90.70)	0.001
Jianliao (SJ 14)	469.70 ± 181.07	572.61 ± 196.71	102.91 (79.85, 125.97)	<0.001	653.64 ± 211.50	81.03 (41.40, 120.67)	<0.001
Jianyu (LI 15)	434.79 ± 140.39	525.98 ± 160.16	91.19 (44.82, 137.56)	<0.001	598.18 ± 181.76	72.20 (10.90, 133.50)	0.02
Jianzhen (SI 9)	453.08 ± 154.54	539.87 ± 167.59	86.79 (56.30, 117.28)	<0.001	627.96 ± 209.67	88.09 (39.88, 136.30)	0.001
Non-acupoints	521.34 ± 147.02	538.67 ± 153.89	17.33 (-36.99, 2.34)	0.08	549.18 ± 143.66	10.51 (-18.87, 39.89)	0.47

Values represented as mean ± SD. The unit of values was Kpa. Abbreviations: SD – Standard Deviation, CI –Confidence Intervals.

PPT comparison of non-acupoint

Figure 2 showed PPT at the non-acupoint. For the patients, the analysis revealed no obvious difference of 17.33 (95% CI, -36.99 to 2.34; p = 0.08) in PPT value on the painful side compared to the non-painful side. Meanwhile, no significant difference of PPT level, which behaved 10.51 (95% CI, -18.87 to 39.89; p = 0.47), was found between the non-painful side of the patients and the ipsilateral side of healthy controls.

Peripheral Sensitization Index

All measured acupoints demonstrated side-to-side difference in the patients. They were used to compute a peripheral sensitization index (PSI). PPT values at acupoints on the painful side below the 25th percentile of the non-painful side indicated peripheral sensitization. The proportion of the patients with peripheral sensitization were 77% at Tianzong (SI 11), 37% at Jianliao (SJ 14), 43% at Jianyu (LI 15) and 60% at Jianzhen (SI 9) (Table 3). Significant correlation (Appendix Table 2) was observed in PSI among Jianliao (SJ 14), Jianyu (LI 15) and Jianzhen (SI 9) ($p < 0.01$). There was no significant association between PSI and other baseline variables ($p > 0.05$) (Appendix Table 3).

Table 3. Frequencies meeting the peripheral sensitization index (PSI) and central sensitization index (CSI) based on PPT response.

Sites	PSI	CSI	PSI×CSI
Tianzong (SI11)	23 (77)	13 (43)	13 (43)
Jianliao (SJ 14)	11 (37)	17 (57)	9 (30)
Jianyu (LI 15)	13 (43)	19 (63)	12 (40)
Jianzhen (SI 9)	18 (60)	18 (60)	15 (50)

Values are individual counts (percentages). Abbreviations: PPT – Pressure-pain threshold.

Central Sensitization Index

The four measured acupoints demonstrated difference between the non-painful side of patients and the ipsilateral side of healthy controls. Therefore, they were used to compute central sensitization index (CSI). The proportion of the patients were 43% at Tianzong (SI 11), 57% at

Jianliao (SJ 14), 63% at Jianyu (LI 15) and 60% at Jianzhen (SI 9) (Table 3). A distinct and significant association (Appendix Table 4) was observed between Jianliao (SJ 14) and Jianyu (LI 15) ($p < 0.01$), Jianyu (LI 15) and Jianzhen (SI 9) ($p < 0.01$) in CSI. Appendix Table 5 showed no statistical significance correlations between CSI and relevant baseline characteristics ($p > 0.05$).

Comparison of Peripheral and Central Sensitization Index

The frequencies of the patients who had both PSI and CSI were 43% at Tianzong (SI 11), 30% at Jianliao (SJ 14), 40% at Jianyu (LI 15) and 50% at Jianzhen (SI 9), respectively (Table 3). Significant correlation was observed between PSI and CSI in measured acupoints ($p < 0.05$) (Appendix Table 6). Table 4 showed that association between subgroups and sex approached statistical significance at the assessment acupoints ($p < 0.001$). No significant association was observed between subgroups and other baseline variable ($p > 0.05$).

Table. 4 Demographic, clinical, and psychological characteristics of sensitization groups.

	Peripheral Sensitization	Central Sensitization	Peripheral and Central Sensitization	No Sensitization	P value
Tianzong (SI11)					
Age (years)	48.78 (43.45, 54.21)	50.38 (44.02, 56.75)	50.38 (44.02, 56.75)	56.57 (48.00, 65.15)	0.47
Sex (%females)	78.28 (56.14, 92.52)	76.92 (46.16, 94.89)	76.92 (46.16, 94.89)	14.29 (3.68, 57.62)	<0.001
Pain duration (weeks)	21.04 (12.91, 29.17)	24.31 (10.23, 38.38)	24.31 (10.23, 38.38)	12.57 (7.16, 17.99)	0.60
VAS	68.04 (61.25, 74.84)	71.15 (62.77, 79.53)	71.15 (62.77, 79.53)	60.00 (52.45, 67.55)	0.34
Jianliao (SJ 14)					
Age (years)	48.09 (38.47, 57.71)	49.59 (43.35, 55.82)	48.89 (38.97, 58.81)	53.27 (46.22, 60.33)	0.78
Sex (%females)	100	82.35 (56.42, 96.62)	100	27.27 (6.01, 60.95)	<0.001
Pain duration (weeks)	22.55 (10.81, 34.28)	23.29 (12.80, 33.79)	22.22 (7.96, 36.49)	11.64 (7.07, 16.21)	0.32
VAS	70.00 (59.17, 80.83)	68.82 (61.14, 76.51)	72.22 (59.60, 84.84)	63.18 (53.41, 72.95)	0.59
Jianyu (LI 15)					
Age (years)	46.92 (38.29, 55.56)	47.47 (41.20, 53.75)	45.42 (36.64, 54.19)	55.10 (48.95, 61.25)	0.32
Sex (%females)	92.31 (63.76, 99.81)	89.47 (66.90, 98.70)	100	20.00 (2.54, 55.41)	<0.001
Pain duration (weeks)	19.69 (9.51, 29.87)	21.89 (12.14, 31.65)	20.67 (9.73, 31.60)	14.80 (9.23, 20.37)	0.76
VAS	68.46 (59.62, 77.30)	66.84 (59.65, 74.04)	66.67 (57.96, 75.38)	62.50 (53.38, 71.62)	0.79
Jianzhen (SI 9)					
Age (years)	48.22 (41.53, 54.91)	48.00 (41.35, 54.65)	46.27 (38.65, 53.88)	53.33 (45.49, 61.17)	0.64
Sex (%females)	83.33 (58.57, 96.44)	83.33 (58.57, 96.44)	93.33 (67.97, 99.83)	33.33 (7.45, 70.06)	<0.001
Pain duration (weeks)	17.11 (9.58, 24.64)	18.22 (10.77, 25.67)	18.67 (9.70, 27.63)	24.00 (6.74, 41.26)	0.78
VAS	67.78 (60.43, 75.13)	66.67 (58.85, 74.48)	68.00 (59.32, 76.68)	65.00 (53.79, 76.21)	0.97

Values presented as % (for sex) or mean with [95% CI]. Abbreviations: VAS – Visual Analogue Acale.

DISCUSSION

The current results were in agreement with a peripherally sensitized state at acupoints which is determined by the side-to-side difference of PPT values in patients with unilateral shoulder pain. Central sensitization at acupoints was conducted by comparing pressure sensitivity in patients with age- and sex-matched healthy controls. No obvious PPT values difference was found at the non-acupoint among the painful side, non-painful side and ipsilateral side. To advance this line of research, association between peripheral and central sensitization at acupoints was examined. We found that the patients displayed a significant association between peripheral and central sensitization at measured acupoints. This finding demonstrated that there existed two patterns of sensitization in acupoints. In addition, three acupoints Jianliao (SJ 14), Jianyu (LI 15) and Jianzhen (SI 9) that are normally used for treating shoulder pain had correlation.

Previous investigations had reported that splanchnic diseases can induce mechanical hyperalgesia on the corresponding acupoints when pressed [24]. Acupoints turned to the "activated mode" or "sensitized mode" from the "silent mode" in pathological conditions. This phenomenon is called "acupoint sensitization". At some acupoints there appeared to be a hypersensitivity of temperature (heat-sensitization) or pain threshold (pain-sensitization) under visceral pain [9, 25]. But unlike those studies, we examined acupoints in patients with musculoskeletal pain in this study.

Central sensitization is challenging clinically, since no standard assessment exists. Some studies recommended the use of various modalities for pain sensitivity at local and distal locations [21, 26]. However, other researches showed that decreased PPTs at the painful and non-painful shoulder, but not at the muscle tibialis anterior [27-29]. According to "Criteria for the Classification of Central Sensitization Pain", patients with diffuse pain distribution, allodynia, and hyperalgesia are more likely to present with central sensitization. One of the patterns of pain distribution is that patients have bilateral pain/mirror pain [17]. In the patients with shoulder pain, the increased sensitivity to mechanical input in the contralateral shoulder would be interpreted as central sensitization [11]. A large number of studies define central sensitization as pain sensitivity at local and distal locations. We chose bilateral pain to define central sensitization, unlike earlier studies, and to determine whether there existed patterns of experimental pain responses at shoulder acupoints.

Collectively, the findings of the study support the alteration in both peripheral and central sensitization at acupoints in patients with musculoskeletal pain. We determined whether peripheral and central sensitization were more likely to occur together or alone. In Table 3, we describe that 19 patients had central sensitization and 13 patients had peripheral sensitization in Jianyu (LI 15). Previous study showed that long-term peripheral sensitization can lead central nervous system changes occurring and resulting in central sensitization [11]. However, there were 6 patients having only central sensitization without peripheral sensitization. This result indicates that peripheral sensitization is not a prerequisite for the presence of central sensitization at acupoints. In PSI or CSI, the strong association was found at three acupoints of Jianliao (SJ 14), Jianyu (LI 15) and Jianzhen (SI 9). From the clinical perspective, Jianliao (SJ 14), Jianyu (LI 15) and Jianzhen (SI 9) are frequently chosen to treat shoulder pain in clinical practice, and have been called "shoulder three acupoints" by acupuncturists [30, 31]. Jianyu (LI 15), Jianliao (SJ 14) and Jianzhen (SI 9), are highly-refined acupoints used to treat shoulder pain in the clinic, and have been proven to be effective at regulating muscle strength and tension of shoulder joint [32]. The strong association among Jianliao (SJ 14), Jianyu (LI 15) and Jianzhen (SI 9) is consistent with the conception of Traditional Chinese Medicine.

To our knowledge, this is the first study to research peripheral and central sensitization at acupoints in patients with shoulder pain. One of the advantages of the study is that the measured acupoints and non-acupoint were marked by an acupuncturist with 24 years of experience in clinical acupuncture treatment. The evaluator who measured PPTs also has extensive experience with using the algometer and without basic knowledge of acupoints. The internal validity is increased by blinding the evaluator who did not know whether the measured sites were acupoints or not during testing. In addition, the evaluator was blinded as to whether the test participant was a patient or a healthy control. The participants were asked to take different positions when different acupoints were measured. For example, the participants were required take a prone position on the examination bed with a suitable pillow under the chest and the arms close to the body when Jianzhen (SI 9) was measured. It increases the reliability of testing PPT over a soft area.

Specifically, the result indicates that peripheral and central sensitization at acupoints is not relevant to pain duration. Moreover, there is no obvious evidence that the pattern of sensitization

has relation with the degree of severity of the clinical condition as measured by VAS. These changes are consistent with the idea of Traditional Chinese Medicine that acupoints are the crucial reflex points of body lesions under pathological circumstance. Hyperalgesia and skin sensitization can occur at corresponding acupoints in the presence of some diseases [33]. Morphological structure studies have reported that the nervous system and blood vessels might have a close relationship with acupoints [34-36]. For example, abundant microvessels existed at the acupoints of Zhongji (RN3) and Zusanli (ST36) in contrast to the surrounding tissues [37]. The acupoints also have a high density of nerve endings including A- and C- afferent fibers [38, 39]. Those characteristics of higher concentration of neural, vascular elements and mast cells could make pain perception more sensitive, and might contribute to peripheral sensitivity. To confirm the specificity of acupoints, we selected a non-acupoint in the infraspinatus muscle. Obviously, no significant difference was found in PPT values among the painful/non-painful side of patients and the ipsilateral side of healthy controls. The finding proved that acupoints become the specifically reflex points that respond to the presence of musculoskeletal pain. Our study provides evidence that there is an association with acupoints sensitization and gender. Pain difference in gender has become an increased topic in recent years, and lower PPT values in women than that in men were found either in healthy subjects or in clinical patients [40]. But, the significant difference may be accounted for by the fact that the majority of the patients is female (63.33%) in the study.

There are limitations that should be kept in mind regarding this study. Pain perception is multidimensional. PPT measurements are just a mechanical and standardized stimulation and are inadequate to describe the complexity of pain perception. The multimodal approach should be used to provide the details of the pain system in both normal and pathophysiological situations, such as different stimulus modalities and quantitative assessment of various pain mechanisms. Second, the non-acupoint was chosen as 2 cm down from Tianzong (SI 11) because the shoulder blade is relatively flat and may reduce the measurement errors between acupoints and non-acupoints. In the clinical trial published recently, the distance was 1 cun (2-3 cm) between the non-acupoints and acupoints, and clinical outcome showed that acupoints treatment alleviated symptoms superior to non-acupoints treatment [41]. The measured sites are adjacent in some studies including PPT measured [20, 42]. The distance is 2 cm between the non-acupoint and Tianzong (SI 11), which is acceptable in our study. For reducing the stimulation effect, there is

an approximately 2 min interval between the repetitions. The probability of a possible stimulation by too close to an acupoint is low but it could not be ruled out completely. In addition, Ashi points also named reflexing points or tender spots. They are the phenomenon acupoints or temporary acupoints, which are dissimilar from acupoints of the fourteen meridians or extraordinary points. Generally, Ashi points have no specific names and definite locations, and will vanish after disease recovered. The aim of our study is to investigate the pattern of experimental pain responses at acupoints, which have specific names and definite locations. For that reason, the Ashi points do not take into account in this study. This study was conducted in a single institution with a small sample size, so the external validity is not clear. This is a cross-sectional study, and it's unknown whether the sensitization at acupoints experiences changes as the disease progresses in longitudinal research. Further studies need to be conducted to confirm the phenomena observed here.

CONCLUSIONS

In conclusion, there exists a mixed presence of sensitization patterns at acupoints in patients with unilateral shoulder pain and a strong correlation among Jianliao (SJ 14), Jianyu (LI 15) and Jianzhen (SI 9). Future research utilizing the multimodal pain approach should be conducted, such as suprathreshold heat pain response, to determine various sensitivity mechanisms.

Legends

Figure 1. The pressure pain assessment sites in the present study. (A) Locations for the pressure pain threshold (PPT) measurement at Jianliao (SJ 14), Jianyu (LI 15) and Jianzhen (SI 9). (B) Locations for the PPT measurement at Tianzong (SI 11) and Non-acupoint.

Figure 2. The values of pressure pain threshold (PPT) at the non-acupoint. Values are mean ± SD.

Footnotes

Contributors Conceived and designed the experiments: Qian-Qian Li, Cun-Zhi Liu. Performed the experiments: Guang-Xia Shi, Qing-Nan Fu, Chao-Qun Yan, Qian-Qian Li. Analyzed the data: Li-Wen Zhang, Xue-Rui Wang. Wrote the paper: Chao-Qun Yan, Shuai Zhang. All authors approved the final manuscript.

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Patient consent Obtained.

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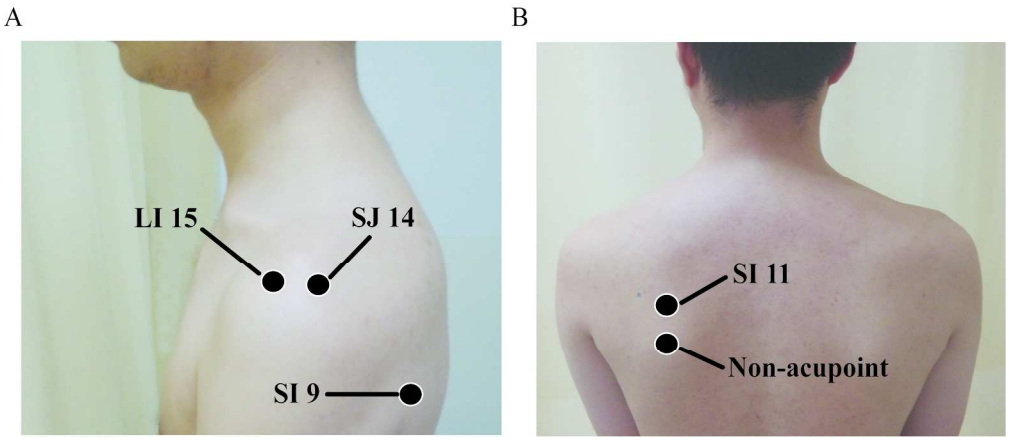

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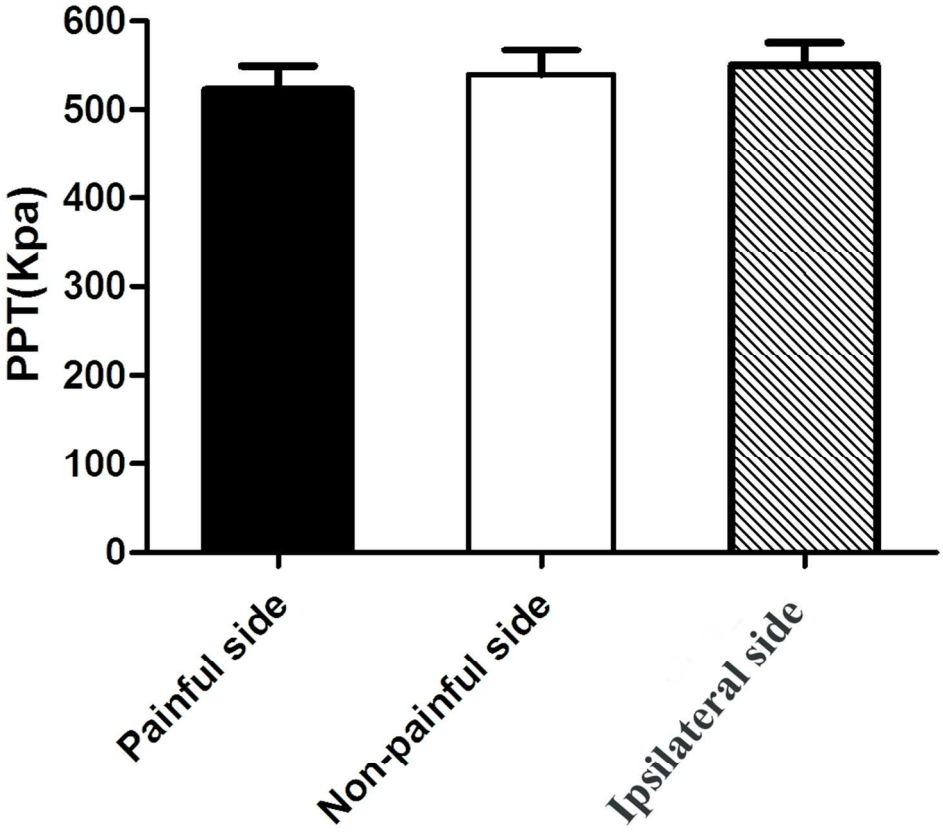

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Appendix Table 1. Locations for the pressure pain assessment sites in the present study.

Points	Location
Jianliao (SJ 14)	In the depression posterior and inferior to the acromion when arm is abducted.
Jianyu (LI 15)	In the depression between the acromial extremity of the clavicle and the great tuberosity of humerus; or when the arm is in full abduction, the acupoint is in the depression at the anterior border of the acromioclavicular joint, and superior to the shoulder joint.
Jianzhen (SI 9)	1 cun directly above the posterior end of the axillary fold when the arm is abducted.
Tianzong (SI 11)	In the depression in the center of the subscapular fossa, at the point 1/3 of the line between the lower border of the mesoscapula and lower angle of capula.
Non-acupoint	2 cm down to Tianzong (SI 11).

Appendix Table 2. Correlation of peripheral sensitization index (PSI) among acupoints.

		PSI			
		Tianzong (SI11)	Jianliao (SJ 14)	Jianyu (LI 15)	Jianzhen (SI 9)
PSI	Tianzong (SI11)	1.00	0.26	0.32	0.35
	Jianliao (SJ 14)	0.26	1.00	0.73*	0.62*
	Jianyu (LI 15)	0.32	0.73*	1.00	0.44*
	Jianzhen (SI 9)	0.35	0.62*	0.44*	1.00

Values are Pearson's correlation. *Significant association between variables ($p < 0.01$).

Appendix Table 3. Correlation between peripheral sensitization index (PSI) and relevant baseline characteristics.

	PSI			
	Tianzong (SI 11)	Jianliao (SJ 14)	Jianyu (LI 15)	Jianzhen (SI 9)
Age (years)	-0.28	-0.13	-0.21	-0.22
Pain duration (weeks)	0.21	0.20	-0.10	-0.20
VAS	0.24	0.16	0.27	-0.12

Values are Pearson's correlation.

Appendix Table 4. Correlation of central sensitization index (CSI) among acupoints.

		CSI			
		Tianzong (SI11)	Jianliao (SJ 14)	Jianyu (LI 15)	Jianzhen (SI 9)
CSI	Tianzong (SI11)	1.00	0.09	0.11	0.17
	Jianliao (SJ 14)	0.09	1.00	0.59*	0.25
	Jianyu (LI 15)	0.11	0.59*	1.00	0.51*
	Jianzhen (SI 9)	0.17	0.25	0.51*	1.00

Values are Pearson's correlation. *Significant association between variables ($p < 0.01$).

Appendix Table 5. Correlation between central sensitization index (CSI) and relevant baseline characteristics.

	CSI			
	Tianzong (SI 11)	Jianliao (SJ 14)	Jianyu (LI 15)	Jianzhen (SI 9)
Age (years)	-0.10	-0.12	-0.32	-0.24
Pain duration (weeks)	0.13	0.29	-0.16	-0.05
VAS	0.33	0.21	0.04	0.01

Values are Pearson's correlation.

Appendix Table 6. Correlation between peripheral sensitization index (PSI) and central sensitization index (CSI).

		CSI			
		Tianzong (SI11)	Jianliao (SJ 14)	Jianyu (LI 15)	Jianzhen (SI 9)
PSI	Tianzong (SI11)	0.48*	0.15	0.40*	0.52*
	Jianliao (SJ 14)	0.17	0.39*	0.58*	0.62*
	Jianyu (LI 15)	0.32	0.36	0.53*	0.58*
	Jianzhen (SI 9)	0.17	0.25	0.65*	0.58*

Values are Pearson's correlation. *Significant association between variables ($p < 0.05$).

STROBE Statement—Checklist of items that should be included in reports of ***case-control studies***

	Item No	Recommendation
Title and abstract	1 **(P1-2)**	(*a*) Indicate the study's design with a commonly used term in the title or the abstract
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found
Introduction		
Background/rationale	2 **(P4)**	Explain the scientific background and rationale for the investigation being reported
Objectives	3 **(P4)**	State specific objectives, including any prespecified hypotheses
Methods		
Study design	4 **(P4)**	Present key elements of study design early in the paper
Setting	5 **(P5-7)**	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls **(P5)**
		(*b*) For matched studies, give matching criteria and the number of controls per case **(P5-6)**
Variables	7 **(P6-7)**	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
Data sources/ measurement	8* **(P6-7)**	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias	9 **(P6-7)**	Describe any efforts to address potential sources of bias
Study size	10 **(P5)**	Explain how the study size was arrived at
Quantitative variables	11 **(P7)**	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding **(P7)**
		(*b*) Describe any methods used to examine subgroups and interactions **(P8)**
		(*c*) Explain how missing data were addressed **(No missing data)**
		(*d*) If applicable, explain how matching of cases and controls was addressed **(No missing data)**
		(*e*) Describe any sensitivity analyses **(NONE)**
Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed **(P8)**
		(b) Give reasons for non-participation at each stage **(No missing data)**
		(c) Consider use of a flow diagram **(NONE)**
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders **(P8)**
		(b) Indicate number of participants with missing data for each variable of interest **(No missing data)**
Outcome data	15* **(NONE)**	Report numbers in each exposure category, or summary measures of exposure

Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included **(P8-11)**
		(*b*) Report category boundaries when continuous variables were categorized **(P11)**
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period **(NONE)**
Other analyses	17 **(P8)**	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion		
Key results	18 **(P14)**	Summarise key results with reference to study objectives
Limitations	19 **(P16−17)**	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	20 **(P14−16)**	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	21 **(P17)**	Discuss the generalisability (external validity) of the study results
Other information		
Funding	22 **(P18)**	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for cases and controls.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.