Clinical Trial/Experimental Study

Medicine

OPEN

Effects of acupuncture on sensory symptoms and motor signs in patients with restless legs syndrome

A crossover randomized controlled trial

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Abstract

Background: Rapid effects of acupoint injection (acuinjection) at 4 acupoints (4P) (ST36, GB41, SP6, and BL60) on legs presenting sensory symptoms and motor signs in restless legs syndrome (RLS) were first described in a case report. This study aimed to confirm the beneficial effects of acuinjection.

Methods: A randomized, controlled, single-blinded, prospective crossover study was conducted during 2018 to 2021. Adult volunteers (age ≥ 20 years) with RLS symptoms for > 2 weeks were included. Eight adults were enrolled and randomized to receive acuinjection (Verum group) or sham injection (Sham group). The effects of acuinjection on discomfort and periodic leg movement (PLM) were evaluated using the suggested immobilization test in a crossover design. The acupoint used was 4P, and normal saline solution (0.1–0.25 mL) was injected in both groups. Leg discomfort was measured using the visual analog scale (VAS), and PLM was measured using an electromyogram. To compare the VAS scores between the groups, repeated measures analysis of variance was used, and the Student *t* test was used to assess the mean discomfort score (MLDS: average of the 6 VAS scores) and PLM index (PLMI) (defined as the degree of PLM/hour).

Results: A significant difference in the VAS score was observed; however, no significant difference was noted in the PLMI between the groups. The MLDS was significantly lower in the Verum group than in the Sham group.

Conclusion: Acuinjection at 4P remarkably inhibited leg discomfort in patients with RLS. Despite the drastic PLM suppression in 1 patient by acuinjection, a statistically significant inhibition of PLM was not confirmed. The results of our study can be applied easily and safely in clinical situations where it is necessary to temporarily reduce or eliminate RLS symptoms.

Abbreviations: 4P = four acupoints, Acuinjection = acupoint injection, ANOVA = analysis of variance, CI = confidence interval, EEG = electroencephalogram, EMG = electromyogram, MLDS = mean leg discomfort score, OSA = obstructive sleep apnea, PLM = periodic leg movement, PLMI = periodic leg movement index, RLS = restless legs syndrome, SD = standard deviation, SIT = suggested immobilization test, VAS = visual analog scale.

Keywords: acupoint injection, acupuncture, periodic leg movement, restless legs syndrome, suggested immobilization test

1. Introduction

Restless legs syndrome (RLS), also known as Willis – Ekbom disease, is a sensorimotor disorder frequently complicated by periodic limb movements (PLMs) during sleep and is a common sleep-related disorder. According to the main diagnostic criteria of the International Classification of Sleep Disorders 3rd Edition (ICSD-3rd),[1] the core symptom of RLS is an urge to move the legs, often combined with uncomfortable sensations in the legs. Based on the abovementioned diagnostic criteria, these symptoms worsen at rest, improve with movement, and predominately occur in the evening or at night.

The prevalence of RLS in adults living in the United States, as determined by phone interviews between 2003 and 2009, with symptoms occurring at least 3 days per week, is 1.8% to 4.5%.[2] Patients without a family history are classified as secondary RLS if they have comorbidities associated with RLS, especially iron deficiency, Parkinson's disease, and chronic renal failure, or as primary RLS if there is no evidence of such comorbidities.[3] Treatment of RLS requires the elimination of inducers,

The authors have no funding and conflicts of interest to disclose.

All data generated or analyzed during this study are included in this published article [and its supplementary information files].

Trial registration: University Hospital Medical Information Network Clinical Trials Registry (https://www.umin.ac.jp/english/); UMIN000030807 - Effects of acupuncture on sensory symptome and motor signs in patients with Restless Legs Syndrome.

Supplemental Digital Content is available for this article.

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How to cite this article: Fukutome T, Murashima K. Effects of acupuncture on sensory symptoms and motor signs in patients with restless legs syndrome: A crossover randomized controlled trial. Medicine 2022;101:51(e32317).

Received: 7 October 2022 / Received in final form: 29 November 2022 / Accepted: 29 November 2022

http://dx.doi.org/10.1097/MD.0000000000032317

including iron deficiency, antidepressants, and caffeine intake, as much as possible. If necessary, pharmacological intervention is recommended. The main treatment options include α2δ ligands and dopamine agonists, although such drugs may not be feasible due to worsening of the RLS or risk of side effects.[4] Side effects include somnolence and dizziness.[5] Worsening or augmentation of RLS symptoms occurs when patients are treated with medications containing a dopamine agonist and includes the onset of symptoms that shift toward daytime hours compared with that before treatment, with an expanding area of discomfort and reduced therapeutic effects.[4] Non-pharmacological treatment is widespread and includes pneumatic compression, near-infrared light spectroscopy of the legs, and massage.[6] However, in cases of severe RLS, the effects of non-pharmacological treatments are insufficient, and in most cases, they are used as support for pharmacological treatment.[6]

Acupuncture that is effective for chronic pain, such as low back pain,[7] is expected to be an effective non-pharmacological therapy for RLS. There are reports showing the effectiveness of acupuncture or electroacupuncture for RLS[8–10]; however, the methods of acupuncture used in these reports were not simple, and/or the clinical effects were not definitive. In the practice guidelines for RLS treatment, acupuncture was not adopted as an effective treatment method[11]; however, recently there has been a tendency to accept the effectiveness of acupuncture.[12]

Acupoint injection (acuinjection) is an acupoint stimulation technique achieved by injecting a solution to treat or prevent illness.[13] Acuinjection is a type of acupuncture that is easy to apply clinically by physicians of the Western world. Patients with RLS in which acuinjection achieved immediate and marked improvement in discomfort (sensory symptoms) and periodic leg movements (motor signs) during wakefulness and sleep were described for the first time in 2018 by Fukutome.[14] In the report, 4 acupoint positions were used (4P: Zusanli [ST36], Zulinqi [GB41], Kunlun [BL60], and Sanyinjiao [SP6]), and the injection included a small dose of normal saline. The purpose of this study was to confirm the hypothesis that sensory symptoms and motor signs in patients with RLS could be inhibited by acuinjections at the 4P region (4P-injection), as assessed using the suggested immobilization test (SIT).[15,16] The primary outcome was alleviating discomfort in the lower legs as indicated by the visual analog scale (VAS) and motor signs as determined by the PLM index (PLMI: defined as the total number of PLMs per hour of SIT).

2. Materials and Methods

The study was approved by the Ethics Committee of the Japan Community Health Care Organization (JCHO) of Kyushu Hospital and complied with the Declaration of Helsinki. The registration ID for this prospective study at the University Hospital Medical Information Network Clinical Trials Registry was UMIN000030807. Written informed consent was obtained from all patients.

2.1. Subjects

Volunteers for this study were recruited through 2 newspaper advertisements in April 2018, and the missing volunteers were recruited among patients who visited the Fukuoka Sleep Clinic. The recruitment was completed in September 2021.

Adult volunteers, aged ≥ 20 years, with suspected RLS who had difficulty falling asleep due to discomfort in the legs, were selected as candidates for this study. The inclusion criteria were as follows: patients who satisfied the diagnostic criteria for RLS according to the International Classification of Sleep Disorders 3rd edition; leg discomfort occurring every night during the previous 2 weeks before enrollment; leg discomfort not accompanied by discomfort in other parts of the body, except for lower back discomfort. The exclusion criteria were the following: patients with a serum ferritin level < 25 ng/mL, which is considered an indicator of iron deficiency anemia[17]; patients with comorbidities associated with RLS, including Parkinson's disease and chronic renal failure, except obstructive sleep apnea (OSA); patients with significant disease in the main organs including chronic renal failure and hemorrhagic diathesis or patients with dementia; patients with traumatic/operative scars at the acupoint site, including 4P; and patients in whom a preliminary SIT was performed before the first SIT that did not correspond to ≥ 20 mm leg discomfort as indicated by the VAS score.

2.2. Study design and data collection

This study was performed at a sleep clinic in a city located in southern Japan. Enrolled subjects were sequentially subjected to the SIT from April 2018 to October 2021.

On enrollment, each of the 8 patients was randomly assigned by a computer to Series A (n = 4) or Series B (n = 4). Randomization was conducted using a computer-generated list of 4 random block sizes, with adjustments to n = 8. In Series A, a sham injection was performed 1 week after the 4P-injection, while in Series B, the order was reversed.

For all patients in Series A and B, a preliminary 30-min SIT was performed 1 hour before SIT, and the onset of leg discomfort was confirmed. The effects of 4P-injection were evaluated using a VAS evaluation, which measured the corresponding leg discomfort observed during the SIT. The VAS evaluation and PLM were compared between patients who received the 4P-injection (Verum group) and patients who received a sham injection (Sham group). The SIT was performed as described by Montplaisir et al[15] and Michaud et al,[16] with some modifications, including the VAS and PLM measurement intervals and the PLM scoring method used.

All 4P acupoints were located in the lower legs. The Zusanli (ST36) was located on the anterior aspect of the leg, lateral to the anterior crest of the tibia at the level of the distal edge of the tuberosity of the tibia. The Zulinqi (GB41) was located on the dorsum of the foot. The Kunlun (BL60) was located in the depression between the prominence of the lateral malleolus and calcaneal tendon. The Sanyinjiao (SP6) was located on the tibial aspect of the leg at the level of the lower fifth of the tibia.

The 4P-injection was performed just before the SIT using a 27-gauge needle. At ST36, SP6, and BL60, the needle was inserted perpendicular to the skin at a depth of approximately 0.5 cm, and 0.25 mL of normal saline was injected. While at the GB41 site, the needle was inserted just below the subcutaneous layer, and 0.1 mL of saline was injected.

Sham injection, containing the same dose of normal saline, was performed on the central lateral skin of the bilateral thigh at the level of the 1/4 cranial part of the femur, which is distant from the paths of the main meridians. Acuinjection, including sham injection, was performed by 1 of the authors.

SIT was started immediately after acuinjection or sham injection. Each SIT started at approximately 21:00 (± 5 minute). During this test, the patients remained in bed and were maintained at a 45-degree angle by reclining and maintaining the legs outstretched. Movement or falling asleep was not allowed. The patients were instructed not to move, talk, or fall asleep during the SIT. The electroencephalogram (EEG), electrooculogram, and chin electromyogram (EMG) were monitored according to the AASM Manual for the Scoring of Sleep and Associated Events: Rules, Terminology and Technical Specifications. Version 2.1[18] to monitor the state of alertness. If the patients fell asleep showing any EEG stages of sleep lasting > 15 seconds during 1 epoch (30 seconds), they were awakened immediately. The EMG of the right and left anterior tibialis muscles was used to quantify leg movement. PLMs were scored according to the

AASM Manual for the Scoring of Sleep and Associated Events: Rules, Terminology and Technical Specifications. Version 2.1,[18] while the inter-leg movement interval for PLM definition was set to 10 and 90 seconds according to World Association of Sleep Medicine (WASM) 2016 standards.[19] The PLMs were counted every 10 minutes up to 1 hour separately for each leg. The PLMI included the number of events of the left and right PLM. If the test was interrupted prematurely due to unbearable discomfort, the PLMI was calculated as the number of PLM × 60/duration of the test (minute).

Leg discomfort, including lower back discomfort, was measured during the SIT using a VAS as described by Michaud et al[16] Every 10 minutes from the start of SIT, the patient was required to estimate and mark their degree of leg discomfort on the VAS, which consisted of a 100-mm horizontal bar. The VAS evaluation also included the discomfort that appeared during the SIT other than in the legs and lower back. Encouraged by the technician who entered the SIT room, the patient recorded the VAS scores with a pen. To minimize the stimulation caused by entering the room and calling out, the recording was made every 10 minute instead of every 5 minute as described by Michaud et al.[16]

The left end point of the bar corresponded to "no discomfort" with a score of 0 mm, and the right end point corresponded to "unbearable discomfort" with a score of 100 mm. If the VAS reached 100 mm in <60 minute, the SIT was discontinued, and all subsequent VAS scores were set to 100 mm. Leg discomfort was recorded for both left and right legs, and the larger VAS was adopted. If there was a large difference in discomfort in the lower legs, the degree of discomfort felt by the patient was not indicated by the VAS calculated as the average of the left and right legs but was a VAS indicative more of discomfort. Therefore, our analysis considered the larger VAS score as an index representing the actual patient condition. The VAS score was indicated by the length to the marked point in mm. The mean leg discomfort score (MLDS) was defined as the average score of these 6 measures.

EEG and EMG were collected and analyzed by authorized sleep technicians of the Japanese Sleep Society. The patient and attending technician were not informed whether the patient was undergoing verum injection or sham injection. The technician saved the analysis results, and the authors of this report received a copy of the analysis results.

In this study, both the patient and sleep technician in charge were blinded for the verum/sham acuinjection, resulting in a double-blinded study. However, at the time of application to the ethical committee, blinding of the technician was expected to be difficult, and thus, the study was registered as a single-blinded randomized control trial.

2.3. Statistical analyses

In the report by Michaud et al,[16] which examined leg discomfort among subjects with and without RLS, the VAS score for discomfort after 60 min of the SIT was 56.7 ± 40.6 (mean ± standard deviation [SD]) in subjects with RLS and 11.4 ± 28.9 in subjects without RLS. From this study, a sample size of 7 patients was estimated to detect a statistically significant VAS difference between the Verum and Sham groups with a power of 0.80 and an alpha level of 0.05. We added 1 patient to obtain an even number for the crossover study.

The group analysis was performed using the independent samples t-test for normally distributed data. The Shapiro–Wilk test was used to analyze the normality of the data. Within group statistical analysis was performed using repeated measures analysis of variance (ANOVA) with a Greenhouse-Geisser correction (factors; time [10–60 minutes], and groups [Verum vs Sham groups]). The results were expressed as means ± SDs. A P value of <.05 was considered statistically significant. All statistical analyses were performed using EZR version 1.5[20] (kanda, https://www.jichi.ac.jp/saitama-sct/SaitamaHP.files/statmedEN.html), a graphical user interface for R 2.13.0 (R Foundation for Statistical Computing, Vienna, Austria).

3. Results

3.1. Study population

Between April 2018 and September 2021, there were a total of 53 applications for subject enrollment, including 33 individuals who applied after seeing a newspaper advertisement and 20 who applied among the RLS patients who visited our clinic. Of these, 26 subjects met the inclusion criteria, and 8 were excluded by the exclusion criteria. As a result, 18 subjects remained as candidates (Fig. 1). All candidates underwent a routine physical examination and standard medical checkup examinations, including biochemical and hematological tests, electrocardiogram, and home sleep apnea testing. Among the candidates, ten subjects who did not show leg discomfort with a VAS score of ≥ 20 mm were excluded from the preliminary SIT performed before the first SIT evaluation. The remaining 8 subjects were enrolled in this study.

3.2. Baseline characteristics

This study completed all scheduled SITs and ended in September 2021. All patients were Japanese adults, and their basic characteristics are shown in Table 1. The mean age of the participants was 62.75 ± 17.83 (mean ± SD) years (range 25–81), the body mass index was 21.91 ± 2.86 kg/m^2, the history of RLS was 2 to 15 years, and the International Restless Legs Syndrome Study Group rating scale score was 22.63 ± 3.74.

The physical examination and electrocardiogram showed no abnormalities in all patients. In 6 patients, the comorbid conditions included hypertension (n = 4), OSA (n = 4, patients were treated with continuous positive airway pressure or an oral appliance, except for 1 patient), dyslipidemia (n = 3), angina (n = 2), hyperuricemia (n = 1), and atrial fibrillation (n = 1). In the remaining 2 patients, no comorbid conditions were identified. Six patients were treated orally with antihypertensive drugs (n = 4), hypolipidemic drugs (n = 3), antihyperuricemic drugs (n = 1), anticoagulant drugs (n = 1), and nitrovasodilators (n = 1). In 2 patients, RLS was treated with pramipexole at a dose of 0.375 mg or 0.750 mg.

Three of the 8 patients were primary RLS without any predisposing or precipitating factors related to RLS. However, 1 of the remaining 5 patients, with a probable indication for continuous positive airway pressure treatment based on a ≥ 3% oxygen desaturation index (ODI3%) equivalent to an Apnea–Hypopnea Index of ≥ 15,[21] had not been diagnosed with OSA. In all of these 5 patients, precipitating factors[1] including OSA and iron deficiency (serum ferritin level ≤ 75 ng/mL) were complications. Thus, the RLS observed in these 5 patients was considered probably secondary, although 2 of the 5 patients also reported a positive familial history.

3.3. Effects of 4p-injection on leg discomfort and PLM

The VAS score increased over time in all patients in the Sham group and was 93.9 ± 16.5 mm after 60 min. Conversely, in the Verum group, the VAS score did not increase with time in 3 patients, and in the remaining 4 positive VAS scores were obtained in the last half of the SIT. As a result, the VAS score after 60 min was significantly lower in the Verum group than in the Sham group. (30.4 ± 3 7.1 vs 93.9 ± 16.5 mm, 95% confidence interval [CI]: −89.73 to −29.73) (P < .001). The MLDS in the Verum group was significantly decreased than that in

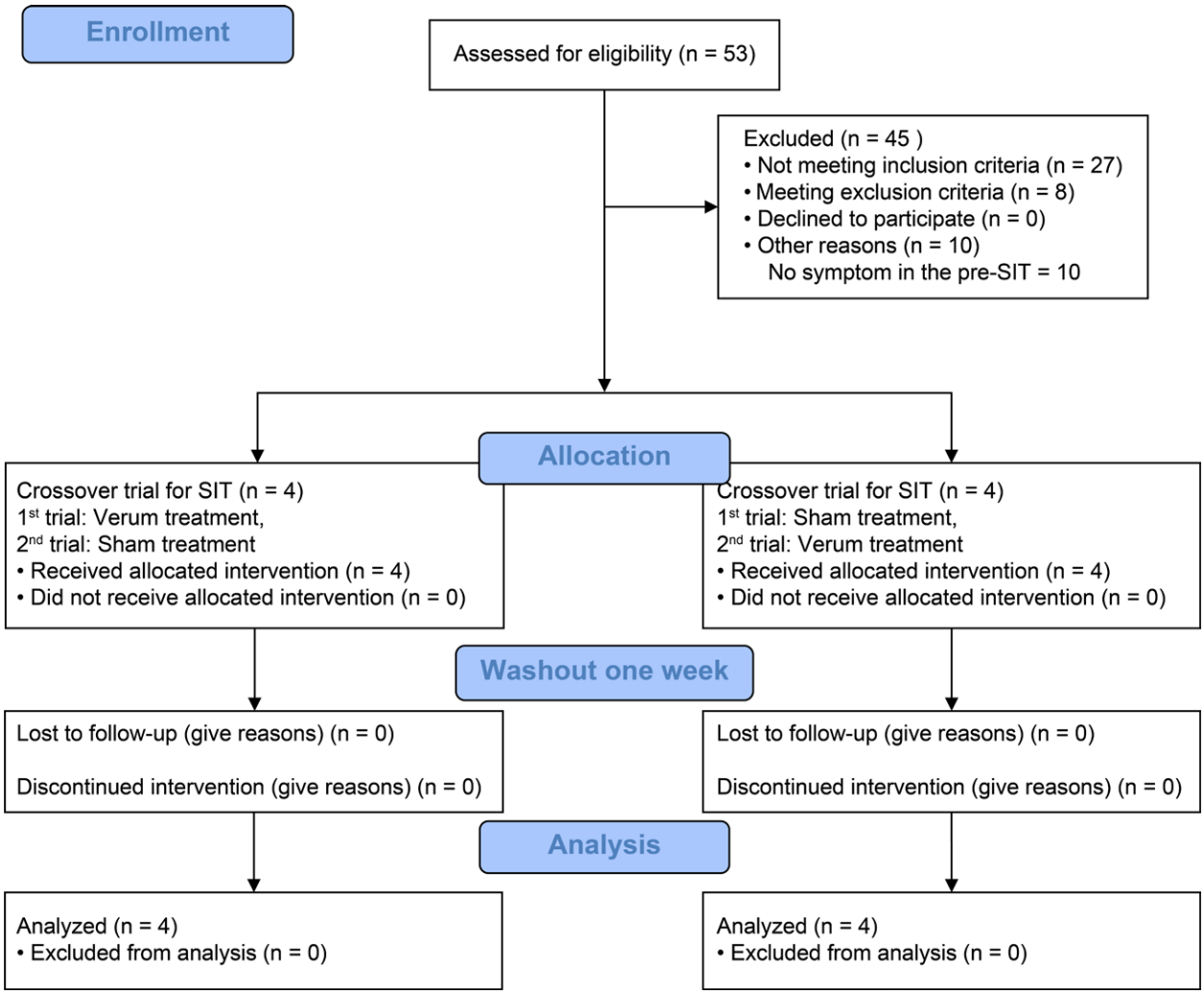

Figure 1. CONSORT Flow Diagram for recruitment of participants in this study and for progression of the crossover study. SIT = suggested immobilization test.

Table 1

Patient characteristics.

Pt. No	SIT series	Age (yrs)	Sex	BMI (kg/m²)	IRLS	RLS history (yrs)	Familial history	ODI 3%/serum ferritin (ng/mL)	Complications
1	A	66	F	25.7	23	16	Yes	33.6/66.5	OSA
2	B	65	F	18.7	19	15	Yes	1/37.4	
3	A	73	F	24	18	2	No	0.5/73.9	OSA (CPAP), HL, HT
4	B	73	M	25.6	23	2	No	7.5/80.4	Af HT, HL, HU Angina
5	B	70	M	19.4	27	2	No	19.7/72.8	HT, OSA (CPAP)
6	B	49	M	20.5	24	34	Yes	1.3/134	OSA (OA)
7	A	81	M	22	19	7	No	6.4/123	HT, HL, Angina
8	A	25	F	19.4	28	9	No	3.2/46.1	

Pt. No.: patient number, SIT series: In A, Verum treatment was the first SIT and Sham treatment was the second SIT, while in series B, the order was reversed.
Af = Atrial fibrillation, BMI = body mass index in kg/m², CPAP = continuous positive airway pressure, Familial history = familial history of RLS. ODI 3%: number of desaturation events ≥ 3% per hour in the home apnea sleep test, HL = hyperlipidemia, HT = hypertension, HU = hyperuricemia, IRLS = International Restless Legs Scale, OA = oral appliance, OSA = obstructive sleep apnea, RLS history = History of RLS symptom (yrs).

the Sham group (15.38 ± 7.95 vs 57.75 ± 18.91 mm, 95% CI: −59.19 to −19.81) ($P < .001$) (Table 2). Repeated measures ANOVA demonstrated a significant difference in the VAS score between groups [$F(1,15) = 18.29$; $P < .001$] and a significant effect over time [$F(6,90) = 23.87$; $P < .001$]. The ANOVA revealed a group by time interaction [$F(6,90) = 7.94$, $P < .01$] (Fig. 2). In both the Verum and Sham groups, PLM was confirmed in 4 patients. There were no significant differences in the mean PLMI between the groups (Verum vs Sham groups: 43.00 ± 69.83 vs 41.63 ± 56.57, 95% CI: −69.53 to 66.78) ($P = .97$) (Table 3).

No significant acuinjection-related complications, except for mild injection-related pain, were observed in either patient.

3.4. Four patients with insufficient results after acuinjection

Patient 1: A 64-year-old woman had been experiencing leg discomfort for 16 years and was receiving pramipexole 0.750 mg

Table 2

Discomfort visual analog scale scores during the suggested immobilization time for the Verum and Sham groups.

	Verum group							Sham group						
Time: min														
Pt. No.	0	10	20	30	40	50	60	0	10	20	30	40	50	60
1	3	19	48	55	64	66	68	0	8	10	13	22	55	98
2	0	0	0	0	0	0	0	0	77	97	100	100	100	100
3	0	1	0	0	0	0	0	1	7	8	34	74	92	100
4	0	0	0	0	0	22	39	0	0	40	97	97	100	100
5	26	27	30	29	30	29	29	8	32	60	69	79	98	100
6	0	3	3	4	4	7	7	0	7	64	93	100	100	100
7	0	0	0	0	42	89	100	0	42	100	100	100	100	100
8	0	5	6	3	0	0	0	0	11	47	47	52	52	53
MLDS	15.38 ± 7.95							57.75 ± 18.91 ($P < .001$)						
60-min-LDS	30.4 ± 3 7.1							93.9 ± 16.5 ($P < .001$)						

Significant differences were noted in the MLDS and 60-min-LDS between the Verum and Sham groups in the 2-sample *t* test ($P < .001$).

60-min-LDS = leg discomfort score after 60 mins (average of the 6 VAS scores), MLDS = mean leg discomfort score (average of the 6 VAS scores), Pt. No. = number of patients, Time = mintime from the start of the suggested immobilization time in mins, VAS = visual analog scale. No discomfort in the legs was set to a score of 0 mm, and unbearable discomfort was set to a score of 100 mm. Leg discomfort was recorded every 10 mins for both left and right legs, and the larger VAS was adopted.

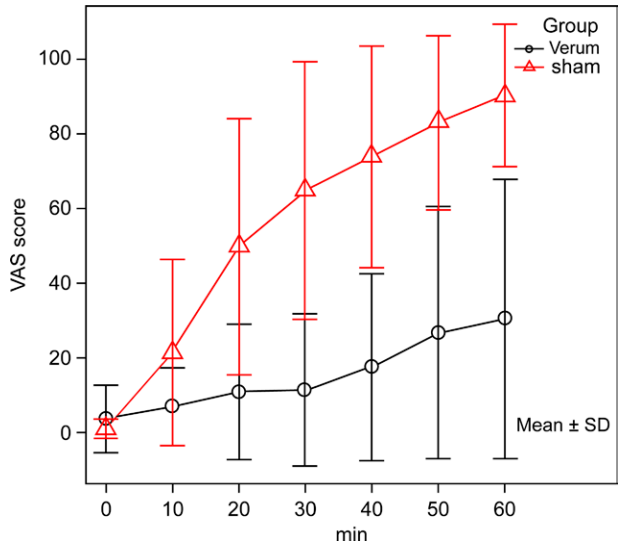

Figure 2. VAS scores of leg discomfort during SIT. The vertical axis depicts the VAS scores, which indicates leg discomfort. The horizontal axis indicates times in minutes after starting the SIT. Verum: Verum group with acuinjections on 4 acupoints. Sham: Sham group with sham injection. Repeated measures ANOVA with a Greenhouse-Geisser correction demonstrated significant difference in the VAS score between the groups [$F(1,15) = 18.29$; $P < .001$] and a significant effect for time [$F(6,90) = 23.87$; $P < .001$ and revealed a group by time interaction [$F(6,90) = 7.94$, $P < .001$]. SIT = suggested immobilization test, VAS = visual analog scale.

per day without complete remission. The home sleep apnea test showed an ODI3% of 33.6, suggesting untreated OSA. After the 4P-injection, discomfort on the right side and PLM in both legs continued. After the SIT, a skin wound far from the ST36 injection site was found, suggesting an incorrect acuinjection. The operator's position on the right side of the sitting patient was speculated to have caused a misidentified ST36 location resulting in a failed acuinjection.

The patient was informed that the injection point of the acuinjection was misaligned and that observation of additional injections would be beneficial in the future for treatment of RLS. Consequently, the patient consented to additional acuinjection.

The operator was repositioned in front of the patient whose knees were flexed at 90° on the bed. An additional acuinjection with 0.25 mL of normal saline on the right ST36 immediately inhibited her discomfort and PLMs of both legs. The operator was positioned in front of the patients for subsequent acuinjections at the ST36 site.

Patient 4: A 73-year-old male had been experiencing itchy discomfort of the legs and lower back at night for 2 years. After the 4P-injection, no discomfort was observed in either leg during the SIT. However, discomfort on the back at the level of the lower edge of the scapula occurred during the second half of the SIT.

Patient 5: A 70-year-old male had been experiencing unbearable itchy discomfort of both lower legs at night for 2 years. With the sham injection, the VAS score increased with time and reached a maximum of 100 mm after 60 minutes. After the 4P injection, mild discomfort was observed in both thighs, with VAS scores ranging from 26 to 30 mm throughout the SIT.

Patient 7: An 81-year-old male had experienced unbearable itchy sensations of the lower legs at night for 7 years and had been treated with pramipexole 0.375 mg daily, without complete remission. In Sham injection, the VAS score increased to the maximum in the first half of the SIT. Although discomfort was inhibited in the first half of the SIT after 4P injection, in the second half, the discomfort of both legs increased to the maximum of the VAS score (Table 3).

The study protocol in Japanese and English are included as Supplementary files, http://links.lww.com/MD/I149.

4. Discussion

This study was conducted to confirm the effects of acuinjection at the 4P site on sensory symptoms and motor signs of patients with RLS. Using the MLDS, we found that the VAS score was significantly lower in the Verum group than in the Sham group. Further, repeated measures ANOVA analysis revealed a statistically significant difference in the VAS scores between the 2 groups. A qualitative interaction was observed between the 2 groups. The 4P-injection clearly inhibited discomfort as a sensory symptom in patients with RLS. Conversely, the PLMI as a motor sign was not significantly different between the 2 groups ($P = .96$), although in 1 patient, additional acuinjection on ST36 inhibited PLMs almost completely. Thus, our hypothesis was partially supported, and confirmation of the effects on motor signs has been left to future study.

4.1. Assessment of the method of acuinjection

Watanabe showed that discomfort, such as pain caused by cancer, could be inhibited by acuinjection using a small dose

Table 3

The periodic leg movement index during the suggested immobilization time for the Verum and Sham groups.

	Verum group							Sham group						
Time: min														
Pt. No.	10	20	30	40	50	60	PLMI	10	20	30	40	50	60	PLMI
1	29	33	30	24	24	26	166	0	0	0	0	4	19	23
2	0	0	0	0	5	0	5	0	16	16	26	23	30	111
3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	27	23	22	26	23	22	143	27	35	30	23	28	26	142
6	0	0	0	0	0	0	0	0	0	0	0	DO	DO	0
7	0	0	0	6	19	DO	30	7	12	DO	DO	DO	DO	57
8	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mean ± SD	43.00 ± 69.83							41.625 ± 6.57, $P = .96$						

PLMs of both legs were counted every 10 min. The PLMI represents the total number of periodic leg movements per hour of the SIT. In case the SIT was interrupted prematurely because of unbearable discomfort, the PLMI was calculated as number of PLMs × 60/duration of the test (min). No significant differences were noted in the PLMI between the Verum and Sham groups in the 2-sample *t* test ($P = .96$). Time: time from the start of the suggested immobilization time (SIT) in mins, Pt. No.: number of patients.

DO = drop out, Mean ± SD = mean and standard deviation of the PLMI, No. = number of patients, PLMI = periodic leg movement index.

of the injecting solution, such as 0.1 mL of normal saline, on the acupoints in the meridian/vessel that pass through the discomfort area.[22] The 4P sites are located along the meridians that pass through the whole side of the leg, including the ST36 along the stomach meridian that passes through the anterior surface of the leg, BL60 along the bladder meridian through the posterior side, SP6 along the spleen, kidney, and liver meridians through the inside, and GB41 along the gallbladder meridian through the outside. In our study, normal saline at a dose of 0.25 mL for ST36, SP6, and BL60 was used as the injection solution, which is the same dose used in previously reported cases,[14] while a dose of 0.1 mL was used for GB41, which was very sensitive within the subcutaneous layer.

In traditional Chinese medicine, many acupoint locations are determined using measurements of the thumb-width[23,24] suggesting that the acupoint is not a point but is spread over an area. Although, in the report of Fukutome,[14] the depth of the acuinjection was not specified, a depth of approximately 0.5 cm at ST36, BL60, and SP6 and a depth just below the subcutaneous layer at GB41 were used. The same depths were used in our study. However, it is possible that the target acupoint was not effectively activated by the depth of the acuinjection and the volume of normal saline used in this study, which may have caused the lack of effect observed by the 4P-injection in the 4 patients in our study.

4.2. Insufficient effects of 4p-injection in 4 patients

In 4 patients, 4P-injection could not inhibit the discomfort in the legs and back during the SIT. In patient 1, an additional acuinjection on the right side ST36 immediately inhibited the remaining discomfort of the right leg and PLM of both legs. This patient's outcome suggested that ST36 was the main acupuncture point for relieving leg discomfort.

In patients 4, 5, and 7, at 30 minutes after sham injection, the VAS score reached 69-100 mm, while the VAS score remained at 0 to 29 30 minutes after verum injection. These findings confirmed the beneficial effects of the 4P-injection, while the effect of the acuinjections used in this study was insufficient to ensure a sufficient effect. During the SIT in patient 4, an itchy discomfort developed on the back at the level of the lower edge of the scapula, where both the bladder meridian (to which BL60 belongs) and the governor vessel (which pass along the midline of the back) pass through. Inhibition of some discomfort in regions other than the legs and lower back may require the activation of a more specific related acupoint other than the 4P.

4.3. Mechanism of acupuncture-mediated inhibition of RLS symptoms

Brain iron imbalance, which is an important underlying pathology of RLS,[6,25] influences dopamine metabolism,[6] causes a disturbance of the descending inhibitory dopaminergic projections from the A11 area to spinal dorsal horn cells, and may result in increased activity in the spinal cord somatosensory tract and preactivation of the subliminal muscle.[6,26] An imbalance in brain iron levels alters glutamine metabolism, causing a hyperglutamic state in the striatum, related to insomnia, and influencing the thalamic activity in the thalamocortical circuit.[27] In addition to dopamine and glutamine, GABA and opioids are involved in the pathology of RLS.[28] Endogenous opioids are believed to be decreased in sensory pathways in patients with RLS.[29]

Such pathological changes in the neural transmitter/modulator can cause modulation of the neural pathway at various levels. Therefore, "RLS should be regarded as a complex sensorimotor disorder in which cortical, subcortical, spinal cord, and peripheral nerve generators[26] are involved" in a network disorder, "resulting in enhanced excitability and/or decreased inhibition."[26]

The "Gate Control Theory" explains some of the mechanisms of pain relief obtained by acupuncture.[30,31] The spinal pathways of impulses coming from the acupoint needling are transmitted through the peripheral sensory nerve and ascend mainly through the spino-ventrolateral funiculus, and a set of nuclei structures involved in the process of pain control are stimulated. These nuclei include the hypothalamus and brain stem nuclei of the periaqueductal gray matter, rostral ventromedial medulla, and nucleus raphe magnus. Then, the processed signal of acupuncture is transmitted downward to the dorsal horn and inhibits pain signals.[30,32] Thus, acupuncture can modulate pain transmission and processing at multiple levels, including the peripheral sensory nerve, spinal cord, thalamus, brain stem, and cortex,[33] resulting in inhibition of pain.

Neurotransmitters and/or modulators of neural pathways associated with the analgesic effects induced by acupuncture include opioid peptides, serotonin, dopamine, glutamate, and their related receptors.[30,34] Among these, opioids and the corresponding receptors in the pathway of the arcuate nucleus-periaqueductal gray matter-nucleus raphe magnus-spinal horn have an important role in the transmission of acupuncture analgesia.[31] Thus, the neural pathway and neurotransmitter/modulators, which are related both to RLS and acupuncture, overlap at multiple levels, especially for opioids. It was assumed that acupuncture inhibits sensory symptoms and motor signs of RLS through these overlapping pathways.

4.4. Clinical relevance of the findings

Ample clinical applications exploit the inhibitory effects of acuinjection on the sensory symptoms of RLS. In treating RLS patients with severe augmentation, discontinuation of the current RLS medication is recommended, followed by introduction of a new drug after a 10-day washout period.[4] 4P injection, the ST36 injection alone, although not definitive, is considered useful for resolution of RLS symptoms during the washout period without affecting the new drug.

In the diagnostic polysomnography of OSA complicated by RLS, PLMS's intervention in apnea events can cause difficulties in assessing respiratory flow waveforms and EEG. In addition, insomnia due to RLS causes shortening of sleep time, thus affecting mandatory sleep time. 4P injection, likely the ST36 injection alone, may solve these problems without significant side effects.[14]

4.5. Limitations of the study and further work

It was unclear whether all 4P acupuncture points were required to inhibit the RLS symptoms, and the most effective acupuncture point should be identified in the future. The duration of the effect of acuinjection using a small dose of normal saline may be too short.[14] Thus, identifying a method of activating acupoints that lasts the duration of a night's sleep and has a powerful activating effect is warranted. The effects of acupuncture on PLM in patients with RLS need to be clarified further.

Interestingly, we found that the effect-duration of the acuinjection with normal saline was no longer than 1 night, and that the patients who received verum acuinjection showed no reduction in RLS symptoms at their return visit 1 week later. However, the duration of the effect of acuinjection was not evaluated in this study. Therefore, the validity of the 1-week period used as the washout interval of verum and sham acuinjection should be verified.

4.6. Future research and conclusions:

These limitations are expected to be resolved in future research. Acupuncture is thought to exert beneficial effects on the neurological pathology of RLS by modulating neural pathways, as suggested by the gate control theory. Thus, acupuncture acts to pinpoint the regions necessary for improving symptoms. Acupuncture is considered to be almost free from side effects, which is in contrast to the side effects often observed by the use of medications. The characteristics of the therapeutic mechanisms of acupuncture may provide useful clues for the ideal treatment of RLS. Research on the mechanisms underlying the beneficial effects of acupuncture is desired.

In this study, 4P-injection could not be statistically confirmed to inhibit PLM. However, since the acuinjection at 4P or ST36 inhibited the PLM in patient 1 and the PLM during wakefulness (PLMW) and PLMS in the report by Fukutome,[14] it is reasonable to assume that the PLMW/PLMS-inhibiting effect of acuinjection is real. PLMS is associated with sympathetic activation, which might be related to cardiovascular diseases.[35] Therefore, the effects of acuinjection on PLMS-related sympathetic activation are of clinical interest.

In conclusion, the study findings strongly suggest that acupuncture, including acuinjection, is beneficial in treating RLS. However, further research is required before acuinjection for patients with RLS can be adopted clinically.

Acknowledgments

We are grateful to Matsuoka H. Minami A. and Minami H. for performing the SIT and data analysis. We also thank Tanamachi N. and all the staff of Fukuoka Sleep Clinic for supporting this study. We thank Editage (https://www.editage.com/) for critically reviewing the statistical methods and analysis of this manuscript.

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