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Original Article

Effect of transcutaneous electrical acupoint stimulation on bone loss for patients with foot and ankle fracture: a pragmatic randomized controlled trial

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Abstract: Objective: The aim for this trial was to preliminarily evaluate the effectiveness and safety of transcutaneous electrical acupoint stimulation (TEAS) for bone loss in patients with immobilization after surgical fixation of ankle and foot fractures. Methods: A total of 80 patients with immobilization after surgical fixation of ankle and foot fractures were randomly divided into an intervention group (n=40) or control group (n=40). The intervention group was given TEAS treatment combined with routine orthopedic treatment, and the control group was given only routine orthopedic treatment. The CT attenuation values, bone turnover markers (ALP, PINP, BGP, CTX, Ca/Cr), bone mineral density (BMD), blood phosphorus, and blood calcium were observed and compared between the two groups at 8 weeks. This was a prospective study. The protocol was registered in the Chinese clinical trial registry (No. ChiCTR2000039944). Results: The CT attenuation values of the intervention group decreased more than those of the control group (P<0.05), however the between group differences in ALP, BGP, Ca/Cr, CTX and BMD (all P>0.05) were not statistically significant. Three mild adverse events were recorded. Conclusion: TEAS treatment may confer additional benefits for bone loss in patients with immobilization after surgical fixation of ankle and foot fractures. Since this was a pilot study, the efficacy of TEAS requires further evaluation through full-scale randomized controlled trials.

Keywords: Ankle and foot fracture, transcutaneous electrical acupoint stimulation, orthopedic treatment, bone loss, randomized controlled trial

Introduction

Bone fracture is one of the most common clinical diseases [1, 2]. When the fracture is healing, patients with foot and ankle fractures receive surgical fixation and a period of fixation treatment [3-5]. This process reduces the mechanical stress on bones but as a result causes bone loss [6, 7]. Studies have suggested that patients who are bedridden after fractures may experience bone mineral loss in their lower extremities, which is related to a reduction of weight bearing [8-11]. Other studies have shown that in individuals with lower extremity injuries, a significant correlation exists between the decrease in bone mineral density (BMD) and an acceleration of bone turnover [12, 13]. Although there are several standard methods to prevent bone loss, such as hormone replacement therapy, bisphosphonates, denosumab, and parathyroid hormone, the inherent side effects of their long-term use have drawn attention [14].

Transcutaneous electrical acupoint stimulation (TEAS) is derived from acupuncture and is characterized as a safe, portable, and simple operation, as an alternative to standard methods. As a result, TEAS is becoming increasingly popular worldwide [15, 16]. Several studies have dem-

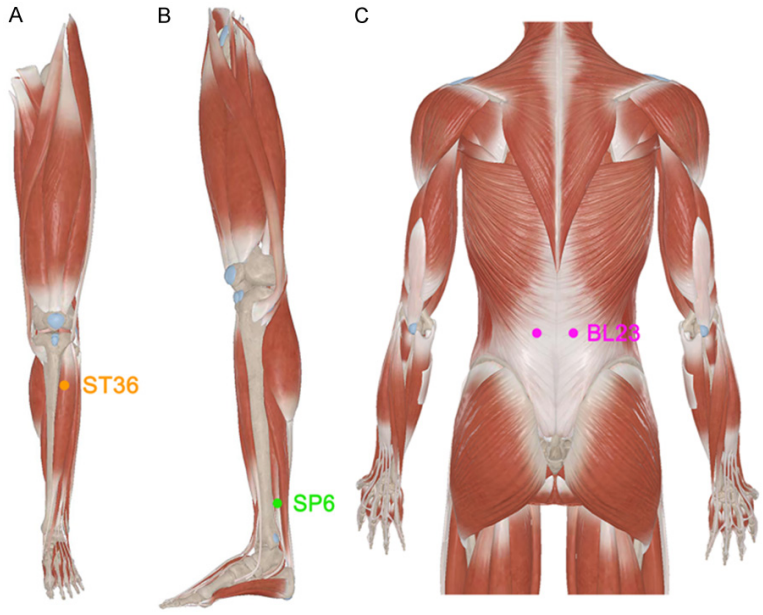

Figure 1. Location of acupoints. Notes: A. ST36: Zusanli. B. SP6: Sanyinjiao. C. BL23: Shenshu.

onstrated that TEAS therapy has similar therapeutic effects to acupuncture in many diseases [17-22]. TEAS has long been recognized as a potentially effective non-drug therapy for fracture treatment, by reactivating bone regeneration and fracture healing [23]. Through appropriate stimulation frequency, TEAS can passively contract muscles, promote osteoblast activity, and inhibit bone resorption, thus achieving the effect of preventing and treating osteoporosis (OP) [24, 25].

TEAS combines pulsed electromagnetic fields (PEMF) with Traditional Chinese medicine (TCM) acupuncture points, which work synergistically [26]. In normal long bones, the metaphysis is electronegative, and the diaphysis is neutral. When a fracture occurs, the metaphysis becomes more electronegative, and the change in electronegativity remains until the fracture heals. Several studies have suggested that PEMF can improve this change in electronegativity, by enhancing the formation of osteoblasts in human bone marrow stromal cells or skeletal stem cells, and ultimately preventing bone loss [27-30]. A large number of studies have suggested that PEMF can also regulate bone metabolism in fracture patients, enhance bone formation, increase BMD, and reduce pain [31-36]. As well, according to previous studies [37-40], and our clinical experience, bilateral Zusanli (ST36), Sanyinjiao (LI11), and Shenshu (BL23) can increase BMD and bone strength, and inhibit the progression of OP. Therefore, TEAS therapy, which combines PEMF with TCM acupoints, could play a positive therapeutic role in OP.

Although it has been proven that the postoperative fixation of ankle and foot fractures can affect bone metabolism and lead to bone loss [41, 42], there is little evidence to show the effectiveness or safety of TEAS in terms of bone loss in patients with postoperative fixation of ankle and foot fractures. Accordingly, we designed a prospective pilot randomized controlled trial, to evaluate the efficacy of TEAS on bone loss in patients with immobilization after foot and ankle fractures. Results from this trial will help inform the design of a future multicenter, large, randomized controlled trial.

Materials and methods

General materials

A total of 80 patients with immobilization after surgical fixation of ankle and foot fractures, receiving treatment in the Beijing Luhe Hospital from April 2021 to April 2022, were randomly divided into an intervention group (n=40) and the control group (n=40). This study was approved by the medical ethical review committee of Beijing Luhe Hospital affiliated to Capital Medical University (No. 2020-LHKY-055-02) and Beijing University of Chinese Medicine (No. 2020BZYLL6011). This was a prospective study. The protocol was registered in the Chinese clinical trial registry (No. ChiCTR2000039944) prior to participant enrollment. This trial obtained the informed consent of all participants. The flowchart of the study is presented in **Figure 2**.

Inclusion criteria: Patients were included if they met the guidelines for the diagnosis and treatment of common diseases in orthopedics and

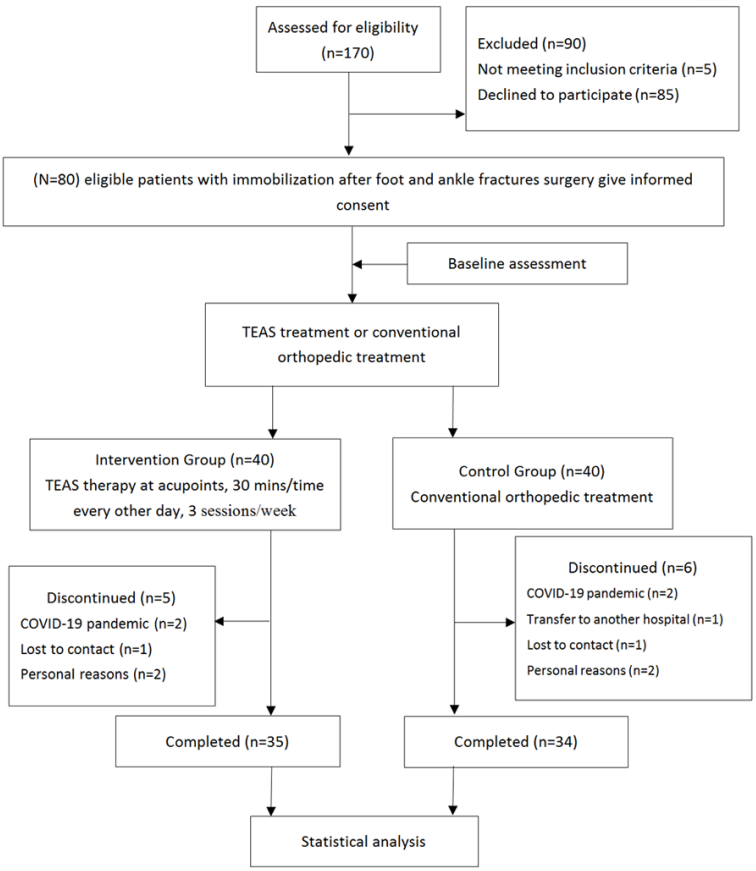

Figure 2. Flow diagram of study design.

traumatology of traditional Chinese medicine [43]; were diagnosed with foot and ankle fractures by imaging and related orthopedics; if imaging examination by X-ray (CT or MRI can be used if necessary) diagnosed patients as having foot and ankle fractures of the distal tibia, fibula, talus, metatarsal and ankle; were aged 18-45 years old (either sex); they satisfied the diagnosis points after surgical fixation of ankle and foot fractures; were fully conscious; the length of internal fracture fixation did not exceed 15 cm above the ankle joint; they agreed to sign an informed consent form.

Exclusion criteria: Patients with endocrine or immune diseases that affect bone metabolism; long-term use of drugs affecting bone metabolism; who had taken calcium, calcitonin, vitamin D and other drugs affecting bone metabolism within 30 days; who had severe organic diseases or mental disorders; who had a history of joint replacement or a stent placed in the body; who had severe infection of limbs, or pathological, old fractures; were pregnant or lactating women; or who were newly diagnosed patients who need calcium intervention. A full list of inclusion and exclusion criteria is provided in Supplement 1.

Methods

Control group

Patients in the control group were provided conventional orthopedic treatment. This consisted of no weight-bearing on the lower limb joint, and muscle training for 8 weeks after operation, including active and passive exercises.

Intervention group

Patients in the intervention group received the same conventional orthopedic treatment, as well as a TEAS treatment by a TEAS apparatus operator (acupuncturist). An electrical apparatus (KWD-808I pulse acupuncture treatment apparatus, Changzhou, Yingdi Medical Co., Ltd, Changzhou, China) was used in this study. The 30-minute treatments were delivered 3 times per week (every other day) for 8 weeks, making a total of 24 treatment sessions over the course of this study. A multidisciplinary team including acupuncturists, rehabilitative physicians, nurses, and physical therapists conducted the trial.

Patients had electrodes from the TEAS apparatus inserted at traditional acupuncture points including bilateral SP6 (Sanyinjiao), BL23 (Shenshu) and ST36 (Zusanli) in **Figure 1**. The acupoint location refers to the P.R. China National Standard "Acupoint Name and Location" (GB/T 12346-2006) [44]. The stimulation frequency was set to 2 Hz/100 Hz, and the fixed current intensity was uniformly 10 mA. For inpatients, TEAS was provided by an acupuncturist in the first week. At the same time, basic orthopedics treatment was completed by a professionally qualified rehabilitation therapist. Before the

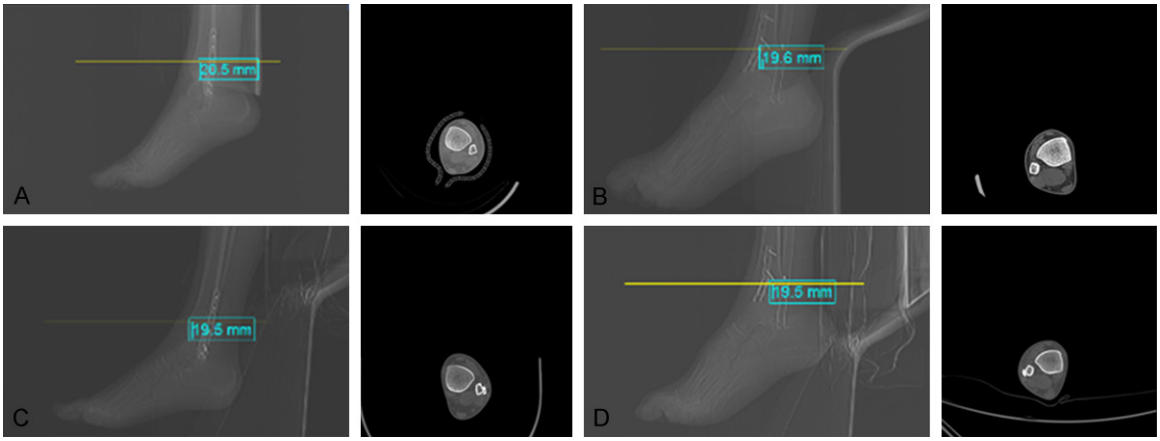

Figure 3. 2 cm above the ankle joint CT-attenuation values on CT scans. Notes: Example of ankle bone CT-attenuation values were shown above the image placing a region of interest (ROI) over an area about 300 mm^2, of 2 cm on the ankle joint, were measured by the same designated radiologist. The four patients, A. CT value of ankle joint before treatment in treatment Group. B. CT value of ankle joint after treatment in treatment group. C. CT value of ankle joint before treatment in control group. D. CT value of ankle joint after treatment in control group. HU, Hounsfield unit; CT, computed tomography.

project was launched, acupuncturists, rehabilitation physicians and nurses were trained in the operation methods and procedures of TEAS, acupoint positioning, and basic orthopedic therapy, to standardize their operation. Discharged patients and their families also received standardized operation process training. Training content was as follows: therapeutic point selection of the three acupoints positioning (each patient had an acupoint positioning guide); operation procedures for TAES (each patient had an instrument operation manual); and basic orthopedic treatment content (such as active and passive weight-bearing lower limb joints, and muscle training). Medical professionals regularly monitored the study to ensure trial quality.

To perform quality control in discharged patients, telephone interviews and WeChat video were adopted to monitor and record the participants' responses after treatment, including: (1) monitoring the operation process of the treatment; (2) monitoring the accuracy of acupoint positioning; (3) inquiring about and recording adverse reactions and treatment instructions (for severe pain, remove the electrode immediately and stand it; for skin redness, reduce power or suspend use in time); (4) the use of the electrode sheet and whether it needed to be mailed; (5) reminding patients of the visit to the hospital for a check after eight weeks.

Outcome measures

Main outcome measures: Computerized tomography (CT) attenuation values (Hounsfield units [HU]), measured by CT scans at week 8, reflected bone loss. This was compared to a baseline measurement by placing a region of interest (ROI) over an area covering 300 mm^2 of 2 cm on the ankle joint (**Figure 3**).

Secondary outcome measures: (1) Change in bone turnover markers (BTM) from baseline to week 8, which was used to predict rapid bone loss and dynamic change processes in bone metabolism [45, 46], with high sensitivity and strong specificity [47]. BTMs can be divided into bone formation markers and bone resorption markers. Bone formation markers include serum alkaline phosphatase (ALP), procollagen type I N-terminal propeptide (PINP), and bone glaprotein (BGP). Bone resorption markers include C-telopeptide of type I collagen (CTX) and Ca/Cr. (2) Change in BMD, which was measured by DXA from baseline to week 8. (3) Electrochemical methods were used to detect blood phosphorus and blood calcium in plasma (general biochemical markers in bone metabolism markers). All the assessments are presented in **Table 1**.

Adverse events

Adverse events were recorded in detail, including severity and its relationship with TEAS. The

Table 1. Locations of acupoints for TEAS

Acupoints	Location
Zusanli (ST36)	3 cun below Dubai (ST35), and one finger-width lateral to the anterior border of the tibia.
Sanyinjiao (SP6)	On the tibial aspect of the leg, posterior to the medial border of the tibia, 3 cun superior to the prominence of the medial malleolus.
Shenshu (BL23)	In the spine area, under the spinous process of the second lumbar vertebra, 1.5 cun lateral to the posterior midline.

1 cun is defined as the width of the patient's thumb or the length of the middle section of the middle finger. Dubai (ST35): on the anterior aspect of the knee, in the depression lateral to the patellar ligament.

possible relationship with TEAS was used to categorize these as treatment-related or non-treatment-related.

Statistical analysis

Given that this was a pilot trial, the sample size was determined to be n=60 participants based on the minimum sample size for a pilot trial [48]. Assuming a 25% dropout rate, a total of 80 patients were required.

Statistical analysis was performed using SAS version 9.4. Continuous variables were represented by mean values ± standard deviation or interquartile range as appropriate. Categorical variables were represented by composition percentage. Analysis of variance or χ^2 test was used to compare demographic data with other baseline data, and the student *t*-test or Wilcoxon rank-sum test was used to compare continuous variables. Categorical variables were analyzed by χ^2 test, Fisher exact test or Wilcoxon rank-sum test. Among these, χ^2 test was used to examine the gender and condition of the affected limb in the demographic data, while the student *t*-test was used to analyze age, height, weight, and BMI. Fisher exact test was used to examine the cause of fractures. Wilcoxon rank-sum test was used to analyze the primary outcome (changes from baseline in CT attenuation values), which showed a non-normal distribution data. The changes from baseline in secondary outcomes including ALP (%), Ca/Cr (%), BGP (%), P1NP (%), CTX (%), and blood calcium (%) and BMD-T values, showed a non-normal distribution and were analyzed by Wilcoxon rank-sum test. A student t-test was used to examine the bloods phosphorus change rate (%) between the two groups. All statistical analysis used two-sided tests, and $P<0.05$ was considered a significant difference.

Results

Clinical data

Between November 2020 and November 2021, a total of 170 participants were screened. Of this 170, 90 participants were excluded by either not meeting inclusion criteria (n=5) or declining to participate (n=85). A total of 80 participants were enrolled and allocated into two groups. 69 participants completed the trial, with 11 (13.8%) participants dropping out (5 in the intervention group and 6 in the control group). Reasons included the COVID-19 pandemic, lost contact, personal reasons, and transfer to another hospital (**Figure 1**). There was no significant difference in age, gender, BMI, ankle fracture, or cause of fracture between the two groups (all $P>0.05$), suggesting that the two groups' clinical data were similar at baseline (**Table 2**).

Comparison of CT attenuation values

Before treatment, no significant difference was identified in CT attenuation values between the two groups. After treatment, the CT attenuation values decreased more in the intervention group than in the control group (-55.2 [-93.6, -37.6] HU vs -27.5 [-79, -6.4] HU, $P<0.05$). This difference in CT attenuation values between the two groups after treatment was statistically significant. See **Table 3**.

Comparison of BTM

(1) Bone formation markers: after treatment, ALP in the treatment group was smaller than in the control group (23.82% vs 28.13%, $P>0.05$). The increase of BGP in the intervention group was less than that in the control group (9.49% vs 21.01%, $P>0.05$); PINP in both groups increased, and the degree of increase in the

Table 2. Baseline patient characteristics

Variable	Intervention group (n=35)	Control group (n=34)	*p*
Sex			0.9516
Men	28 (80.00)	27 (79.41)	
Women	7 (20.00)	7 (20.59)	
Age at fracture, mean (SD), y	32.97 (5.91)	32.56 (5.61)	0.7672
Height, mean (SD), cm	169.91 (6.45)	172.41 (6.33)	0.1091
Weight, mean (SD), kg	71.59 (10.18)	73.51 (14.78)	0.5314
BMI, mean (SD), kg/m^2	24.77 (3.00)	24.71 (4.64)	0.9510
Ankle fractured			0.7166
Left	18 (51.43)	16 (47.06)	
Right	17 (48.57)	18 (52.94)	
Cause of fracture			0.8112
Crushing	2 (5.71)	2 (5.88)	
Sporting injury	4 (11.43)	3 (8.82)	
Ground falls	13 (37.14)	13 (38.82)	
Falling from height	5 (14.29)	6 (17.65)	
Road traffic Incident			
Car	6 (17.14)	6 (17.65)	
Electric bicycle	3 (8.57)	2 (5.88)	
Motorbike	2 (5.71)	1 (2.94)	
Bicycle	0 (0.00)	1 (2.94)	

Abbreviations: TEAS: Transcutaneous Electrical Acupoint Stimulation; BMI: Body Mass Index; y: Year; cm: Centimeter; kg, Kilogram; m: Meter.

intervention group was greater than in the control group (49.17% vs 47.18%, *P*>0.05). However, there was no significant difference in bone formation markers (ALP, BGP and PINP) between the two groups.

(2) Bone resorption markers: after treatment, the increase of Ca/Cr in the intervention group was less than in the control group (1.65% vs 27.07%, *P*>0.05). The increase of CTX in the intervention group was greater than in the control group (17.14% vs 4.62%, *P*>0.05). However, there was no significant difference in bone resorption markers (Ca/Cr and CTX) between the two groups. See **Table 4**.

Comparison of blood phosphorus and blood calcium

After treatment, blood phosphorus in the intervention group decreased by -3.1%, and did not change in the control group. The difference between the two groups was not significant (*P*>0.05). Blood calcium increased in both groups, and the increase in the intervention group was higher than in the control group (8.48% vs 6.73%, *P*>0.05). See **Table 5**.

Comparison of BMD

(1) DXA was used to measure the lumbar spine and greater trochanter of the femur, and after treatment it was found that the BMD (T-score) of the control group had decreased by 0.1 compared to the intervention group. Thisdifference was not significant (*P*>0.05). See **Table 6**.

(2) After treatment, the BMD (g/cm^2) of the lumbar spine in both the control group and the intervention group both increased by 0.02. The difference was not statistically significant (*P*>0.05). The decrease in the femoral neck BMD (g/cm^2) value in the intervention group was higher than in the control group (0.0195 vs 0.0105, *P*> 0.05). The decrease of BMD (g/cm^2) of the trochanter in the control group was significantly higher than in the intervention group (0.0165 vs 0.007, *P*> 0.05). Lastly, the decrease of total body BMD (g/cm^2) value of the control group was higher than in the intervention group (0.0225 vs 0.0025, *P*>0.05). See **Table 7**.

Adverse events

A total of three adverse events occurred: one in the intervention group (infection in ankle) and two in the control group (infections in lower leg). There were no serious adverse events, and the events that occurred were mild and irrelevant to TEAS. See **Table 8**.

Discussion

In our trial, we found that CT attenuation values in the intervention group decreased more than in the control group after 8 weeks of treatment. These data show that TEAS action is desirable and prevents bone loss. We conclude that TEAS may be recommended for patients after surgical fixation of ankle and foot fractures.

To our knowledge, this is the first trial evaluating the effects of TEAS on bone loss in patients after surgical fixation of ankle and foot frac-

Table 3. Primary outcome for intervention group and control group

Variable	Intervention group (n=35)	Control group (n=34)	*p*
CT-attenuation values			0.0303
Median (interquartile range, IQR), HU	-27.5 (-79, -6.4)	-55.2 (-93.6, -37.6)	

Abbreviations: CT: Computed Tomography; HU: Hounsfield Units.

Table 4. Differences in bone metabolism markers between observation group and the control group at 8 weeks after treatment

Changes of variables	Intervention group (n=35)	Control group (n=34)	*p*
ALP (%)			0.4616
Median (interquartile range, IQR)	23.19 (7.14, 39.24)	28.13 (5.88, 44.29)	
Min, Max	-70.35, 163.83	-18.82, 96.00	
Ca/Cr (%)			0.7037
Median (interquartile range, IQR)	1.65 (-39.67, 97.42)	27.07 (-44.10, 85.27)	
Min, Max	-87.84, 420.82	-86.76, 638.12	
BGP (%)			0.3574
Median (interquartile range, IQR)	9.49 (-7.07, 36.08)	21.01 (2.12, 39.94)	
Min, Max	-21.7, 180.16	-22.06, 264.03	
P1NP (%)			0.6855
Median (interquartile range, IQR)	49.17 (2.00, 83.61)	47.18 (11.57, 89.82)	
Min, Max	-19.36, 690.51	-61.64, 330.68	
CTX (%)			0.7966
Median (interquartile range, IQR)	-17.14 (-31.37, 45.71)	-4.62 (-22.22, 15.22)	
Min, Max	-60.00, 190.16	-59.57, 114.81	

Abbreviations: ALP: Serum Alkaline Phosphatase; PINP: Procollagen Type I N-terminal Propeptide; BGP: Bone Glaprotein; CTX: C-telopeptide of Type I Collagen.

Table 5. Differences in blood calcium and phosphorus between observation group and the control group at 8 weeks after treatment

Change invariable	Intervention group (n=35)	Control group (n=34)	*p*
Blood calcium (%)			0.5436
Median (interquartile range, IQR)	8.48 (4.31, 12.62)	6.73 (1.8, 14.81)	
Min, Max	-6.09, 21.97	-30.12, 31.94	
Blood phosphorus (%)			0.1786
Mean (SD)	-4.56 (20.26)	1.80 (18.16)	
Min, Max	-38.6, 35.16	-33.71, 57.78	

tures. Moreover, this trial innovatively explores whether BMD changes continuity after just 8 weeks of TEAS, whereas BMD is shown to change in 6 months in other studies. In this two-arm randomized clinical pilot trial, we analyzed the effectiveness of TEAS on bone loss in patients with immobilization after surgical fixation of ankle and foot fractures. Interestingly, regarding the secondary outcomes (BTM, blood phosphorus, blood calcium) there were no statistically significant differences between the two groups. We speculated that the reason was due to a lack of statistical power because of the inadequate sample size and small differences in between-group effects.

There are some limitations in this study. First, since patients are only hospitalized for one week after orthopedic treatment and then discharged, the post-discharge treatment could only be performed by patients and their families. To compensate for this limitation, in the future, professional acupuncturists and orthopedic doctors will train patients in a rigorous

Table 6. BMD (T-score) by DXA between study groups at 8 weeks changed from baseline

Change variable	Intervention group (n=35)	Control group (n=34)	*p*
BMD-T			0.3555
Median (interquartile range, IQR)	0 (-0.3, 0.1)	-0.1 (-0.3, 0.1)	
Min, Max	-3.4, 0.4	-1, 1	

Abbreviations: BMD: Bone Mineral Density; DXA: Dual Energy X-ray Absorptiometry.

Table 7. BMD (g/cm^2) by DXA between study groups at 8 weeks, change from baseline

Change in variable	Intervention group (n=35)	Control group (n=34)	*p*
Lumbar spine BMD, g/cm^2			0.9839
Mean (std)	0.02 (0.03)	0.02 (0.03)	
Median (q1, q3)	0.017 (-0.003, 0.04)	0.01 (-0.013, 0.042)	
Min, Max	-0.069, 0.072	-0.037, 0.102	
Femoral neck BMD, g/cm^2			0.8983
Mean (std)	-0.03 (0.11)	-0.02 (0.05)	
Median (q1, q3)	-0.0195 (-0.057, 0.02)	-0.0105 (-0.045, 0.012)	
Min, Max	-0.555, 0.097	-0.225, 0.048	
Trochanter BMD, g/cm^2			0.5812
Mean (std)	-0.03 (0.08)	-0.02 (0.05)	
Median (q1, q3)	-0.007 (-0.059, 0.02)	-0.0165 (-0.051, 0.006)	
Min, Max	-0.376, 0.08	-0.145, 0.091	
Whole-body BMD, g/cm^2			0.1965
Mean (std)	0.01 (0.18)	-0.02 (0.04)	
Median (q1, q3)	-0.0025 (-0.035, 0.021)	-0.0225 (-0.041, 0.003)	
Min, Max	-0.436, 0.9012	-0.127, 0.046	

Table 8. Adverse events

	Intervention group (n=35)	Control group (n=34)
Total	1	2
Serious adverse events	0	0
Redness of the skin	0	0
Pain	0	0
Infection	1	2

Notes: Adverse reactions were recorded for all subjects, and an adverse reaction event that occurred continuously in the same subject was recorded as an adverse reaction event.

and standardized way during the week of hospitalization, on both TEAS and orthopedic treatments. Throughout, WeChat video and voice call will be used for quality monitoring, and patients with adverse reactions will be asked to come back to the hospital for a checkup. Secondly, the patients could not be blinded due to the nature of TEAS treatment, however all the outcomes were objective and obtained from laboratory tests or machine measurements. Therefore, the absence of blinding was not considered as a factor affecting the results of this trial. Thirdly, we could not judge the subjective feelings of patients due to the lack of subjective outcomes. Lastly, this is an exploratory study with a small sample size, which results in low statistical power.

However, there are some notable strengths. The measurement of CT attenuation values, for patients admitted to hospital with foot and ankle fractures, allows for bone density measurements without additional radiation exposure and costs. Previous research suggests that CT attenuation values can be used to identify patients' bone loss [49-50], and the value (HU) from CT scans of the wrist is a new way to measure low BMD [49]. Additionally, it was noted that patients with osteoporosis had significantly lower CT attenuation values at all vertebral levels. Therefore, another benefit of using CT attenuation values are that it can accurately identify patients with osteoporosis, who have not been screened by

DXA, where a lower value (HU) indicates less bone density. Moreover, when comparing CT and DXA scans, it was found that 50% of patients with CT measurement results showed a risk of osteoporosis and vertebral compression fractures. However, the BMD results from DXA were non-osteoporotic (DXA showed false negative results) [51]. Most importantly, we observed significant differences in the primary outcome (CT attenuation values), which demonstrated the addition of TEAS was able to prevent bone loss more than basic orthopedic treatment alone.

In order to understand the dynamic change processes of bone metabolism, we needed to evaluate the bone metabolism markers. Measuring bone turnover markers is helpful to predict rapid bone loss. In addition, subsequent changes in BMD can be predicted based on the changes in bone turnover within 4 weeks, which can provide early and effective prognostic measures [52]. However, in our study, there was no statistical evidence that bone metabolism (BTM, blood calcium and phosphorus) differed between groups. We speculate that no significant effects were found in bone metabolism due to two factors. One is that the small sample size resulted in insufficient testing power, so that the small treatment effects were missed. On the other hand, there are many influencing factors for bone metabolism markers in hematuria such as food intake [53], exercise status [54], renal dysfunction [55], effects of drugs (such as heparin [56], glucocorticoid [57]), and the coupling relationship between bone resorption and bone formation [58], that each can affect the results.

In addition, the International Federation of Clinical Chemistry and Laboratory Medicine recommends using serum PINP as a bone formation marker, and serum CTX as a bone resorption marker, making it a reference analyte for bone turnover markers in clinical research [59, 60]. In our study, after 8 weeks of treatment, the serum CTX results of the intervention group and the control group decreased, and the value of the intervention group was more than that of the control group (17.14% vs 4.62%, $P>0.05$). Moreover, serum PINP in the intervention group and the control group increased after the treatment, and the increase in the intervention group was higher than in the control group (49.17% vs 47.18%, $P>0.05$). However, the difference was not statistically significant. The P1NP and CTX changes from baseline (%) showed positive trends, and we hypothesize that between the groups significant effects were not found primarily due to insufficient sample size. In the future, sample size should be expanded to verify this.

Conventionally, BMD measured by DXA is the gold standard for the diagnosis of osteoporosis [61]. It is simple and fast to operate and does little harm to the body [62]. However, the DXA vertebral body BMD measurement is used only to predict the overall fracture risk, and therefore is difficult to use to predict the absolute risk in some individuals [63, 64]. As well, several studies suggest that BMD may change after about 6 months [65-67]. This is the reason CT attenuation values were used as the primary outcome measure for this study, with BMD as a secondary outcome measure. Additionally, it was hoped to explore whether bone density changed within the 8-week treatment period. DXA was used to scan the participants' L1-L4 and femurs, to record and analyze any changes in BMD [68]. According to the results, the difference between the two groups was not statistically significant, so it cannot be concluded that there was a difference in bone density between the two groups. We hypothesize that no significant effects were found in BMD, due to an insufficient test period (our trial was an 8-week treatment), but that the BMD will change in 6 months [69, 70]. Consequently, expanding the test period may be of value in future trials.

Conclusion

In conclusion, 8 weeks of TEAS may represent a treatment for bone loss in patients with immobilization after foot and ankle fracture surgery. CT attenuation values as a primary outcome were feasible in this study, negating the need for additional radiation exposure and cost. Since this was a small sample size pilot study, the efficacy of TEAS may require confirmation by an adequately powered trial.

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Disclosure of conflict of interest

None.

Abbreviations

TEAS, Transcutaneous electrical acupoint stimulation; BTM, Bone turnover marker; BMD, Bone mineral density; PEMF, Pulsed electromagnetic fields; HU, Hounsfield units; CT, Computed tomography; DXA, Dual energy X-ray absorptiometry; ALP, serum alkaline phosphatase; PINP, Procollagen type I N-terminal propeptide; BGP, Bone glaprotein; CTX, β-cross-linked C-telopeptide of type I collagen.

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Supplement 1

The inclusion criteria were as follows:

(1) age 18-45 years old (either sex);

(2) satisfied with the above-mentioned key diagnosis points and immobilization needs of foot and ankle injury;

(3) the admitted patient has a clear consciousness;

(4) the length of internal fracture fixation should not exceed 15 cm above ankle joint;

(5) the patient is informed and agree to sign an informed consent form.

Exclusion criteria were included:

(1) patients with endocrine diseases (thyroid, parathyroid, gonad, adrenal diseases and etc.) that affect bone metabolism, immune diseases such as rheumatoid arthritis, digestive tract, liver and kidney diseases that affect calcium and vitamin D absorption, and malignant diseases such as multiple myeloma;

(2) long-term use of glucocorticoids, antiepileptic drugs, estrogen, heparin, progesterone and other drugs affecting bone metabolism, various congenital and acquired bone metabolic disorders;

(3) patients who took calcium, calcitonin, vitamin D and other drugs affecting bone metabolism within 30 days;

(4) patients with severe infection of limbs, or pathological, old fractures or other fractures of limbs, and severe heart, liver, kidney, lung, blood, and endocrine system diseases;

(5) history of severe digestive system disease, kidney disease, connective tissue disease, malignant tumor, no history of other affecting bone metabolic disease;

(6) pregnant or lactating women;

(7) patients with a stent placed in body or a history of joint replacement;

(8) patients with known heart disease and patients with pacemakers;

(9) patients who have been treated by this study within the past 30 days;

(10) patients with allergies, poor compliance, or mental disorders who cannot cooperate with the study;

(11) newly diagnosed patients who need calcium intervention.